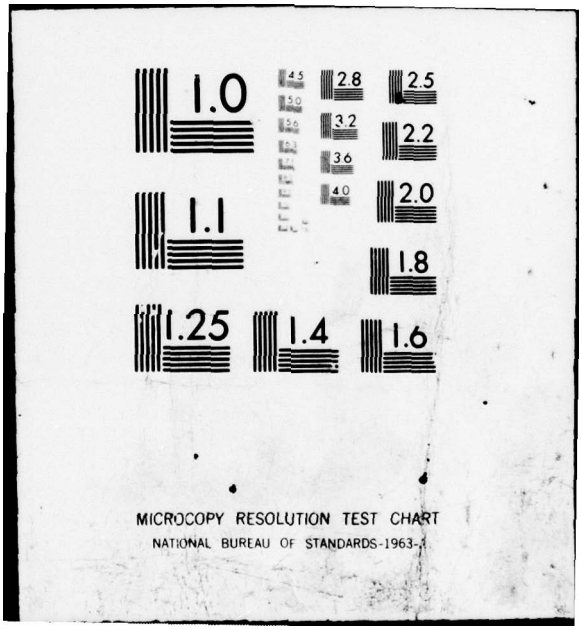
AD-A080 030 CALIFORNIA UNIV SAN FRANCISCO F/G 6/5
STUDIES ON MOLLUSCICIDAL AND OTHER PROPERTIES OF THE ENDOD PLAN--ETC(U)
JUN 79 A LEMMA, D HEYNEMAN, H KLOOS N00014-76-C-0218
UNCLASSIFIED NL

1 OF 6
AD
A080030


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

STUDIES ON THE MOLLUSCICIDAL AND OTHER PROPERTIES OF THE ENDOD PLANT *PHYTOLACCA DODECANDRA*

(12)

LEVEL II

Compiled and edited by
Aklilu Lemma, Donald Heyneman and Helmut Kloos

(9) Final Report.

(11) June 1979

(6) # STUDIES ON MOLLUSCICIDAL AND OTHER PROPERTIES OF THE ENDOD PLANT, PHYTOLACCA DODECANDRA.

With Special Emphasis on the Epidemiology of Schistosomiasis In Ethiopia and the Possibility of Localized Control Using Endod as a Molluscicide on a Community Self-Help Basis

Source: University of California, San Francisco

(10) AKLILU LEMMA, DONALD HEYNEMAN and HELMUT KLOOS

(12) 537

Prepared for:

MICROBIOLOGY PROGRAM
CODE 443
OFFICE OF NAVAL RESEARCH
DEPARTMENT OF THE NAVY
ARLINGTON, VIRGINIA 22217

Attention: DR. ARTHUR J. EMERY
PROGRAM DIRECTOR, MICROBIOLOGY
BIOLOGICAL AND MEDICAL SCIENCES DIVISION

(15) CONTRACT - N00014-76-C-0218
PROJECT NO. - NR 204-011

I. PREFACE

Donald Heyneman

In past years attempts to control schistosomiasis and other trematode diseases of mankind and domestic animals have chiefly focused on destruction of the snail intermediate hosts. These efforts often were undertaken as well organized team operations conducting the molluscicide activity, followed by a period of confidence based on the observation of dramatic subsidence of snail populations and disease transmission. Too often a sense of disappointment and frustration later developed with the rapid resurgence of snail populations after cessation of molluscicide operations, followed by a return of human infection to precontrol levels. Any well planned intensive focal molluscicide effort, usually a relatively costly operation, can indeed reduce snail populations dramatically and bring down incidence of transmission and ultimately reduce the prevalence of disease. However, cost-effectiveness constraints have in the past restricted application of long-term molluscicide efforts to valuable and environmentally controllable localities, such as highly developed irrigation schemes for sugar or cotton production, or to regions of sustained experimental study, such as the Rockefeller Foundation-supported program in St.Lucia in the Caribbean.

With the recent development of promising new anti-schistosome chemotherapeutic compounds, such as oxamniquine against S. mansoni, metrifonate against S. haematobium, and praziquantel against all three human species (as well as cestodes), a new confidence based on chemotherapeutic efficacy is on the rise. The role of environmental control has also received impetus from evidence of success in China using snail habitat alteration, in which canals were filled and replaced and banks modified to prevent breeding of the amphibious vector snails, in addition to massive, sustained use of molluscicides (Trop. Med. Hyg. News 28(3): 1-62, 1979. Suppl.). Improved standards of living and health education have proved in Puerto Rico to be effective means to reduce infection levels of snails and human contact with infected water, chiefly by improved sanitation and water supply (Negrón-Aponte and Jobin, Am. J. Trop. Med. Hyg. 28(3): 515-525, 1979). Another area receiving much emphasis currently is the effort to develop a prophylactic or therapeutic anti-schistosomal vaccine, for which there are grounds for "guarded optimism ... for ... experimental vaccination against schistosomiasis by the early 1980's ..." (Clegg & Smith, Adv. Parasit. 16, 1978).

Snail-borne trematode disease, especially schistosomiasis, has thus been marked by varying approaches to its control but a nearly unvarying pattern of disease spread in Africa and elsewhere. Although no single method is considered sufficient for disease control in most endemic areas,

the preferred approach has seen wide fluctuations in popularity. Currently, confidence in chemical snail control has fallen to a low ebb. "The control of schistosomiasis by molluscicíding, ... has proven to be essentially ineffective" (Kagan, Am. J. Trop. Med. Hyg. 28(3): 429-439, 1979). Molluscicíding is being relegated to a secondary or a highly localized role as concern over possible human or animal toxicity and other environmental effects (especially fish kills) have risen, along with a sense of helplessness in the face of the astonishing ability of snail populations to reestablish themselves in endemic areas.

Snail control requires continuous operations so long as infected humans or animal reservoirs are available to reseed the infection at water contact sites. Molluscicíding efforts have also grown more costly with sharply rising hard-currency prices of the imported synthetic products generally used (Bayluscide® and Frescon®), with the continuing need to train, equip, and maintain the molluscicide delivery teams. This has become an increasingly oppressive burden on the host country, as many of the developing world's most impoverished nations are included in endemic areas.

The developed nations, working through WHO and other international organizations, largely support these projects. But they too are unable or unwilling to sustain them indefinitely. The term project focuses on the problem. So long as snail control is part of a short-term schistosomiasis project, organized and funded from outside the country or the endemic area, there will be an initial success, but then a need to maintain a long-term, continuing control plan, infrastructure, and financial support.

A technique therefore is needed to link external aid with a local, self-sustained means to control snails. It is one such approach to autonomous schistosomiasis control, discovered in 1965 in Ethiopia by Aklilu Lemma, that has been supported by the international research community. Under Lemma's energetic leadership an impressive series of collaborative studies both in Ethiopia and abroad has been undertaken, resulting in an extensive series of publications, several patents, and a number of published studies. It is to this area of research that the following Final Report is addressed. The report consists of an introduction and historical review of studies conducted and findings, followed by a compilation of published materials and unpublished documents and reports dealing with this innovative and promising approach to long-range schistosomiasis control. Though tested successfully on a relatively small scale in Adwa, it is still untried on a regional basis or as a major commitment for long-term disease containment. Inclusion of previously published material (with permission - see Acknowledgements section) along with other documents and information brings together literature on this topic that is now scattered in journals of restricted distribution or specialized subject matter not often available to health officials, epidemiologists or biomedical researchers in the field. It is hoped that the availability of such a compilation will focus attention on this approach and renew interest in further research on the use of endod or other natural products

as a possible means to control host snails at moderate cost or even local profit, based on a self-help methodology that directly involves the people who are most affected and would have most to benefit.

The stimulus of Lemma's work on this problem has galvanized health and parasitic disease research in Ethiopia. It led initially to the establishment and development of an Institute of Pathobiology under his directorship as a focus for the conduct of this work. The Ethiopian Science Foundation (ESF) was established in 1972 as a result of patents generated from research on endod done at Stanford Research Institute in California and Tropical Products Institute in London. The ESF was intended to serve as an independent national organization for funding scientific and technological investigations in Ethiopia. It has since been absorbed in a more comprehensive, higher level governmental organization, the Ethiopian Science and Technology Commission, to which Lemma has made significant contributions during his service as Dean of the Faculty of Science of the Haile Sellassie I University in the mid-1960's and in his more recent role and direct contribution as Chief Advisor on Science and Technology to the Chairman of the Provisional Military Government of Socialist Ethiopia during 1974 and 1975. Lemma's discovery of the molluscicidal and other properties of endod, a saponin compound concentrated in the dried berries of the soapweed plant, Phytolacca dodecandra, has led to many interdisciplinary studies and a variety of international collaborations, hopefully a model for the study of other such natural products.

This common endemic shrub is familiar to many peoples of East Africa and has been recognized in particular by dwellers of the Ethiopian highlands as a readily available and useful laundry soap. The astonishing range of activites and potential application of this natural product emphasizes its economic potential beyond that of snail control. However, for the purpose of this report attention is focused on endod as a natural plant product, locally grown, that potentially could be employed as an inexpensive molluscicide processed as a village-level activity by residents of schistosomiasis-endemic areas. The possibility of such a locally-administered, self-help means to keep snail populations under continuing control is the compelling aspect of this approach. A number of problems must first be solved, however, to make the method feasible: large-scale cultivation of selected high-yield insect-resistant strains of the plant; low-cost (labor but not capital intensive) means to process the berries and formulate an effective product; a safe, simple method of dispensing the molluscicide; and a centralized method of monitoring the results and controlling the use of endod. Leads to the possible solution of each of these problem areas have been investigated. Discovery of many possible additional uses for endod have been made along the way, which may prove to be important sources of revenue and economic justification for continuation of the research program initiated and sustained under ONR support.

This is the potential. Fulfillment of the promise will require multiple research effort and multiple research support. Schistosomiasis control is a key to human development in Africa. This attempt to involve local populations in their own health improvement is of particular interest. It gives villagers in disease-endemic, impoverished areas an opportunity to contribute directly and personally to the control of an infectious agent that erodes their health and energy, especially among the children, reducing their chance to fulfill their genetic and social potential. Synthetic chemical molluscicide with imported products and centrally controlled teams involve little local initiative or participation. This has been considered an advantage in terms of speed and efficiency. The plan can be designed and implemented centrally without village agreement, understanding, or participation. But that has also proved to be its greatest weakness. Only when village participation is a necessity can we hope to see the long-term continuity of effort that is required. If villagers can become responsible for control of snails in their own streams or irrigation canals (as occurred in China under vastly different circumstances), one can envisage significant social improvement, health awareness and involvement. Snail control based on village participation could possible become a pattern leading to a new sense of regional responsibility and pride. Perhaps far more than snail and even schistosomiasis control may be involved.

II. ACKNOWLEDGEMENTS

The work reported here is a product of the efforts of many individuals and organizations, truly an international collaborative undertaking. We are grateful to each contributor, each researcher and each participant in the many projects and activities that have been part of this program over a 15-year span. We would like to give our thanks to each person individually, but must be content with this general statement of acknowledgement and our expression of gratitute to the hundreds of persons involved at every level of paticipation and responsibility. We believe that the more persons who become personally involved in work of this sort, especially among the villagers and residents of the endemic areas, the greater the final impact and the better the chance to develop a local means of continuous, self-sustaining disease control.

Specific acknowledgements for individual studies are indicated in each of the articles reproduced in this report. We would like, however, to express our special appreciation and thanks to the administration and supporting staff of the former Haile S lassie I University (now Addis Ababa University); the Stanford Research Institute in Menlo Park, California; the G.W. Hooper Foundation, University of California Medical Center in San Francisco; the World Health Organization; the U.S. National Institutes of Health; the Rockefeller Foundation; the United Kingdom Ministry of Overseas Development; the Swedish and Netherlands governments' foreign assistance officers; and the United States Office of Naval Research.

We particularly wish to express our most sincere thanks and appreciation to Dr. Arthur J. Emery, Director of the Microbiology Program of the ONR, for his continuing scientific support and concern, made especially pleasant by his warm friendship and unfailing interest over a period of eight years of ONR sponsorship. Without his personal involvement and continued belief in this approach to disease containment, we would not have been able to advance our work to its present stage.

Since most of the articles assembled and reproduced in this report have previously been published, we have obtained permission from the authors and/or journal publishers for their inclusion here. We would like to acknowledge with thanks the permission of the following authors or publishers to reprint articles on endod or on associated epidemiological investigations on schistosomiasis in Ethiopia that resulted from the funded studies encompassed in this Final Report. The articles are listed here chronologically under general categories. The full references, alphabetically arranged within similar categories, including patents and unpublished reports, can be found following Chapter IV (Research on Endod and the Epidemiology of Schistosomiasis in Ethiopia) on pages 50-61. Material reprinted in the present Final Report is listed in the Table of Contents, pages ix-xiv. The editors are grateful to the authors and publishers for permission to reprint these here.

TABLE OF CONTENTS

V. ARTICLES, PUBLISHED AND UNPUBLISHED PAPERS, PATENT DESCRIPTIONS AND CORRESPONDENCE REPRINTED IN THIS FINAL REPORT:

page

page

page

III. INTRODUCTION

AKLILU LEMMA

1 THE SCHISTOSOMIASIS PROBLEM

1.1. Global Significance of Schistosomiasis

Schistosomiasis is now generally considered to be one of the most important and rapidly spreading parasitic diseases, a continuing threat to large-scale agricultural development in many tropical and subtropical parts of Africa, the Middle East, the Far East, some of the Caribbean Islands and many parts of South America. An estimated 200 million people are affected by this debilitating disease, with another 400 to 500 million exposed.

Schistosomiasis (known as bilharziasis in much of Africa) is a typical example of a man-made disease, Most developing countries in Africa and elsewhere depend on agriculture for their economic survival and advancement. Well-intentioned agricultural projects requiring irrigation canals have often resulted, unfortunately, in the creation of new breeding habitats for the snails in which these blood flukes multiply and from which they swarm to invade human skin. Infected and noninfected people brought together to work in these areas must drink from, bathe and labor in the same canals. All too often, human wastes are also excreted, washed, or drained into the same water. This deadly combination is all that is needed for rapid spread of schistosomiasis, especially among highly vulnerable children.

Large-scale agricultural development schemes indeed are needed, but settlers and laborers must remain healthy to be able to realize their economic potential. Schistosomiasis, with its debilitating effects on heavily infected individuals, can result in serious losses of manpower and less debilitating but more widespread energy loss as well as direct pathology. The frequent result is that some of the envisaged economic gains are not realized. Instead, increasing misery and social disruption of life patterns may become the chief by-products. No large-scale agricultural development plan should be carried out in endemic countries without first giving proper consideration to the economic and socioeconomic impact of diseases that may be an accidental by-product. Multifaceted schistosomiasis control and prevention efforts should be an integral part of the initial development plan, rather than the far more costly and difficult catch-up effort to cure disease and prevent its further spread after it has been established.

---

*Presently on secondment from the Ethiopian Government to the United Nations serving as Principal Scientific Coordinator of the United Nations Conference on Science and Technology for Development in New York.

National and international authorities concerned with land utilization and intensive development have only recently begun to be concerned about the increase in schistosomiasis prevalence following creation of irrigation schemes in most endemic areas of Africa, South America (particularly Brazil) and Asia. World Health Organization surveys and studies in Africa and the Middle East indicate that increased incidence of this disease is closely related to development of water resources and human habits associated with use of the water. Heightened incidence is not necessarily confined, however, to areas of expanding irrigation. Increased illness may also occur in relatively dry areas where man is augmenting the amount of water stored for future use, especially where sociocultural customs fit the epidemiology of schistosomiasis.

Because of the slow development of tissue granulomas and the chronic nature of the disease, the extent of damage or the burden imposed on infected individuals often is not fully realized until irreversible destruction to liver and other tissues has occurred. Although schistosomiasis is a calamitous private and societal imposition (frequently shortening the life span or imposing its pathological consequences without killing outright), its severe damage can also cause mortality, especially among children. Besides eliciting tissue damage, schistosomiasis also tends to reduce resistance to other infections. Perhaps its most insidious impact is the loss of energy which, along with the weakening effect of other parasitic infections, produces a state of lethargy--an all-too-common sight in tropical regions.

To convince public officials of the economic importance of this disease, attempts have been made to measure its effects in terms of socio-economic loss. These are based on quantification of reduction of work output by infected individuals and the general cost of treatment and control of the disease. The resulting statistics, usually rough and necessarily conservative estimates, measure only the obvious clinical effects, without considering the costs of social disruption and misery. The annual economic loss due to schistosomiasis in the Arab Republic of Egypt, for example, is estimated at 80 million Egyptian pounds; in Iraq, 6 to 10 million Iraqi dinars; Japan, $18 million; and the Philippines, 13 million pesos. (These figures are for the period 1964-1967. They are clearly outdated but no others are available.)

Numerous examples can be cited to illustrate the public-health importance of schistosomiasis. In the Egyptian province of Aswan, the incidence of Schistosoma haematobium (the bladder form of the disease) increased tenfold within three years from the introduction of perennial irrigation; its prevalence has now extended to affect large numbers of the resident population. The ecological and epidemiological impact of the Aswan Dam has often been referred to in the popular and scientific press. A similar level of infection with schistosomiasis has occurred in the Gezira area of the Sudan. The disease is on the rise in the newly developing Awash Valley of Ethiopia, where large-scale irrigated agricultural development involving sugar cane, bananas, and cotton production is underway. When the prevalence of schistosomiasis began to reach alarming proportions in Rhodesia's Umshandige Irrigation Scheme, the project had to be abandoned after 10 years of construction costing $10 million.

1.2. Schistosomiasis in Ethiopia

Until recently, schistosomiasis was assumed to be nonexistent in Ethiopia. Many parasitology texts, some still in use, ruled out the disease there on the assumption that the low temperatures of the highland areas would prevent the snail hosts from becoming established. Though true in part (see section 3 of this report), studies carried out by different teams over the last 10 to 20 years have shown that both the intestinal and urinary forms of the disease are widely distributed in different parts of the country. Generally speaking, intestinal schistosomiasis is widely spread in patches of isolated foci in most of the highland areas, while urinary schistosomiasis is more restricted to lower and warmer areas, such as the Awash, Wabi-Shebelle and Genale Valleys. (See enclosed distribution map for more details.)

Schistosomiasis in Ethiopia does, however, appear to be a relatively new disease, chiefly in isolated patches that would appear to lend themselves to relatively easy control measures. Nonetheless, its very wide distribution in the country, the abundance and capability of vector snails to breed and transmit the disease in different parts of the country, large scale agricultural developmental activities involving irrigation (particularly in the valleys), improvement of highways and the development of resort areas, and especially the rapid movements and migrations of infected and uninfected people are making this disease an increasingly important one that has elicited the serious concern of the Ethiopian government.

2. POSSIBLE CONTROL METHODS

2.1. Mass Treatment

Important recent progress has been made in developing effective, relatively nontoxic drugs for schistosomiasis, such as metrifonate, oxamniquine and praziquantel. Yet, mass treatment of schistosomiasis is still unavailable at an economically tolerable cost, and the need for sustained treatment and careful supervision of patients, especially those with untreatable tissue damage, further complicates the picture. However, even if ideal drugs become available, they would be of limited value in effectively controlling the disease so long as treated individuals would be promptly reinfected while their source of infection remained unchecked.

2.2. Vaccination

Although progress is also being made in immunological studies that eventually may lead to development of an appropriate vaccine for mass immunization against schistosomiasis, the possibility of having an effective prophylactic vaccine still lies far in the future. The immunological problems involved are immense. Unlike the single-celled bacterial or viral organisms for which reliable vaccines have been developed, schistosome worms are large and complex organisms, with multiple antigens of unidentified and varying immunological potency and protective effect. Current research in schistosomiasis is giving

top priority to this problem but even its staunchest advocates do not anticipate success in less than a decade and many others are far less sanguine.

2.3. Health Education and Sanitary Improvements

Health education and sanitary improvements should help immensely in the continuing struggle to control schistosomiasis. Considering the low level of education and living standards of the hundreds of millions exposed to infection, the unavoidable human dependency on water contact in many of the endemic regions, and the inevitable increased water contact in agricultural development with use of irrigation water, control of schistosomiasis through health education and improvement of sanitation alone is simply unrealistic. It is, of course, an essential component of a multiple control effort.

2.4. Improvement in Standard of Living

In Puerto Rico incidence and prevalence of schistosomiasis among the rural population was reduced significantly as a result of improved standards of living. A clean water supply to individual houses, effective health education with warning signs for children not to swim in infected waters, and provision of better sanitary conditions all aided in the island-wide effort. This was significantly augmented by a general rise of income at the individual level--perhaps the key to control of any infectious agent.

However, dramatic improvement in the standard of living of many developing countries is not expected in the near future, with burgeoning populations and increased economic pressures. It is clear that something must be done that can reach out to the village level where transmission occurs and where the problems of poverty and continual reexposure are greatest.

2.5. Snail Control

In spite of disappointments and serious problems (see Preface), a still widely employed approach to schistosomiasis control is by interrupting the life cycle of the parasite through killing its vector snails. Several potent snail-killing chemicals, molluscicides, have been developed and variably used in different parts of the world for over 50 years. The oldest and perhaps most widely used have been copper sulphate and sodum pentachlorophenate. These are still being used in China, but in recent years more effective and efficient molluscicides, such as Bayluscide® and Frescon®, have come on the market and largely replaced the older compounds.

None of these molluscicides is free of problems, as we have learned to anticipate. Copper sulphate, less potent that the others, is readily absorbed in water by both organic and inorganic matter; sodium pentachlorophenate is an irritant to handlers and is quickly inactivated by the sun's ultraviolet light. Frescon®, the most potent molluscicide currently available, is not ovicidal in concentrations sufficient to kill adult snails, is absorbed by organic and inorganic matter in water, and is effected by pH of the water. Bayluscide®,

despite partial inactivation by solar ultraviolet rays, genarally is considered to be the molluscicide of choice. Cost, danger to humans who handle the substance or are exposed to the treated water, lethality to other aquatic organisms, especially fish or food for fish, duration of potency, and relative efficacy are all critical considerations.

Practically speaking, any of these chemical products can be used to control snails. The key to their successful use, however, is through appropriately repeated application, reliable dosage and frequent monitoring, made possible only by diligent effort by a well-trained cadre supported by sufficient funds. Successful use also implies continuing purchase of large quantities of imported molluscicides over an indefinite period for treatment of large volumes of water in different and often remote parts of the affected country--at significant cost and demand upon scarce technically proficient personnel as well as the perennial scarcity or higher priority elsewhere of hard currency. The continued budgetary demand by foreign purchases of these molluscicides is particularly burdensome and inevitable, as has been repeatedly demonstrated by the rapid return and reestablishment of large snail populations and the virtual impossibility of their permanent extirpation.

As a result of the low priority of molluscicidal purchases and the lack of convincing proof of the "dollar benefit" from schistosomiasis control and because of the competing demands for the limited foreign currencies available, little has been done to prevent the rapid spread of this debilitating disease. The lack of an assured market for new molluscicides discourages industry from investing in research and development of new snail-killing agents. Since it seems unlikely that this situation will soon change, an alternative solution is required. To this end, the "best" molluscicides might well be those produced from local products, distributed locally and used as needed at the village level by a permanent cadre of appropriately-trained villagers. If other uses could be found for such a product, its advantages would be multiplied.

2.6. The Chinese Approach

According to Yokogawa (1972), there were an estimated 110 million people suffering from schistosomiasis in China during the prerevolution years. The leaders of the revolution, who envisaged this disease to be a potential threat to their rural development, decided to launch a major campaign against it as an integral part of their early agricultural reconstruction. Chairman Mao himself initiated and promoted the campaign against schistosomiasis. His poem of 1958, urging his people to new efforts to "stamp out the scourage," and the resulting campaign are described in Joshua Horn's "Away with All Pests" (1969).

By a concerted national effort, millions of people were involved in the campaign to eliminate snails from the Yangtze river and tributary water of China. They did this by all conceivable means, including removing individual snails by hand or chopsticks, by crushing them under soil fill as new ditches were dug and old ones filled, as well as by diverting waterways to destroy snails by desiccation. These enormous efforts, followed by continued village-level surveillance,

led to a significant reduction in transmission and prevalence of the disease in China.

Although the Chinese approach was successful in that country, how many developing nations now affected by this disease can mobilize such numbers of people and undertake such a vast snail control campaign on a continuing basis?

2.7. Japanese and Israeli Approaches

In Japan, Schistosoma japonicum and other snail-borne diseases were successfully controlled by massive use of sodium pentachlorophenate as a molluscicide and by lining newly constructed irrigation canals with concrete to prevent growth of vegetation on the banks to eliminate snail breeding and feeding sites.

Israel, which depends on irrigation of its arid land for citrus and other agricultural development, has protected itself from the problem of schistosomiasis by using sprinkler or soakage systems which avoid snail breeding possibilities and prevent schistosome transmission.

Unfortunately, neither of these approaches can be implemented in most developing countries where extreme scarcity of resources, funds, and trained personnel, and a multiplicity of administrative problems prevent use of such capital-intensive methods.

3. NEW TRENDS IN SCHISTOSOMIASIS RESEARCH

3.1. Learning to Live with the Parasites

As was noted, severity of the disease depends on the number of parasitic worms harbored and of eggs held in the tissues. Quantitative stool examination of heavily infected people and autopsies of individuals who were presumed to have died from the disease have shown that some individuals were infested with up to 4000 pairs of these worms. Infection with less than 100 worms is generally considered to be tolerable for a normal adult. According to recent studies by researchers of the British Medical Research Council, such low levels of infection have the added advantage of maintaining a degree of concomitant immunity which prevents subsequent infection by new schistosome larvae (cercariae).

The two commonly used ways to reduce disease is to eliminate established worms by chemotherapy or to eliminate intermediate host snails to prevent reinfection or new infections. Whereas most of the widely used drugs such as Hycanthone, Niridazole, Stibocaptate, or Tartar emetic are toxic when used in the quantities needed to expel all worms from an infected individual, lower doses that could expel 60 to 80% of the worms may be a useful route for periodic mass therapy that can safely be administered. Recent studies by the Rockefeller team working in St. Lucia in the Caribbean have demonstrated that worm burdens can indeed be suppressed and kept at very low levels by continued and regular administration of low doses of Hycanthone.

Such a "deworming" process, however, requires a well organized medical team to conduct regular surveys to determine the intensity of infection and to administer the drug under close supervision.

Laboratories in various countries are now actively engaged in the searching for and testing drugs for use in such mass treatment efforts and the newer compounds, such as oxamniquine, metrifonate and praziquantel, are most promising. However, this method alone will not be sufficient to control the disease.

Equally important for the foreseeable future is the prevention of infection of the snails that transmit the disease and control of their numbers. Regular application of molluscicides in disease transmission (water-contact) sites will keep the snail population low and thereby reduce the number of infected snails and subsequently the number of newly infected people and ultimately the number of worms per infected individual. The latter occurs from a combination of normal parasite death, continued attack on the parasites by the host's immune mechanism, and reduction of the number of new parasites available to enter human skin. The real problem, as was discussed earlier, is the high cost and hard currency requirement of available molluscicides.

This, then, led to a search for another molluscicide approach: development of a compound that could be produced locally and used, as needed by the village or community affected.

3.2. The Example of Endod

With this in mind, the molluscicidal property of endod, a natural plant product, has been actively studied in Ethiopia, the United States, the United Kingdom and elsewhere since 1964. Some of the promising results obtained suggest that this or similar locally produced plant products can be used to control schistosomiasis and other snail-borne diseases on a sustainable, community self-help basis. Endod is the Ethiopian name for the pokeweed plant, Phytolacca dedecandra. The berries of this plant have been used for centuries in Ethiopia and other parts of Africa as soap for washing clothes.

While making an ecological study to determine the distribution of schistosome-transmitting snails in a small stream in northern Ethiopia, Lemma in 1964 observed windrows of dead snails piled up at spots immediately downstream from sites where local people had done their laundry using endod. Areas farther up and downstream from these sites still had an abundance of live snails. This led to the discovery of the molluscicidal property of endod, on which extensive studies have since been made and some promising results obtained.

Some aspects of the multidisciplinary approach followed, both the encouraging results and the numerous problems encountered, are included in this report as examples of research and development problems and prospects in a developing country such as Ethiopia.

3.2a Endod as a Molluscicide

The molluscicidal property of endod has been investigated over the last 14 years in the Pathobiology Institute of the Addis Ababa University of Ethiopia, the Tropical Products Institute (TPI) in London, the Stanford Research Institute (SRI) in Menlo Park, California, the G.W. Hooper Foundation of the University of California, San Francisco, the Harvard School of Public Health, Boston, the U.S. Public Health Service laboratory and field stations in Puerto Rico, the U.S. Naval Medical Research Unit Number 3 (NAMRU-3) in Cairo, and at other laboratories in different parts of the world.

Over 40 scientific articles have been published on studies to date and several patents secured on different aspects of the processing of this plant product. Most of these publications were in research or medical journals of limited distribution, such as the Ethiopian Medical Journal, so work with this compound did not receive the wide circulation needed for a broad range of scientists and potential users to conduct additional studies and tests to advance more rapidly development of a product suitable for mass use. Nonetheless endod berries even without extraction and concentration of the active elements, have proven to be potent molluscicides to all species of snails tested, including those that transmit schistosomiasis, fascioliasis (liver fluke disease) and other snail-borne infections of animals and humans. The molluscicidal activity appears to remain stable under various physicochemical conditions and has proven to be safe to animals and humans at molluscicidal concentrations.

Birds are known to feed on the berries of the wild growing plants, though this aspect has not been studied in the laboratory. Aqueous suspensions of ground berries applied to economically important crops appear to serve as a useful fertilizer. As with most molluscicides, fish are affected at molluscicidal concentrations. However, since schistosome-transmitting snails usually breed in small streams or near shores of lakes that are relatively free from edible and economically important fish, the application of endod to control snails does not appear to affect significantly economic fish populations, and studies to date do not suggest that their food chains are involved, though this must be studied further.

In a five-year schistosomiasis pilot control study using endod in Adwa, the northern part of Ethiopia where the molluscicidal property of this substance was first discovered, the prevalence of S. mansoni in children between the ages of 1 to 5 was reduced from 50% at the start of the control project in 1969 to 7% after continuous control for five years (1973), a reduction of about 85%. Overall prevalence of the disease among all ages and sexes in Adwa (17,000) dropped from an initial 63% to 34% by the end of the five-year period. This was achieved by systematic application of crude ground endod berries collected from the immediate neighborhood of Adwa.

Annual disease surveys during the five-year control period (1969-1973) showed a progressive reduction both in prevalence and incidence of the disease in Adwa, while comparable figures remained more or less constant in the untreated nearby comparison village of Inticho, suggesting that the action was primarily due to application of endod to reduce the snail population. Ecological observations during these five years also indicated that endod had no obvious adverse affects on the microflora and fauna of the treated streams.

3.2b. Chemical Studies

Studies to isolate and identify the active principle in the endod berries led to the discovery of a new compound, oleonolic acid glucoside, which Stanford Research Institute chemists have named Lemmatoxin. Three procedures were developed to extract the active ingredients from the endod berries. In chnonological order, these are, first, a relatively involved method based on methanol extraction, followed by potassium hydroxide hydrolysis (two steps). This was first developed and patented by Tropical Products Institute chemists in 1971. Second, a simpler method, involving a one-step butanol extraction from an aqueous suspension of dried berries, was developed and patented by Stanford Research Institute investigators in 1972. Both of these procedures give an extract about 10 times more concentrated than the crude berries, but both have the disadvantage of depending on imported alcohols which again demand hard currency. This shortcoming was overcome by the third and perhaps most promising extraction procedure, based on fermentation techniques. It was discovered by an Ethiopian chemist working in his small laboratory at the Institue of Pathobiology in Addis Ababa (Tesfaye Lemma, 1975).

The fermentation-based extraction procedure depends on very practical and simple techniques, using yeast cells that are part of the normal flora of the berries. When ground endod berries are soaked in water and left in a warm place, they ferment rapidly and eventually separate into a clear supernatant which contains the active principle and a residue, which contains yeast cells and debris. The supernatant can easily be separated from the residue, and then can be concentrated by evaporation or dried into a fine powder, using small locally produced solar drying chambers. The resulting product can be prepared as a fine powder for dusting over water, flakes that can be prepared either to sink or float on water to attack specific snail habitat targets, emulsion concentrates for spraying, and briquettes of different hardness (by varying compression) for slow release in water. In the case of the briquettes, for example, farmers and villagers can easily be shown how many briquettes of endod and how often per week or per month they should be applied at predetermined spots, based on the water volume to be treated and the degree of hardness of the briquette needed to kill snails varying distances downstream. The simple drying and processing apparatus for this can be constructed by neighborhood high school or college students, or by the local blacksmith. Such apparatus should be simple enough for operation by village-based community health workers. The necessary background studies needed to refine and standardize these methods have not yet

been undertaken. This will require an interdisciplinary team of experts to test and develop approprite procedures.

The yeast cells that result from the fermentation process can then be washed, sun dried and used as high protein supplements for animal feed, particularly as additives for chicken feed, though, again, these prospects have yet to be fully developed.

Agronomic studies are also required to select endod strains with high molluscicidal potency, superior yields and higher resistance to insects, disease, and drought. Initial studies have been underway since 1972. Strains of the plant 2 to 3 times more potent than the original varieties have been developed by selective crossings and search for additional plants. These strains are now being selectively propagated for domestication and mass cultivation. However, again, more research is needed to intensify the work and develop an international or regional approach for selection, propagation and distribution of appropriate plant stocks.

Greenhouse studies on nutritional requirements and other biological characteristics of the endod plant also are being undertaken by agronomists in Ethiopia. Artificially induced mutations for selection of improved strains are part of this ongoing research. These studies, however, are very modestly funded and require both financial and intellectual support.

A major difficulty encountered is high susceptibility of the Phytolacca plant to certain Drosophila grubs which bore through the stem, selectively killing young shoots of the plant. Various systemic and topical insecticides are being investigated to control these fly larvae.

Most of these studies are still preliminary, in need of in-depth multidisciplinary investigation.

4. OTHER USES OF ENDOD

4.1. Detergent and Foaming Properties

Pursuing the traditional use of endod as a laundry detergent, the butanol-extract has been tested with a detergiometer, to compare it with available commercial detergents. It was found to be an effective clothes cleaning agent. Further studies should find ways to make this extract serve as a supplement to other detergents and a substitute for some of them. Endod has the special advantage of being harmless to delicate fabrics (such as fine cotton, linen and wool), unlike some corrosive chemical detergents. It also leaves the clothes non-compressed and is biodegradable, with no apparent deleterious effect on the environment (suggested also by the centuries of its use in streams).

The very high foaming property of endod could be modified for use in lightweight concrete and in foam rubber preparations. It can also be used as a dispersant agent in perfume manufacture. Limited preliminary studies have given some promise for these commercial applications, though of course much more work is needed for their full development.

4.2. Larvicidal Property

While testing the comparative toxicity of endod and other compounds to various elements of stream flora and fauna, studies at Harvard showed that mosquito larvae are particularly susceptible to the lethal effect of endod. This led to other investigations that demonstrated that in addition to killing mosquito larvae, larvae of the notorious black fly (Simulium spp.), which transmit the river blinding disease, onchocerciasis, are also susceptible, as well as larvae of the domestic house fly (Musca domestica). The active principle appears to serve as a stomach poison to a number of feeding insects. Further development of endod for possible application as an insecticidal agent for village-level use could become of significant public health value. Since snail and malaria-transmitting mosquitoes breed in the same type of environment, control of snails with endod may provide a side benefit of reducing mosquito populations, just as spraying for mosquito control often has the unanticipated added benefit of controlling sandflies (Phlebotomus spp.), vectors of leishmaniasis.

4.3. Hirudinicidal Property

The aquatic leech (Lymnatis nilotica) a major animal pest causing much suffering and damage to livestock in many tropical countries, long has been known to be susceptible to the lethal action of endod. In Ethiopia, endod has for centuries been used for the control of this pest. This traditional use now should be improved upon for more effective protection of domestic animals from this seriously destructive ectoparasite.

4.4. Trematode Larvicidal Property

Schistosome cercariae and other trematode larvae are highly susceptible to the lethal action of endod. Miracidia that hatch from schistosome-laden stools, cercariae released by the hundreds or thousands from infected snails, and the intramolluscan stages of trematode parasites, all are susceptible. Infected waters can be rendered safe for several days by the application of small quantities of endod. The active ingredient can also be prepared in an ointment form for possible topical application on the exposed skin of workers in irrigation canals as a prophylaxis against cercarial penetration. This has been tried with some success using the tails of test mice which were coated with endod ointment and immersed in cercaria-containing water. However, here again, far more work is needed.

4.5. Spermicidal Properties

As a result of a systematic biological screening of the activities of the butanol extract of endod, it was found to be one of the most active of any biological agents against human sperm, suggesting its possible use as a locally produced, topically applicable birth control agent employed as a vaginal foam. In this connection, it is worth noting that endod has long been known and still is widely used by traditional societies in Ethiopia and other parts of east Africa as an abortifacient. Recent laboratory studies have shown it to have strong uterine contraction properties. Intrauterine injection of small quantities of endod extract in pregnant mice causes sterile and apparently harmless abortion. Its topical application therefore may, in addition to prevention of pregnancy, prove to have a useful purpose as a "day after" pill.

4.6. Other Snail-Killing Properties

In addition to being effective against schistosome-transmitting snails, as discussed above, endod is also effective against other snails that transmit important human and animal diseases. Laboratory and field studies have indicated that Lymnaea spp. are extremely susceptible to endod. These are the snail hosts for the important cattle and sheep liver fluke causing fascioliasis. Spraying pastures with relatively low concentrations of endod kill snails, eggs and infective larvae of the parasites without affecting the animals or vegetation on which it is sprayed. In view of the world-wide distribution of fascioliasis, development of endod to control this disease could benefit not only developing but also developed countries, in many of which the disease is prevalent, and, in fact, abundant.

4.7. Fungicidal Properties

Biological screening tests have further revealed that endod has a selective toxicity to dermatophytes, the fungi that cause a variety of skin conditions, such as athletes' foot, ringworm, etc. The possible use of endod for treatment of these diseases needs further investigation.

This retinue of cidal properties may suggest that endod is being promoted as an old-fashioned cure-all for all the afflictions of mankind from the common cold to over-population. It of course is not a panacea, but nonetheless it possesses an astonishing range of potentially useful attributes. We do feel that there is a potential here worthy of careful investigation--a target for collaborative efforts of scientists from developing and developed countries. We also feel that encouragement of small-scale industry and trade opportunities involving the affected peoples and villages directly is worthwhile both for their health and economic improvement. The degree to which it involves people directly may be the measure of its continuing value and promise for the future.

5. OTHER SPINOFFS FROM STUDIES ON ENDOD

5.1. Institute of Pathobiology

The establishment and development of the Institute of Pathobiology (IPB) of the Addis Ababa University (formerly Haile Selassie I University) was one of the useful by-products of studies on endod and the epidemiology of schistosomiasis in Ethiopia. The Parasitology Research Unit (PRU) of the Faculty of Science, predecessor of the IPB, was established in 1965 following the 1964 discovery of the molluscicidal properties of endod. As a result of a variety of research grants generously provided by the U.S. National Institutes of Health (NIH), the U.K. Ministry of Overseas Development, the World Health Organization, the Rockefeller Foundation, the U.S. Office of Naval Research, the former Haile Selassie I Foundation, and others for studies on endod, schistosomiasis and leishmaniasis in Ethiopia, the PRU expanded rapidly and became a semi-independent research institute. In 1970, the Faculty Council and the Board of Governors of the University formally granted the Institute independent research institute status under a newly appointed interfaculty Board of Directors. In 1973, the IPB moved to its present location in the Southern Campus of the University and acquired excellent facilities for expansion of its research programs. The Institute was sustained by a budget from multiple sources, over 45 full-time staff, and many research and teaching activities. Support through a research contract from the U.S. Office of Naval Research was a critical factor at that time in providing seed money for research that stimulated much of the subsequent interest in endod and support for its investigation.

5.2. Ethiopian Science Foundation (ESF)

Following development of new extraction methods for concentrating the active ingredient in endod, wide interest in exploitation of this product developed. As a safeguard to maintain control of possible commercial application, two of the extraction procedures were patented (one, as previously noted, was based on the method developed at the Tropical Products Institute in London in 1968, the other at the Stanford Research Institute at Menlo Park, California, in 1970). All royalties accrued from these patents were to be given to the Ethiopian Science Foundation, a private nonprofit organization specifically created to raise funds for promotion of science and technology research in Ethiopia. The ESF has since been superseded by the National Ethiopian Science and Technology Commission, established by government decree in 1975.

5.3. Relationship With Other Institutes

Because of the multidisciplinary nature of research involving endod and schistosomiasis, various studies were begun in which different research institutes and departments collaborated, both within Ethiopia and abroad. Such collaborations within the University of Addis Ababa and various governmental bureaus, involved, for example, the Chemistry and Biology Departments of the Faculty of Sciences, the Chemical and

Civil Engineering Departments of the College of Technology, the Institute of Development Research, the Ethiopia Nutrition Institute, the College of Agriculture, the College of Public Health, the Ministry of Health, and the Ministry of Agriculture. Collaboration for more sophisticated chemical and biological studies were initiated with the Stanford Research Institute in California, where the active ingredient was determined; the University of California, San Francisco, where many of the biological tests were conducted; Harvard University School of Public Health, where the larvicidal properties of endod were discovered; the Tropical Products Institute in London, where a procedure for continuous extraction of the active ingredients was developed; C.W. Post College of Long Island University, where the mode of action of endod on insects was studied.

These collaborative studies involved many staff and students and had valuable educational benefits as well as direct contributions to the study of endod.

6. PURPOSE OF THE PRESENT REPORT

The principal purpose of this Final Report is to combine the scattered results of studies on endod and on the epidemiology of schistosomiasis in Ethiopia. Some of these studies were published in journals with limited circulation, some were reported in the popular press, and many of the background studies have not been reported elsewhere. Combining this information into a single volume, with a brief review of the status of the work and current research leads, was felt by the principal investigators to be a useful consolidation of information on a potentially significant product. More important, it may provide stimulus for refocused interest in much-needed research on this and related topics.

Primary emphasis of the studies reported here is development of the endod plant as a locally produced molluscicide for use in the control of schistosomiasis and other snail-borne diseases on a community self-help basis. Other natural products should also be sought and developed for similar purposes. To this end, we hope that the collaborative approach taken in the endod research program might serve as a model for the study of other local products, whether for molluscicidal or other purposes. The advantages of its wide distribution, easy cultivation, and diverse properties make endod particularly worthy of such investigations. Should the nearly 15 years of assorted studies and experience with endod lead to its widescale production and use, those efforts will have been justified both for the specific results achieved and the ripple-effect toward other efforts to stimulate local or regional research for self-contained and self-sustained public health efforts.

BACKGROUND AND POPULAR ACCOUNTS OF ENDOD:

Lemma, A. (1964). Ethiopian Medical Journal 3 (4): 1-2 (editorial).

Lemma, A. (1966). Science Faculty Monograph, Haile Selassie I University (now Addis Ababa University), Addis Ababa, Ethiopia.

Lemma, A. and Duncan, J. (1968). Institute of Pathobiology Monograph Haile Selassie I University (now Addis Ababa University), Addis Ababa, Ethiopia.

Anonymous (1971). Medical World News 3 (19 Feb.).

Anonymous (1971). Life Sciences Research Reports. A Special Report (Stanford Research Institute) 3 (2): 1-5.

Anonymous (1973). World 4 (24): 41-42.

Lemma, A. (1973). Naval Research Reviews 26 (6): 1-10.

Anonymous (1974). Intercom (Stanford Research Institute) 219 (11 Jan.).

Anonymous (1974). The Sidney Morning Herald, Nov. 28, p. 24; Nov. 29, p. 14.

Muller, M. (1974). New Scientist 3 (904): 14-15 (4 July).

Muller, M. (1975). In Brief (The Johns Hopkins University School of Hygiene and Public Health), 11th Alumni issue pp. 2-3.

Subcommittee on Molluscicide Control of Vector Snails and Control Projects, Ethiopian Ministry of Health (1975). Recommendations of the Meeting of 22-23 Oct.

Anonymous (1975). The Ethiopian Herald 30: 1,7 (5 Jan.).

Anonymous (1975). Science News 107 (25): 402 (21 June).

Anonymous (1978). Research Resources Reporter 2 (10): 15-16.

AGROBOTANICAL STUDIES ON ENDOD:

Lugt, C.B. (1978). Ethiopian Medical Journal 16: 140-141.

CHEMISTRY OF ENDOD:

King, T.A., Jewers, K., Richardson, H., and Falshaw, C.P. (1968). 5th International Symposium on the Chemistry of Natural Products, London, July 1968. Abstract F-43.

CHEMISTRY OF ENDOD (continued):

Powell, J.W. and Whalley, W.B. (1969). Phytochemistry 8: 2105-2107.

Parkhurst, R.M., Thomas, D.W., Skinner, W.A., and Cary, L.W. (1973). Phytochemistry 12: 1437-1442.

Parkhurst, R.M., Thomas, D.W., Skinner, W.A., and Cary, L.W. (1973). Indian Journal of Chemistry 11: 1192-1195.

Parkhurst, R.M., Thomas, D.W., Skinner, W.A., and Cary, L.W. (1974). Canadian Journal of Chemistry 52: 702-705.

Parkhurst, R.M. (1975). Indian Journal of Chemistry 13: 757-758.

PATENTS AND REGISTRATION:

Ethiopian Government, Ministry of Interior (1972). Certificate of Registration of Association. Addis Ababa.

Jewers, K. and King, T.A. (1972). The Patent Office, London. Patent No. 1277414.

Lemma, A., Parkhurst, R.M., and Skinner, W.A. (1974). United States Patent Office, Patent No. 3,813,383.

Parkhurst, R.M. and Stolzenberg, S.J. (1975). United States Patent Office, Patent No. 3,886,272.

TOXICOLOGY AND MOLLUSCICIDAL PROPERTIES OF ENDOD:

Lemma, A. (1965). Ethiopian Medical Journal 3: 187-190.

Lemma, A. (1970). Proceedings, OAU Symposium on Bilharziasis in Africa, Addis Ababa (Nov.).

Lemma, A. (1970). Bulletin, World Health Organization 42: 597-612.

Yohannes, W.L. (1970). Proceedings of the OAU Symposium on Schistosomiasis, Niamey, Niger. OAU/STRC. Publications Bureau.

Lemma, A. (1971). Ethiopian Medical Journal 9: 113-118.

Yasuraoka, K. (1971). WHO mimeographed Document WHO/SCHISTO/71.8.

Baalawy, S. (1972). Bulletin, World Health Organization 47: 422-425.

TOXICOLOGY AND MOLLUSCICIDAL PROPERTIES OF ENDOD (continued):

Lemma, A., Brody, G., Newell, G.W., Parkhurst, R.M., and Skinner, W.A. (1972). Journal of Parasitology 58: 104-107.

Spielman, A. and Lemma, A. (1973). American Journal of Tropical Medicine and Hygiene 22: 802-804.

Lemma, A. and Yau, P. (1974a.) Ethiopian Medical Journal 12: 109-114.

Lemma, A. and Yau, P. (1974b.) Ethiopian Medical Journal 13: 115-124.

Lemma, A. and Ames, B.H. (1975). Transactions of the Royal Society of Tropical Medicine and Hygiene 69: 167-168.

Lugt, C.B. (1978). Ethiopian Medical Journal 16: 140-141 (Abstract).

FIELD APPLICATION OF ENDOD:

Lemma, A. (1965). Ethiopian Medical Journal 3: 84-92.

Lemma, A. and Duncan, J. (1970). Journal of Parasitology 56: 438-439.

Flemings, M.B. and Goll, P.H. (1972). Ethiopian Medical Journal 10.

Lemma, A., Goll, P., Duncan, J., and Mazengia, B. (1978). Proceedings of the International Conference on Schistosomiasis, Cairo, Egypt, Oct. 18-25, 1975. pp. 415-436.

OTHER USES OF ENDOD:

Stolzenberg S.J. and Parkhurst, R.M., (1974). Contraception 10: 135-143.

Stolzenberg, S.J., Parkhurst, R.M., and Reist, E.J. (1975). Federation Proceedings 34 (3).

Stolzenberg, S.J., Parkhurst, R.M., and Reist, E.J. (1976). Contraception 14: 39-51.

EPIDEMIOLOGY OF SCHISTOSOMIASIS IN ETHIOPIA:

Lemma, A. (1964). Ethiopian Medical Journal 3: 1-2.

Lemma, A. (1965). Ethiopian Medical Journal 3: 84-92.

EPIDEMIOLOGY OF SCHISTOSOMIASIS IN ETHIOPIA (continued):

Lemma, A., Demisse, M., and Mazengia, B. (1968). Ethiopian Medical Journal 6: 61-71.

Lemma, A. (1969). Ethiopian Medical Journal 7: 147-176.

Brown, D.S. and Lemma, A. (1970). Annals of Tropical Medicine and Parasitology 64: 533-538.

Duncan, J. and Goll, P. (1970). Proceedings of the OAU Symposium on Bilharziasis in Africa. Nov.

Duncan, J., Lemma, A., and Mazengia, B. (1970). Ethiopian Medical Journal 8: 17-25.

Lo, C.T. (1970). Proceedings of the OAU Symposium on Schistosomiasis, Addis Ababa, Nov. pp. 85-88.

Brown, D.S. and Wright, C.A. (1972). Journal of Zoology (London) 167: 97-132.

Lo, C.T. (1972). Malacologia 11: 225-280.

Lo, C.T., Fleming, M.B., and Lemma, A. (1973). Ethiopian Medical Journal 11: 271-278.

Kloos, H. and Lemma, A. (1974). Ethiopian Medical Journal 12: 157-173.

Polderman, A.M. (1974). Tropical and Geographical Medicine 26: 170-177.

Lo, C.T. and Lemma, A. (1975). Annals of Tropical Medicine and Parasitology 69: 375-382.

Polderman, A.M. (1975). Acta Leidensia 42: 1-193. (Ph.D. dissertation).

Duncan, J. and Lemma, A. (1976). Ethiopian Medical Journal 14: 3-15.

Hiatt, R.A. (1976). American Journal of Tropical Medicine and Hygiene 25: 808-817.

Hiatt, R.A. and Gebre-Medhin, M. (1977). American Journal of Tropical Medicine and Hygiene 26: 473-481.

Kloos, H. (1977). Ph.D. dissertation. University of California, Davis, 284 pp. Dissertation Abstracts International. 38 (6).

Kloos, H. and Lemma, A. (1977a.). American Journal of Tropical Medicine and Hygiene 26: 899-908.

EPIDEMIOLOGY OF SCHISTOSOMIASIS IN ETHIOPIA (continued):

Kloos, H. and Lemma, A. (1977b.). Ethiopian Medical Journal 15: 161-168.

Kloos, H., Polderman, A.M., DeSole, G., and Lemma, A. (1977). Tropical and Geographical Medicine 29: 399-406.

DeSole, G., Lemma, A. and Mazengia, B. (1978). American Journal of Tropical Medicine and Hygiene 27: 928-930.

Kloos, H., Lemma, A. and DeSole, G. (1978). Annals of Tropical Medicine and Parasitology 72: 461-470.

Teklehaimanot, T. and Goll, P.H. (1978). Ethiopian Medical Journal 16: 115-127.

Fuller, G.K., Lemma, A., and Haile, T. (1979a.). Transactions of the Royal Society of Tropical Medicine and Hygiene 73 (1): 121-122.

Fuller, G.K., Lemma, A., and Haile, T. (1979b.). American Journal of Tropical Medicine and Hygiene 28 (3): 526-530.

Lemma, A., DeSole, G., Polderman, A.M., Mazengia, B., and Kloos, H. (1979). Ethiopian Medical Journal (in press).

IV. RESEARCH ON ENDOD AND THE EPIDEMIOLOGY OF SCHISTOSOMIASIS IN ETHIOPIA

Helmut Kloos

1. PAST STUDIES ON ENDOD

1.1 Folk uses

Phytolacca occurs in most of tropical Africa and in parts of Asia and South America (48). In Ethiopia, where P. dodecandra (endod) grows, individual wild bushes are retained around dwellings (Fig.1). Its fruits (Fig. 2) have been used for centuries as a laundry soap and other parts of the plant as a medicine and poison. The first reports of its use as a soap in Ethiopia come from Portuguese Jesuits in the early part of the 17th century (47). Its traditional medicinal uses are described in several medieval Ethiopian medical texts written by scribes of the Orthodox Church (79) and present folk uses of endod in Ethiopia, including its use as purgative, soap, hirudinicide (leechicide) and abortifacient, were described by Lemma (55) and Kloos (51). Watt and Breyer-Brandwijk (80) described uses as taenicides, emetic, abortifacient, stimulant, tonic, purgative, veterinary medicine, toxin and vermicide in other African countries. It is locally believed in Ethiopia that clothes and human hair washed with endod become free of lice and ticks. Lemma (54) first discovered the molluscicidal property of endod in Adwa, in northern Ethiopia, while observing that the dried berries used by native people for washing clothes in streams caused high snail mortality immediately downstream of these laundering sites. These initial observations led to the extensive and intensive investigations on endod, especially as a molluscicide, that were to follow during subsequent years and are summarized in this report.

1.2 The first scientific studies

Schistosoma mansoni, S. haematobium, S. bovis, Fasciola spp. and possibly other snail transmitted human and animal infections are endemic in Ethiopia (see Section 3), providing a stimulus for research on the molluscicidal properties of endod and pointing out the need to do other research on endod in that country. Preliminary laboratory and field studies conducted at the Institute of Pathobiology in Addis Ababa between 1964 and 1968 showed that endod is indeed an effective molluscicide. One hundred percent kill of the vector snails Biomphalaria pfeifferi and Bulinus truncatus sericinus was achieved with a 10-30 ppm aqueous suspension of sun dried, crushed endod berries after a 24-hour exposure period, compared to 1,000 ppm for manufactured soap (Fig. 3). Other parts of the plant were found to be less potent. No toxic effects were noted in mice, rats, chickens, sheep and rhesus monkeys given high dosages of endod and no skin irritation was reported in animals and man, giving an initial indication that its use as a molluscicide was probably safe for workers (55). Likewise, no permanent phytotoxicity was found to develop during continuous application of high concentrations to different local plants grown experimentally (81).

The potency of endod was found to remain stable over a wide range of pH values (5-9), temperatures, and ultraviolet radiation. It retained its toxicity in various concentrations of organic and inorganic river mud (in contrast with copper sulphate), although it still proved to be

biodegradable. Nevertheless, the berries can be stored for more than 5 years without loss of potency (65). Like the other molluscicides presently in use, crude endod kills fish, tadpoles and schistosome miracidia and cercariae at molluscicidal concentrations. However, it is nonovicidal for Bulinus and Biomphalaria. Only niclosamide ethanolmine salt, (Bayluscide®, kills the eggs of these snail species. Endod is also toxic to leeches, which constitute an economic and public health problem in many parts of the tropics (57). These observations, made comparatively with major synthetic molluscicides now in use, showed that ground endod berries do possess characteristics that ranks it among the important molluscicides--not yet as toxic as very low doses of the commercial synthetics, but with many favorable features and a potential for increased molluscicidal potency.

Preliminary field trials with endod followed these promising laboratory experiments. A 5-km stretch of the Assam stream in Adwa, a 5-km section of an irrigation canal in a Wonji Sugar Estate in the Awash Valley (Fig. 4), and a 200-m strip of Lake Hora Abjiata (near Debre Zeit) were freed of schistosome host snails by application of 50-100 ppm of crude endod during an exposure of 3-6 hours (58).

1.3 The Adwa schistosomiasis control project

The successful preliminary field trials, tied to earlier reports of unusually large numbers of S. mansoni infections in Adwa, prompted development of the 5-year Adwa Schistosomiasis Control Project (60). The goal for this undertaking was to test endod under natural conditions within the framework of a community self-help program. The project was carried out between 1969 and 1974, under the guidance of Dr. Aklilu Lemma and the staff of the Institute of Pathobiology. It therefore was continued by the community itself, supported largely by the provincial government of Tigre.

In Adwa, a town of about 20,000, S. mansoni is transmitted by B. pfeifferi in two streams bisecting the town, the main sources of domestic water supply. All age groups and both sexes come into frequent intimate contact with stream water. The economy of Adwa centers around subsistence grain farming, administrative services and service-connected industries and crafts for use in the hinderland (54).

Before and during precontrol studies on schistosomiasis prevalence, snail population dynamics and human water contact patterns, the cooperation and assistance of community and provincial leaders was obtained, through public lectures, door-to-door visits, film presentations, demonstrations to the public and appeals to religious leaders and government officials to inform the population of the urgency of the project and to involve local residents in the control efforts. Endod berries picked in the surrounding mountains and bought in local markets were dried, crushed and periodically applied in the town's streams by the staff of the Institute of Pathobiology and local people. Weekly snail surveys were made to monitor snail occurrence (66).

An interim evaluation of the project made two and a half years after its commencement releaved that endod had been effective in controlling the B. pfeifferi population. Continuous application of this molluscicide eliminated B. pfeifferi from the streams treated but had no apparent effect on the flora and fauna of the rivers (49).

Attempts to reduce the amount of human water contact through health education, fencing off of major transmission sites, and placement of

guards proved to be fruitless (Fig. 5), although assistance in stream-cleaning operations and endod application was generously given by the town's residents (66,see also figs. 6-9).

Comparable parasitological data obtained in 1969 and 1974 (5 years later) showed a decrease in S. mansoni infection rates in Adwa from 63% to 33% in the general population and 50% to 7% in the 1 to 6 age group. In the nearby control village of Inticho, by contrast, infection rates in the general population decreased from 22% to 17% during the same 5-year period (66).

The conclusion appears warrented that the observed decrease in parasitism in Adwa was primarily due to the control of B. pfeifferi, the concomitant interruption of the S. mansoni transmission cycle, and the cercaricidal and miracididal action of endod. This is also suggested by the failure to reduce the amount of human exposure to infected water during the study period. A major factor in the success of the Adwa self-help project was the active participation by members of the community (59).

The undertaking of the Adwa control project and the active laboratory research both at the Institute of Pathobiology and small local laboratories in the 1960's and early 1970's drew the attention and support of the Ethiopian authorities and some international organizations which provided a focal point of discussion at several international schistosomiasis symposia (Fig. 10). Upon presentation of the final report of the Adwa study at the International Conference on Schistosomiasis in Cairo, October 18-25, 1975 (66), strong recommendations were made by that group for further research and studies on molluscicides derived from natural products, such as endod, for use on a community self-help basis.

1.4 The chemistry of endod

The endod plant provides material of special interest to organic chemists involved in saponin chemistry--partly because of the high percentage by weight of crude saponins in the dried berries, and partly because of the chemical complexity of the materials involved.

Most of the chemical work on endod until now has concentrated on the saponins of dried berries. This work has been rewarded with interesting chemical findings and valuable biological discoveries. However, according to Parkhurst (23), it appears that we still are seeing only the tip of the iceberg in developing the chemical potential of the endod plant.

Chromatographic separation of the crude saponins in endod demonstrates the dozens of compounds present, of which only a few have been characterized, to which one must add that the nonsaponin portion of the dried berries still amounts to 75% of the total weight. The nonsaponin fraction may be broken down into petroleum-soluble lipids, water-soluble sugars, starches, pectins and gums, and a water-insoluble fraction. Very little is known about the chemistry or potential uses of these fractions. Abundant as the berries are, they represent a small fraction of the plant, which may stand as a full and tall shrub with an equally extensive root system--ample material for future chemical study.

With the development and refining of endod, particularly valuable byproducts were obtained, some of which may prove to be as valuable as the main product. During isolation of the crude endod saponins, described in detail later, a water-soluble fraction containing sugars

and various polysaccarides was obtained, the future importance of which only further work can establish. New pectins, starches, thickening agents, material for fermentation to alcohols, sources of rare sugars with industrial importance, all may be found after sufficient study of the large amount of plant material made available from the cropped berries.

One also obtains a water-insoluble fraction, which has not yet been investigated, which may prove to be useful in animal feed, as a fuel, or as a soil additive.

From the petroleum extraction of endod, a green lipid material is obtained. While most of this material is composed of palmitic, oleic, and stearic acids, 12.5% consists of a nonsaponifiable bright orange waxy material containing squalene and a complex mixture of high molecular weight alcohols, i.e. phytosterols and/or triterpenols.

As the value of the endod saponins is established and production rises, large-scale development and additional uses for the saponin will follow and more byproducts should become available--an intriguing invitation to creative chemists to investigate this "wonder weed of Africa" (23).

The need to determine the chemical nature of endod and extract its active principle to obtain a more potent or concentrated molluscicide was apparent. However, existing facilities in Ethiopia did not permit such elaborate laboratory research. Investigators in the USA, England, St. Lucia, Brazil, Egypt, Tanzania, and Japan studied the molluscicide and helped to characterize its biological activity (35-38,46,56-58,62,63). Its chemical structure was studied by Horton (4) and Powell and Whalley (72). The specific molluscicidal saponins identified at the Stanford Research Institute (SRI) were named lemmatoxin-A, lemmatoxin-B and lemmatoxin-C (6,69,70,71), all derivatives of oleanolic acid. Recent studies by Parkhurst (23) showed that Phytolacca dodecandra contains larger percentages of molluscicidal saponins and has a different chemical structure than do the other species of Phytolacca.

Two endod extraction processes were developed. King and co-workers (56) extracted the glycosides of oleanolic acid with a methanol-ethanol solvent at the Tropical Products Institute in London. Lemma and co-workers (65), working at the Stanford Research Institute, developed a butanol extraction process which increased the potency of endod 7- to 10-fold over the crude endod. Snail kills at 3 to 4 ppm were obtained without significant loss of molluscicidal stability when tested under different physico-environmental stresses (varied sunlight and ultraviolet light, presence of organic and inorganic matter at different pH levels) (62,63,65)(Fig. 11). Endod proved to be much more stable under sunlight and ultraviolet light than were the major commercial molluscicides tested. Eight species of Schistosoma- and Fasciola-transmitting snails were about equally attacked by endod (63). Equally important for use in self-help control projects, the butanol extract constituted less than 10% of the total endod berry weight and proved to be safe to handle (11). Schistosome cercariae and miracidia were killed at 10 ppm (10). Although not ovicidal to Bulinus sp. and Biomphalaria glabrata, the butanol extract does kill eggs of Lymnaea sp. at molluscicidal concentrations (63). Mosquito larvae and leeches are also susceptible to endod (65,75). This suggests, together with more recent findings (see Section 2.5), that concurrent control of medically important arthropods, chiefly the anopheline malarial vectors, may be achieved in the same habitats.

Experiments with monkeys, dogs and cats showed that endod extract is also a potent emetic (65).

The two extraction processes were patented (30-32) to protect them against possible unauthorized exploitation.

Upon his return from the USA to Ethiopia in 1972, Dr. Lemma, together with Dr. Sheldon Crane, constructed a pilot-scale extraction plant designed to produce sufficient quantities of butanol extract to permit quantitative field evaluations of this material at the Institute of Pathobiology (2). However, the butanol extraction process was abandoned in 1974, before this goal could be obtained, owing chiefly to the difficulty of recovering the powdered molluscicide during the drying phase. The high cost and complexity of the butanol extraction process were other factors against this approach (8).

Another pilot extraction plant was built at the Institute of Pathobiology with the assistance of Professor R. Bisanz, former Dean of the College of Technology of Addis Ababa University, and his colleagues (8). This followed the discovery by the organic chemist of the Institute of Pathobiology, Mr. Tesfaye Lemma, that endod berries soaked in water for a few days rapidly ferment and use up all the free sugars and fats, leaving only the active principle in the aqueous solution. Thus, butanol is not needed for defatting and extraction in this simple water fermentation process (figs. 12-15), which, though still in a developmental stage, has yielded extracts that kill host snails at 6-8 ppm (11) and more recently at 4 ppm (16). The water extract of the defatted berries is thus about 7 times more toxic to the snails tested than the original crude endod berries (16).

An Ethiopian Science Foundation was established in 1972 through the Ethiopian Government Ministry of Interior (29) by Dr. Aklilu Lemma to receive all proceeds that may accrue from any development of the various patents for the endod extraction processes and from other endod products. All proceeds are dedicated to the promotion of scientific and technological research in Ethiopia.

1.5 Agrobotanical studies

While research on the extraction, chemistry and application of endod has been progressing rapidly since the discovery of its molluscicidal properties in 1964, studies on the agronomical aspects have lagged behind the more spectacular initial finds. Detailed investigations of the agronomic aspects of endod production only began in 1972. A major goal of these studies has been to select and breed plants for favorable growing characteristics and productivity (small bushes are more productive than large ones) and high potency of berries. Endod is a dioeceous plant but can be propagated readily from berries and cuttings (Fig. 16). Some agrobotanical data on plant ecology, nutrition, germination, spacing and irrigation have been gathered during the past few years and studies on the ecology of endod and its pests are now underway (see Section 2.2).

The great climatic and edaphic diversity of Ethiopia apparently gave rise to several geographical strains of Phytolacca dodecandra apparently with varying growth characteristics, yields, insect resistance and molluscicidal properties. Lemma (57) noted that 2 principal varieties of endod grow in Ethiopia, based on color of berries. More

recently, 2 varieties with distinct growth characteristics (erect bushes and prostrate vines) were identified among over 60 samples collected in 7 administrative regions (provinces) of Ethiopia (24). Lemma (57) found no significant differences in potency among some strains he tested, but Dr. Taye Bizuneh, Mr. Yemanu Tekie and Mr. Engida Assefa found remarkable differences (8). Recent studies, therefore, have only begun to investigate the botanical diversity that characterizes endod in Ethiopia. It is apparent that for efficient use of locally-grown and processed endod at the village level, high-potency varieties will have to be adapted to local areas and cultivated. The availability of wild berries in local markets, which represent several ecotypes, was all that was available for the Adwa project. Consequently, use of berries with an application that followed a predetermined standardized chemical assay could not be used. Such quantitative studies were begun elsewhere in 1976-77 (see p. 28).

On the largest endod farm in Ethiopia, in Debre Zeit at the edge of the Ethiopian Plateau at 1,800 m elevation, 50 km south of Addis Ababa, several dozen geographical strains of endod have been planted from cuttings and berries for field and laboratory testing (Fig. 17). A greenhouse, constructed for experiments on endod cultivation under controlled conditions, was completed at the Institute of Pathobiology in 1975 and a nursery in Sebeta (2,300 m) to supply seedlings and rooted cuttings in the proposed plantation there.

Although endod is confined in distribution in Ethiopia to the highlands above about 1,800 m, it was found to grow well and bear fruit on experimental plots in the hot, arid lowlands at the Melka Worer Experimental Farm (750 m) and at the Melkasa Experimental Farm (1,400 m), both in the Awash Valley. Since many schistosomiasis mansoni and all schistosomiasis haematobium transmission foci in Ethiopia are in the lowlands, where the irrigation schemes are also located, successful growth at lower elevations is important. High-potency berries have already been harvested at Melkasa (24).

2. ONGOING AND PROPOSED STUDIES ON ENDOD

2.1 Precontrol studies in Tensae Berhan

The Adwa Control Project was an initial trial to study (in part) the feasibility of endod application with a community on a self-help basis to control schistosomiasis (66). It soon was apparent that a reliable source or a large-scale production of endod using simple technology was required, with a need to quantify endod application and to determine its toxicological effects. It also became clear that project communities smaller than Adwa and nearer to Addis Ababa should be selected, to facilitate more detailed studies.

Tensae Berhan, a town of 8,000 located just below the escarpment of the Southern Ethiopian Plateau at 1,700 m in the Awash Basin and only 200 km from Addis Ababa, was chosen by the Institute of Pathobiology in 1974 as an excellent site for the second schistosomiasis control project. The high endemicity of schistosomiasis mansoni had been established earlier during routine stool examinations at the local clinic. Three small rivers that pass by Tensae Berhan, the Arba Dima and its two tributaries, the Ferekasa and the Homba, provide all domestic and recreational water for the community and are intensively used by the local population (14).

The main objective of the study in Tensae Berhan is to demonstrate the molluscicidal effectiveness of the water extract of endod in breaking the Schistosoma mansoni transmission cycle and to obtain sustained control of schistosomiasis, fascioliasis and other snail-borne infections, using local village resources (14). Development of this project might serve as a model and an inspiration for other Ethiopian communities to control their snail-borne infections.

The 3-year pretreatment study (1975-78) consists of (1) a parasitological baseline survey of the town's population, (2) clinical investigations (3), biweekly malacological surveys (14), (4) 2 water contact surveys (5) and (5) a socioeconomic survey, all of which were completed in 1976. Results of these studies are summarized below. The treatment phase is expected to extend from 1979 to 1982.

As in Adwa, a 10% random sample of the town's total population was parasitologically studied. Nearly 35% of the total study population of 796 were found to be infected. Significantly more males than females shed S. mansoni eggs. Age-distribution of infection showed the characteristically steep peak in the 6-15 year age group. Mean egg output corresponded with the age and sex distribution of infection rates. A relationship between clinical symptoms and egg output was discerned, although concomitant infections with other helminths and protozoa tended to interfere and complicate the symptomology--a never-solved and almost universal finding in the tropics. No definite relationship was found between socioeconomic status and level of parasitism, except that professional water carriers were among the most heavily infected, and merchants and administrators who employ the water carriers for hauling household water and the servants for laundering, were the least infected (14).

Two water contact studies were undertaken, one in 1975, a second in 1976. Age- and sex-adjusted infection rates were found to be correlated with water-contact patterns. Distinct hourly, diurnal and seasonal distribution patterns were observed. Recovery of S. mansoni-infected B. pfeifferi over the whole length of the test streams (see below) and the common use of the boulder-strewn river beds and bushy slopes for defecation suggest that infections are acquired at all water-contact points. About 90% of all water contacts in Tensae Berhan occurred at 4 major contact points. Their location within a 2-km stretch of the Ferekasa and Arba Dima rivers should facilitate control operations by permitting concentration of molluscicidal treatments to that section of the river (5).

Studies undertaken between 1974 and 1976 on snail population dynamics in the 3 main streams show a distinct seasonal distribution of B. pfeifferi. This species, Bulinus sp. and Lymnaea natalensis are flushed out of their habitat by flood water during the 3 rainy months (July-September) and remain absent during the 3 following dry months (October-December). B. pfeifferi repopulates the local rivers from upstream sources after December, and toward the end of the dry season (June) large snail populations are present. These seasonal fluctuations in snail numbers suggest that 3 treatments of endod should be made annually to control schistosomiasis in Tensae Berhan (14).

A socioeconomic study was undertaken to provide information on economic indicators, demographic patterns, and mobility status of the local population (14). Another study at the end of the project may reveal subsequent changes in population structure, agricultural

practices, income sources and levels, amount of education, water supply and delivery systems, and water-contact behavior of the town's population. Such studies, if supplemented with appropriate data on the ecological impact of endod on disease, mosquitoes, and other vectors and pests (i.e. leeches), may permit an evaluation of the possible range of beneficial effects of endod and the project. Parallel studies in the reference village of Dolchia should also contribute to that objective.

The Tensae Berhan population, including administrators and public health officials, showed considerable interest and cooperated eagerly during the baseline studies, as did the Adwa residents a decade earlier. They anxiously await becoming cured of schistosomiasis and protecting their children from the disease. Further community participation and support is therefore anticipated. This should take the form of assistance with endod production, its extraction, and application. With outside assistance, development of a health education program, improvement of water supply, and regulation of water contact is also a reasonable objective, with ultimate takeover of the project by the community, a primary goal.

Interviews with persons seen along the rivers in Tensae Berhan revealed that a considerable number of these individuals came from surrounding rural areas on foot and crossed the schistosome-infested rivers, particularly on weekly market days. Some farmers and pastoralists came from nearby rural areas to water their livestock at the main water-contact points in Tensae Berhan (14). It is clear that interruption of transmission in these rivers will benefit not only the population of Tensae Berhan but the rural population in nearby areas as well.

2.2 Agrobotanical studies

In 1977-78 the highest priority in agrobotanical studies of endod was still in the Awash Valley, but the designation of endod as one of Ethiopia's most important crops by the Ethiopian National Agricultural Research Association (26) is encouraging agronomic research at the national level. The relatively high cost of the endod used in Adwa (66) will very likely be reduced after large-scale cultivation begins in future project communities.

A Dutch agronomist, Dr. Charles B. Lugt, joined the Institute of Pathobiology staff in 1976 to help in this project. He found that endod berries are most potent during the green stage (19), an important finding bearing on production, as loss of crop to birds and other animals may be prevented and the harvesting time extended (19).

Dr. Lugt also began the first phytochemical studies of endod at the Institute of Pathobiology in 1977, yielding 8 different components with different snail-killing properties. Also in 1977 the first projected figures were calculated for large-scale endod production for molluscicidal use. Without selection for high-potency and high-yielding strains, the endod needed to treat the canals employed for a 1,200 hectare plot of sugar cane can be raised annually on a 2 ha plot. However, with selective breeding only a 0.5 ha endod plot would be required to treat the irrigation system supporting this acreage of sugar cane (67).

The high incidence of infested endod plants at all the experimental stations prevented some cultivating trials, and it was suggested that priority be given to pest control (24). Gitona pauliani larvae of the order Hymenoptera, stem borers and leaf miners kill and damage many

endod plants. Several other pests, including another, still unidentified species of Gitona as well as plant nematodes are suspected to cause extensive damage to the shoots and roots of plants grown at the various experimental stations (22). Laboratory studies toward control of pests, including use of insecticides, predators and cultivation of more pest-resistant varieties of endod and of alternate host plants were begun in 1977. Natural pest resistance was found in some plants, which were selected for further study (19,22). The taxonomy of the unidentified pests is being studied by Dr. L. Tsacas of the Museum National, De Histoire Naturelle, Entomologie Generàle et Appliqueé, Paris, France, and field studies on the life cycle are carried out in the Institute of Pathobiology and at the various agricultural experiment stations (22).

Extensive cultivation of improved strains of endod, development of technology for preparation of the purified product at the village level and diversification of its uses (see below) should encourage local industries and large-scale schistosomiasis control programs (9).

2.3 The extraction process

The fermentation-based extraction process is being improved upon and a solar-based drying process is to be developed at the Institute of Pathobiology to further simplify these operations and reduce operating costs. Defatting of the endod berries, a procedure that is required in all extraction processes because of the unfavorable interaction between the fat and glycoside components of the berries (68), is achieved more easily and cheaply by the fermentation procedure than by the butanol extraction process (16). Research continues at the Institute of Pathobiology to modify the present extraction and formulation procedures with the goal of making the extract less toxic to nontarget organisms and to further increase the potency of endod. Briquettes, pellets, and powder are to be produced for slow release in different habitats (9).

2.4 Development of a chemical assay

A colorimetric method for quantitative assay of the active principle of endod in treated bodies of water was developed by Mr. Tesfay Lemma (15) in the Institute of Pathobiology. During field tests made in Chuahit (in the Lake Tana basin) and in Tensae Berhan in 1976-77, molluscicidal concentrations of fine powdered crude endod could be maintained over most of the treated stream courses for 24 hours after application in the various rivers under investigation. In Chuahit, 50% snail kills were obtained for Bulinus truncatus exposed to a concentration of 6 ppm for 24 hours, and 100% mortality using concentrations ranging from 8-100 ppm. Comparable results for Biomphalaria pfeifferi in Tensae Berhan were 10-15 ppm and 25-100 ppm. The biodegradability of endod could be reduced and the molluscicidal activity maintained over longer periods of time, through the use of antibiotics and disinfectants and by adjusting the pH of the river water (15,16).

2.5 Insecticidal, insect repellent, larvicidal, bactericidal and other biological application studies

Studies of insect repellent, insecticidal and larvicidal action of endod on the common housefly (Musca domestica), a major domestic pest in all tropical countries, anopheline mosquito larvae, blackfly larvae (Simulium spp.), and other insects are now in progress at the Institute of Pathobiology. Preliminary findings show that the larvae of

Anopheles gambiae are more resistant to endod at molluscicidal concentrations but are more suceptible than those of A. aegypti and Culex fatigans (1,3,12). One possible mode of larvacidal action of endod was described by Flemings (146). Cockroaches (Blatella germanica) and army worms (Spodoptera exempta) were unaffected by endod (12) but black flies (Simulium sp.) and house flies were found to be highly susceptible (1).

Recent comparative toxicity studies of endod, in comparison with Frescon(R) and Bayluscide(R), at the Institute of Pathobiology indicate that endod and Frescon do not lethally affect most microfloral and microfaunal forms at molluscicidal concentrations. Leeches, however, were much more susceptible to endod than were snails. Tadpoles were sensitive to all 3 molluscicides, but fish (Tilapia, carp and catfish) were susceptible to all 3 at molluscicidal concentrations (3), corroborating earlier studies on other fish species (56,57,67), although susceptibility levels varied with the species of fish. Dr. Ephraim Mamo (20) found that sheep and dogs tolerated (water/fermentation extract) endod dosages up to 200 mg per kg of body weight when it was administered orally, but dogs given lower doses intravenously succumbed to its toxic effects. Nevertheless, possible ecological effects of short- and long-range ecological effects of continuous application of endod on the flora and fauna of treated bodies of water should be studied (9).

Low concentrations of endod were found to have antidermatophyte activity (10). Bactericidal, antiviral and anthelmintic action of endod extract could not be demonstrated, but encouraging results were obtained on the protozoa Trichomonas vaginalis, T. foetus and Trypanosoma congolense. Studies continue on fractionation of endod (necessary to overcome its hemolytic action on red blood cells). In vivo tests in experimentally infected animals are being made to develop treatment regimes, and the toxicity of endod further studied in mammals with the ultimate goal of employing it in drugs for human consumption (10,27).

Concern over possible mutagenic activity of endod has led to studies that have shown that at the levels tested this molluscicide so far demonstrated no mutagenic effect when tested in an in vitro system using Salmonella spp. (64).

2.6 Development of antifertility and abortifacient agents

Studies undertaken on rats at the Stanford Research Institute indicate that derivatives of oleanolic acid from endod have spermicidal and blastocidal properties that may be suitable for the development of new contraceptive preparations (76-78). Not only was endod found to produce immediate and clean abortions in rats, with minimum side effects, but was also 10 times more effective than the material used commercially in the USA as a spermicide for use in vaginal contraceptives (23).

2.7 Synthetic analogs

Searches for synthetic analogs from oleanolic acid from sources other than endod are being continued at the Stanford Research Institute with the objective of developing compounds that are less toxic to fish. Some promising results have already been obtained (78).

2.8 Industrial uses of endod

Some experiments are also underway, in collaboration with the Indo-Ethiopian textile factory, on the development of endod as a surfactant for wetting and soaping materials. At present, Ethiopian textile manufacturers must import these chemicals from Europe (13).

Work is continued on the detergent property of endod, with the objective of soap manufacture and as an additive to synthetic products (13). Preliminary experiments have shown that endod may also be a useful foaming agent in the manufacture of light concrete. Studies in these areas are being conducted by chemical engineers at the College of Technology, Addis Ababa University.

2.9 Schistosomiasis control projects in irrigated farms

Extension of irrigated areas and enlargement of farm labor populations (which would almost certainly include some infected individuals) in the Awash Valley and other lowland areas in Ethiopia suitable for large-scale irrigation greatly increases the danger of spread of schistosomiasis into localities hitherto unaffected by this infection. The feasibility of growing endod on irrigated plots as a cash crop and applying the community self-help concept in these commercial farms is presently being explored. The use of this molluscicide is to be regarded as only one measure in a comprehensive approach to the control of this disease, which must also include health education, a safe water supply, latrine construction, mass treatment, and proper irrigation engineering and maintenance for snail control. Such a comprehensive program is to form an integral part of the National Schistosomiasis Control Program (see Section 2.11). Farm management and the laborers themselves are becoming increasingly aware of the danger of schistosomiasis in irrigation schemes and are eager to support control projects. B. pfeifferi has already become established in all farms studied in the upper Awash Valley and S. mansoni is being transmitted in at least 3 of those 8 farms. Longitudinal malacological studies (52,53) have shown that the occurrence of B. pfeifferi in most irrigation schemes is limited to certain canals. This suggests that spot treatment with endod molluscicide could be a highly localized and therefore feasible increment of a combined schistosomiasis control program.

2.10 Extension of endod studies to Egypt

A collaborative program between the Ethiopian and Egyptian governments calls for studies to determine the feasibility of cultivating endod under different environmental conditions in Egypt (7). In addition to these adaptation trials, biological studies on the effects of endod on micro-organisms, plants and animals, are to be carried out in the laboratory and in the field in Egypt. Development of a quantitative assay for endod, and testing of the efficacy and field application of different endod extractions and formulations are to follow the biological studies. If successful, an attempt will be made to determine the potential use of endod over a 5-year period in a defined endemic area in Egypt. The Institute of Pathobiology and the Institute of Research in Tropical Medicine of the Egyptian Ministry of Health have taken initial steps toward collaborative studies in this endeavor but further arrangements and support are necessary before the actual project can begin.

2.11 National Schistosomiasis Control Project

A proposal to develop a National Schistosomiasis Control Program in Ethiopia involving the Institute of Pathobiology, Ministry of Health, Valley Development Agency, Ministry of Agriculture and Ministry of National Resources has recently been approved by the Ethiopian government, the World Health Organization and United Nations Development

Programme (26). This program should eventually involve the cooperative efforts of farmers organizations and other community organizations and all health units in all communities in endemic areas. This multidisciplinary approach will permit research into the epidemiology of schistosomiasis and effective control measures including large-scale cultivation of endod and its toxicity on biological systems, training of indigenous health workers and development of viable control operations.

2.12 Blood cholesterol-lowering property of endod

The most recent addition to the rapidly growing list of actual and potential uses of endod is its ability to lower blood cholesterol, as shown by studies by Dr. R. Malinow on rats at the National Oregon Primate Research Center (34). Interest in this area of endod research dates back to the observation made by Dr. R.M. Parkhurst in Ethiopia that cholesterol (in butter) and the active components of endod mutually counteracted the effects of each other (23). Dr. Malinow found that single doses of crude endod saponins (crude butanol extract) at the 20 mg/kg level mixed with cellulose in a ratio of one to four and administered orally effectively reduced the absorption of cholesterol in six rats. The percent cholesterol absorption was 54% of the control group. This result compared favorably with alfalfa saponins at 51% and Indian soap root and Chinese bitter melon saponins at 77% and 92% respectively (34). Doses of 0.3 and 0.6% (w/w in food) of endod saponins given over a one week period failed to reduce plasma cholesterol in ten rabbits in comparison to control (34). Monkey studies originally planned have not yet been carried out.

2.13 CONCLUSION

The development during the past 10 years of new uses for the Ethiopian folk plant endod (Phytolacca dodecandra) for various public health and industrial applications illustrates the feasibility and the opportunity to develop and utilize products indigenous to developing countries within these countries. At a time when accelerated rates of world-wide inflation, shortage of resources and rising costs of energy rapidly increase the price of products and goods imported from industrial and oil-producing nations, the development of endod and other natural products in developing countries for internal use has considerable economic importance and ecologic implications.

As a molluscicide, endod already ranks with the leading synthetic products. The abundance of endod in parts of the tropics and subtropics, its versatility, biodegradability and suitability for local production and application, and the inexpensive simple technology associated with it are perhaps its outstanding features. Developing countries where schistosomiasis and other trematode diseases are widespread should benefit greatly both from endod used as a molluscicide and from the array of other activities of this remarkable plant. However, the recent discovery of its abortifacient and anticholesterol properties point up potential uses of endod in the industrialized countries as well.

With the completion of many of the basic studies on chemistry of the active principle of endod, its extraction methodology and agrobotany, and with successful field testing of the effectiveness of endod, research should now focus on development of large-scale production of berries and their use in disease control projects. However, the cooperation of administrators, public health workers, and local citizen

groups at all levels of government is essential for the success of this effort. The very nature of endod as an indigenous plant locally processed primarily to be used in self-help projects makes community understanding and acceptance essential. With "... the flow of resources increasingly being redirected from the urban and modern to the rural and traditional ones..." (21), and with development of concepts of "appropriate technology" (73) becoming an important element in revised national development strategies, tropical parasitic disease control programs in many countries can now test the development and application of endod as one of the new approaches to internally directed and sustained health-improvement projects.

3. THE EPIDEMIOLOGY OF SCHISTOSOMIASIS IN ETHIOPIA

The study of schistosomiasis in Ethiopia presents many challenges from the epidemiological, ecological and control points of view. Past studies indicate that variations in elevation, topography, climate, the distribution of the snail intermediate hosts and the economies and life ways of the population have resulted in a discontinuous, highly localized distribution of schistosomiasis in Ethiopia. Schistosoma mansoni, S. haematobium, S. bovis and at least 2 other snail-transmitted parasites of man and his livestock are endemic in this country. Research on schistosomiasis in Ethiopia began during the Italian occupation in the 1930's but intensive studies have been made only since the 1950's, beginning with Ayad's (85) extensive survey, followed by the discovery of high infection rates in the northern province of Tigre (94,116), in some eastern lowlands (118,136), in the town of Harar (113) and in the Lake Tana area (85). Ayad (85), Lemma (118) and Kloos and co-workers (110) reviewed the literature on schistosomiasis in Ethiopia.

Climatic patterns in Ethiopia have been studied in depth by Schaller and Kuls (137) and their influence on the occurrence and distribution of schistosomiasis has been analyzed by Aram (84), Kloos (106) and Kloos and co-workers (110).

The Ethiopian population is concentrated on the two well watered high plateaus, where agriculture supports densities between 50 and 300 persons per square kilometer. The lowest densities (1-10 persons/sq km) are found in the arid lowlands, where pastoral nomadism and simple gathering prevail (49).

Most Ethiopians continue to use streams and other undeveloped or marginally developed water resources for their daily needs. Wells and piped potable water supplies are confined to larger towns and a few irrigation schemes. Many communities, including some with more than 5,000 inhabitants, continue to depend solely on streams or rivers (116, 120,131).

Although irrigation has been practiced for about 2,500 years in Ethiopia, it is still relatively unimportant. Large irrigation schemes are confined largely to parts of Eritrea and the Awash Valley, with small irrigated plots scattered widely on the humid highlands. However, increased urbanization, extension of road and transportation systems, spread of modern technology and education and the control of malaria are opening up remote parts of the country to mechanized agriculture, hydroelectric development and other industries. These developments result in increased population movements and creation of new snail habitats.

3.1 SCHISTOSOMIASIS MANSONI

Both Schistosoma mansoni and S. haematobium are endemic in Ethiopia but the former is more widely distributed than the latter and the areas of their occurrence do not overlap. S. mansoni infections have been reported by medical facilities from all 14 administrative regions (provinces) (see Map). The mapping of comparable prevalence data for locally born children(p.464)shows that endemic schistosomiasis mansoni is most prevalent at intermediate elevations (1,200-2,000 m) and is absent from the hot lowlands below 900 m in eastern and southern Ethiopia and above 2,000 meters elevation. Prevalence rates above 50% were reported from the Lake Tana Basin, in most localities surveyed in the northern part of the Ethiopian Plateau (in Tigre and Eritrea provinces) and in several villages in the foothills of the Ethiopian and Somali plateaus (110).

The recent discovery of high prevalence rates among locally-born persons in villages in the eastern foothills of the Ethiopian Plateau (110), in the remote Nile Gorge of western Wollega (103), and among humans, baboons (Papio anubis) and grivet monkeys (Cercopithecus aethiopica) in Mui National Park in southwestern Ethiopia (122), and in the Lake Tana Basin (133) indicates that many endemic foci at intermediate elevations in Ethiopia are still unknown.

3.1.1 Intermediate hosts of S. mansoni

B. pfeifferi occurs widely in the Ethiopian highlands (83,87,88, 110,144) and has been found at elevations up to nearly 3,000 meters (84, 87,106). This species occurs seasonally in a great variety of habitats (streams, pools, irrigation canals and swamps), reaching its highest densities toward the end of the dry season of October-March (3,10). B. pfeifferi may be absent from areas below 700 m in the lower Awash Valley (108), the Red Sea coast and apparently southern Ethiopia (85,87,110). The few earlier records of Biomphalaria in the western and southwestern parts of Ethiopia (87) have not been confirmed.

Live B. sudanica have been found only in the Rift Valley lakes Ziway and Abaya, and empty shells were recovered from Lakes Awassa and Abyata (83,88). That B. sudanica transmits the Ethiopian strain of S. mansoni is suggested by high infection rates in the human population around Lake Ziway (110).

3.1.2 Environmental influences on transmission

In view of the widespread occurrence of B. pfeifferi, the concentration of the human population and the low prevalence of schistosomiasis mansoni in the Ethiopian highlands, it appears that temperatures are too low for completion of the S. mansoni cycle above 2,000-2,200 m. A similar upper altitudinal boundary of S. mansoni transmission was proposed for the East and South African highlands, based on field and laboratory studies (86). Mean annual air temperatures at the 2,000 and 2,200 m contours in Ethiopia are 16-18°C(103). Aram (84) recorded mean annual water temperatures of 13-15°C in three streams between 2,000 and 2,750 m on the Ethiopian Plateau. Laboratory studies show that S. mansoni miracidia and cercariae become almost motionless at around 10°C and gradually more active with increasing temperatures, up to 40°C. Optimum temperatures for penetration of the larvae lie between

18°C and 35°C. Similarly, B. pfeifferi seldom reproduces at temperatures above 28°C (86). Snail and parasite distribution in the Awash Basin show the effect of the temperature barrier. Lesser factors inhibiting transmission include water chemistry, high stream velocity and shade (106).

Extensive surveys of the chemical composition of stream and lake water show that water chemistry does not significantly limit the occurrence of intermediate hosts in Ethiopia, except in some salt water lakes, including Abyata, Langano, Shala and Awassa, and the numerous hot springs (83,106). It is of interest to note that Bulinus sp. naturally infected with S. bovis were recovered from Lake Awassa (128). Stream velocity is locally high enough during the two rainy periods to flush out B. pfeifferi and prevent its recolonization for 5 months or more (84, 120). Shade has also been associated with the absence of Biomphalaria in some parts of Ethiopia (87) but large forests producing sufficient shade to prevent establishment of this genus have withstood deforestation by man mostly in small areas in southwestern Ethiopia (135).

The large amounts of silt carried by the Ethiopian rivers and streams during the rainy periods may contribute to impoverish the snail fauna in the lowlands. Mean annual silt load of the Awash River is more than 25 times higher at Dubti, in the lower part of the Awash Valley, than at Wonji, in the upper valley (107). Brown and Lemma (91) predicted that B. pfeifferi will not spread into the lower parts of the Awash Valley. Malek (129) associated the seasonal decline of B. pfeifferi in the Gezira cotton scheme to corresponding increases in silt load of the Blue Nile, which also originates in the Ethiopian highlands.

Extreme temperatures and other physical barriers to S. mansoni spread are not necessarily permanent and may be modified with further developments of water resources. B. pfeifferi remains absent in all rivers, lakes and overflow swamps in the middle Awash Valley but invaded irrigation canals in the Melka Sadi banana plantation and in the Amibara cotton scheme shortly after their establishment (107,108). Although the water supply and sanitary conditions were improved in some schemes after the study by Bruijning (93), schistosomiasis control programs in Ethiopia are still confined to Wonji and Metahara estates and conditions in the other irrigated farms remain conducive to the transmission and spread of the parasite by migrants (106,107).

3.1.3 Age, sex and socioeconomic factors

Although little is known about the epidemiology of schistosomiasis mansoni in different parts of Ethiopia, nearly all past studies show the characteristic age distribution curve on infection, with the highest rates in the 5-20 group and a gradually declining prevalence toward old age (94,104,105,106,123). Higher rates in males than females have been reported from different highland farming communities (105,120) and from most irrigation schemes (108). The observed sex differences of infection in irrigated areas are primarily the result of occupational differences, since only men work in the fields and women stay at home. In the highlands sex-linked transmission is less well understood. Whereas Polderman (134) found no association between frequency of water contact and infection in males and females in the Lake Tana area, Lemma and co-workers (120) found a significant correlation between frequency, duration and intensity of water contact and infection rates in different age groups in both sexes in Tensae Berhan. Water contact and infection among different social and

age groups in Ethiopia is far from understood and more intensive epidemiological studies are needed before long range schistosomiasis control programs can be effective in different parts of the country.

Past studies show that the presence of many linguistic, religious and economic groups in Ethiopia and the ecological diversity of the country present strong gradients in the epidemiological significance of water use along cultural, social and geographic lines (106,120,123,127,134).

3.2 SCHISTOSOMIASIS HAEMATOBIUM

Much less is known about S. haematobium than S. mansoni in Ethiopia, partly due to the scarcity of studies in the lowlands, where this schistosome is endemic (Map). The scarcity or absence of infection in most localities surveyed in spite of widespread occurrence of potential intermediate hosts of the genus Bulinus has puzzled many parasitologists and malacologists and various explanations have been given. Brown and Burch (90) and Brown and Wright (92) suggested that a combination of factors, including scarcity of B. truncatus (the main transmitter of S. haematobium in North Africa and Southwest Asia), poor communication, low water temperatures, and low population density prevent B. truncatus-borne strains of the parasite from becoming established in the Ethiopian highland area.

It is becoming increasingly clear that absence of appropriate snail hosts and low water temperatures are the main factors accounting for the absence of endemic schistosomiasis haematobium in central Ethiopia. Extensive population movements that could have resulted in diffusion of the parasite over large parts of the country have taken place in Ethiopia for several thousand years (132,140), continuing at an accelerated rate in the present. The high population densities in the highlands normally should result in situations suitable for transmission.

Endemic schistosomiasis haematobium has been reported only among Afar (Danakil) pastoralists from the middle and lower parts of the Awash Valley, where infection rates are 10-64% (112,118,136). However, recent surveys revealed a high prevalence (20-42%) among farmers in the lower Webi Shebelle Valley (99) and supposedly similar rates in the lower Genale Valley (Dolo Health Center, unpublished data) (Map). Sporadic cases have occasionally been reported from the Ethiopian highlands (85,87) but most of them were thought to have originated outside of Ethiopia. Epidemiological surveys among 901 persons in various villages in the highlands and Rift Valley failed to find persons with schistosomiasis haematobium and only 2 cases were reported among 30,000 by 5 hospitals and health centers in those areas (105,106).

3.2.1 Intermediate host of S. haematobium

Recent progress in snail classification has helped to clarify taxonomic problems in the Ethiopian snail fauna. It is now generally agreed that there are at least four genetic taxa of Bulinus in Ethiopia, each apparently with different levels of susceptibility to infection. They include the diploid B. abyssinicus, B. africanus and B. tropicus, each with 18 chromosomes (n = 18), the tetraploid B. sericinus, often referred to as B. truncatus/sericinus or B. truncatus (n = 36), the hexaploid B. hexaploidus (n = 54) and the octoploid B. octoploidus (n =

72) (90,92,95,96,141). Burch (96) included the latter two species in the newly erected subgenus Isidora. Brown and Wright (92) consider variations in climate among different altitudinal zones to have been instrumental in the evolution of B. hexaploidus and B. octoploidus as indicated by the present restriction of these two higher polyploid snails to elevations above 2,100 m and the diploid and tetraploid forms to regions below 2,100 m. Cross-breeding experiments may show whether the latter four species interbreed and produce progeny. Such studies may thus help to answer the question whether B. sericinus is a distinct species rather than a race of B. truncatus (141,142,144).

B. africanus (n = 18) which, like B. abyssinicus, belongs to the africanus group, and includes the most important transmitters of S. haematobium in Africa, has been found in the western and northern parts of the Ethiopian Plateau (88,112) and in the Mui Game Reserve in southwestern Ethiopia (122). Brown and Burch (90) consider snails of the africanus group to be rare in Ethiopia, although Brown and Wright (92) emphasized that the presence of many morphologically intermediate forms of the four basic genetic species in Ethiopia requires further taxonomic revisions.

3.2.2 Intermediate host-parasite relationships

The only experimentally and epidemiologically confirmed intermediate host of S. haematobium in Ethiopia is B. abyssinicus (108,124), which is limited in its distribution to some lowlands of Somalia and Ethiopia (130). In Somalia, too, B. abyssinicus is the sole transmitter of S. haematobium (82), but whereas this snail has been found only in fresh water swamps and lakes in Ethiopia, it has also become established in irrigation canals in Somalia. The confinement of high infection rates in man to areas of the freshwater swamps and lakes in the middle and lower Awash Valley, the only known habitats of this species in Ethiopia (110), and to the swamps in the Webi Shebelle (99), also points up the importance of B. abyssinicus in transmission.

All other bulinine snails challenged with the local strain of S. haematobium proved to be refractory. Lo (126) failed to infect B. tropicus with an Egyptian strain of S. haematobium but achieved low infections in B. octoploidus. B. tropicus rarely transmits S. haematobium within its range of occurrence in eastern and southern Africa (97,126). Brown and Wright succeeded in infecting B. "truncatus" (n = 36) from Wonji irrigation scheme with Egyptian S. haematobium but not B. tropicus or B. octoploidus. Brown (89) found B. "truncatus" from Wonji to be refractory to a strain from Kenya. Wu and Burch (143) found Ethiopian B. sericinus to be highly susceptible to the Egyptian strain of S. haematobium and moderately susceptible to the Iranian strain, both of which pass through B. truncatus, and Lo (126) reported infections in B. sericinus from Aden with the Egyptian strain. Wu and Burch (143) obtained low infection rates in B. sericinus with the Liberian strain, which uses an africanus snail, prompting them to state that "B. sericinus may be a potential transmitter of eastern Ethiopian [Awash Valley] schistosomiasis haematobium." Kloos and Lemma (108), however, found B. "truncatus/sericinus" from several irrigation schemes in the Awash Valley to be completely refractory to the local strain of S. haematobium, and Kloos and Lemma (107,108) found no natural infections in B.

"truncatus" from those schemes.

It may be concluded that the Ethiopian sericinus (or truncatus/sericinus or truncatus) and tropicus complexes are unable to transmit the local strain of S. haematobium, which passes through the africanus snail B. abyssinicus, but that introduction of Egyptian or other exotic strains of the new parasite, as a result of population movements, may establish the transmission cycle in suitable localities where B. sericinus already occurs. This view is supported by the situation on the Kano Plain in Kenya, where local B. truncatus is refractory to the local strain of S. haematobium, which uses a snail of the africanus group. Interestingly, the Kano Plain B. truncatus, like the Ethiopian B. sericinus and B. "truncatus", is compatible with the Egyptian strain of the parasite (138). Lack of a high degree of local snail/parasite specificity was also observed by Lo (126), who found that snails of the truncatus and africanus groups from the Gambia, Iran, Western Aden and South Africa were all more susceptible to Ethiopian S. haematobium than the local B. truncatus.

The altitudinal barrier in the highlands suppresses transmission of S. haematobium similarly to S. mansoni. Several laboratory studies (86) showed that the optimum temperature for infection of B. truncatus and other species is 20-30°C and that no infection may occur at water temperatures of 10°C or below.

3.2.3 Age and sex distribution of infection and aspects of human ecology

Fairly extensive and representative epidemiological data on schistosomiasis haematobium exists only for the Afar in the Awash Valley. Infection in that ethnic group describes the same general age distribution curve as for other peoples in endemic areas (99,112,118) but is rather unique with regard to the high prevalence in Afar females. Lemma (118), who first noted the higher rates in females, found twice as many females as males above age 20 infected but this did not appear to be the case in the lower age groups. The more recent surveys by Kloos et al. (112) showed that infection in females is three times that of males, due mainly to the seasonal collection of several aquatic and semiaquatic food plants by females in swamps.

The extension of the irrigated areas in the Awash and Webi Shebelle valleys will result in the growth of the already large migrant farm labor populations near these endemic centers where they may become infected for the first time. Development of an irrigation scheme in one Awash swamp already has resulted in a sharp increase in S. haematobium infection among migrant laborers (108).

3.3 BOVINE SCHISTOSOMIASIS AND FASCIOLIASIS

Schistosoma bovis, Fasciola hepatica and F. gigantica are also important in Ethiopia. S. bovis is widely distributed in Ethiopia, having been found in 7 of the 14 Ethiopian provinces (128). Infection rates between 3 and 10% were found by P.H. Holmes (unpublished) and one human case of S. bovis was found in the lower Awash Valley (Institute of Pathobiology, unpublished). S. bovis, which is commonly transmitted in Africa by the same snail hosts as S. haematobium (86), is compatible with more species of Bulinus in Ethiopia and is adapted to lower temperatures than S. haematobium, also noted in other parts of Africa (86). Lo and Lemma (128) found B. abyssinicus naturally infected with S.

bovis in the Awash Valley endemic center. They found Bulinus sp. in Lake Awassa, Bulinus "truncatus" (n = 36) in Adwa, and obtained laboratory infections in Ethiopian B. abyssinicus, B. africanus, and other species of Bulinus.

F. hepatica and F. gigantica are apparently transmitted by Lymn natalensis and L. truncatula. The former snail is widespread throughout Ethiopia, the latter being common only in areas above about 1,200 m elevation (88). P.H. Holmes (unpublished) reported a total of 326 of 490 cattle stools in 8 highland villages positive for either F. hepatica or F. gigantica. Fasciola sp. has often been found in surveys of human populations as well (127, 131, 147).

3.4 CONCLUSION

The focal distribution of infection and the apparent confinement of endemic Schistosoma mansoni to intermediate altitudes and of S. haematobium to some lowland river valleys in the eastern and southern parts of the country encourages concentrated efforts in schistosomiasis control. Low temperatures inhibit S. mansoni transmission in large areas in the densely populated highlands, and high temperatures in some lowlands. Noncompatibility between several widely distributed Bulinus species and the local strain of S. haematobium apparently limit schistosomiasis haematobium occurrence to some lowland swamps, infested with the only known intermediate host, B. abyssinicus. Application of molluscicides such as endod, when used together with health education and water supply programs is a promising control strategy. Without new and innovative control methods and approaches schistosomiasis is likely to become a major disease in some parts of Ethiopia. The continued reliance of many rural communities exclusively on rivers, lakes and reservoirs for all their daily water needs and scarcity of schistosomiasis control programs have assured the maintenance of high transmission levelsin suitable localities to this day. Higher S. mansoni infection rates in children, farmers and other groups with more frequent water contact than populations with little exposure and increasing population movements indicate that water resources development in rural areas at intermediate and lower altitudes will cause a further increase in disease prevalence. The higher prevalence of S. haematobium infection in females than males of pastoralists in the Awash Valley and the confinement of its intermediate host snail to several swamps and lakes should be used advantageously in control projects. Perhaps the major advantages of using endod in disease control projects are its broad spectrum toxicity to mollusc, arthropod and other insect vectors and various infectious agents, as well as its abundance and biodegradability. The widespread occurrence in Ethiopia of S. bovis, Fasciola hepatica and F. gigantica may thus also be effectively dealth with in the future.

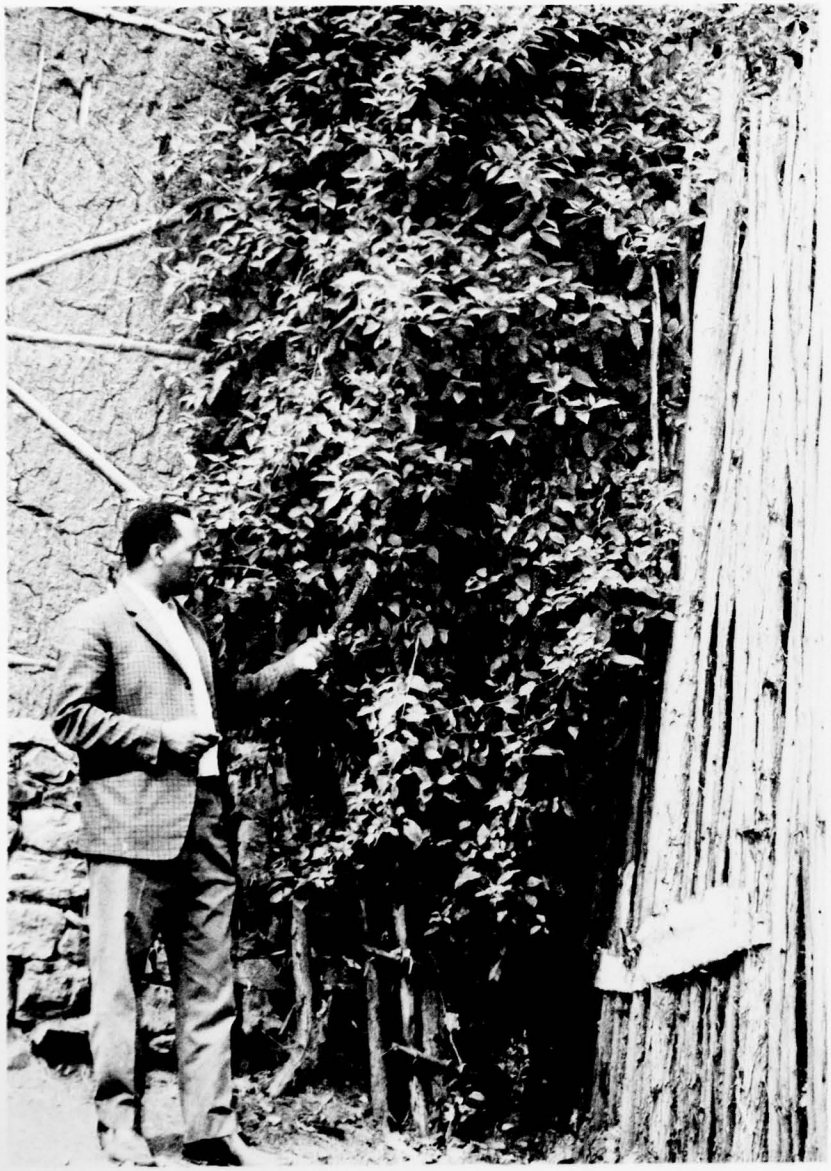


Figure 1. A endod bush growing in a Ethiopian garden

Figure 2. Mature endod berries

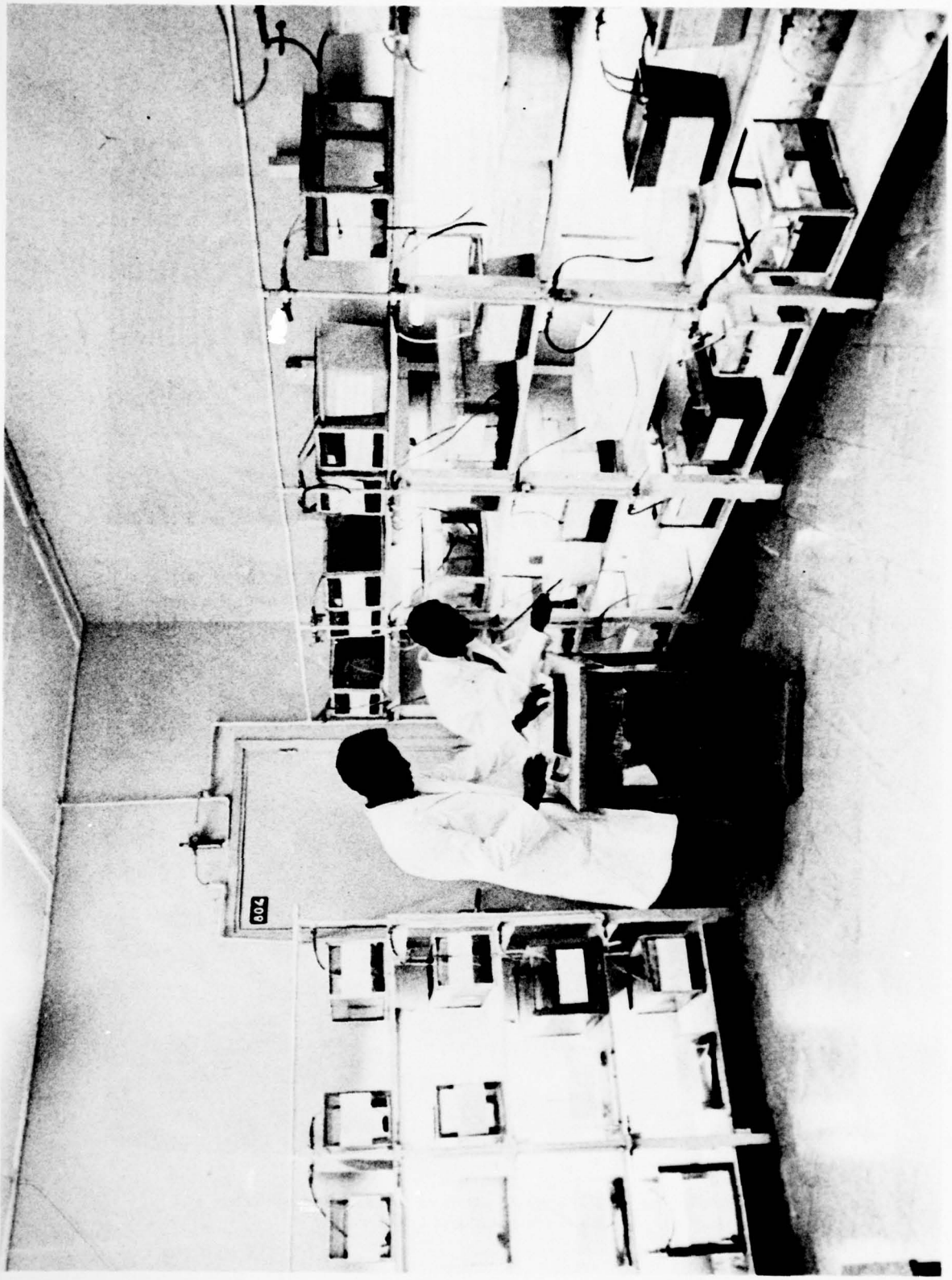


Figure 3. The snail breeding room in the Institute of Pathobiology

Figure 4

MEMBERS OF INSTITUTE OF PATHOBIOLOGY STAFF EXAMINE AN IRRIGATION DITCH IN WONJ, PRIOR TO MOLLUSCICIDE APPLICATION

Figure 5

A SCENE IN ADWA, showing exposure to schistosome-infected waters—with an unused bridge nearby

Figure 6. The Adwa Schistosomiasis Control Project

Figure 7. Community participation in Adwa

Figure 8. Clearing of a stream in Adwa before endod application

Figure 9. Endod application in Adwa

Figure 10. A recent WHO schistosomiasis symposium at which the use of endod was discussed

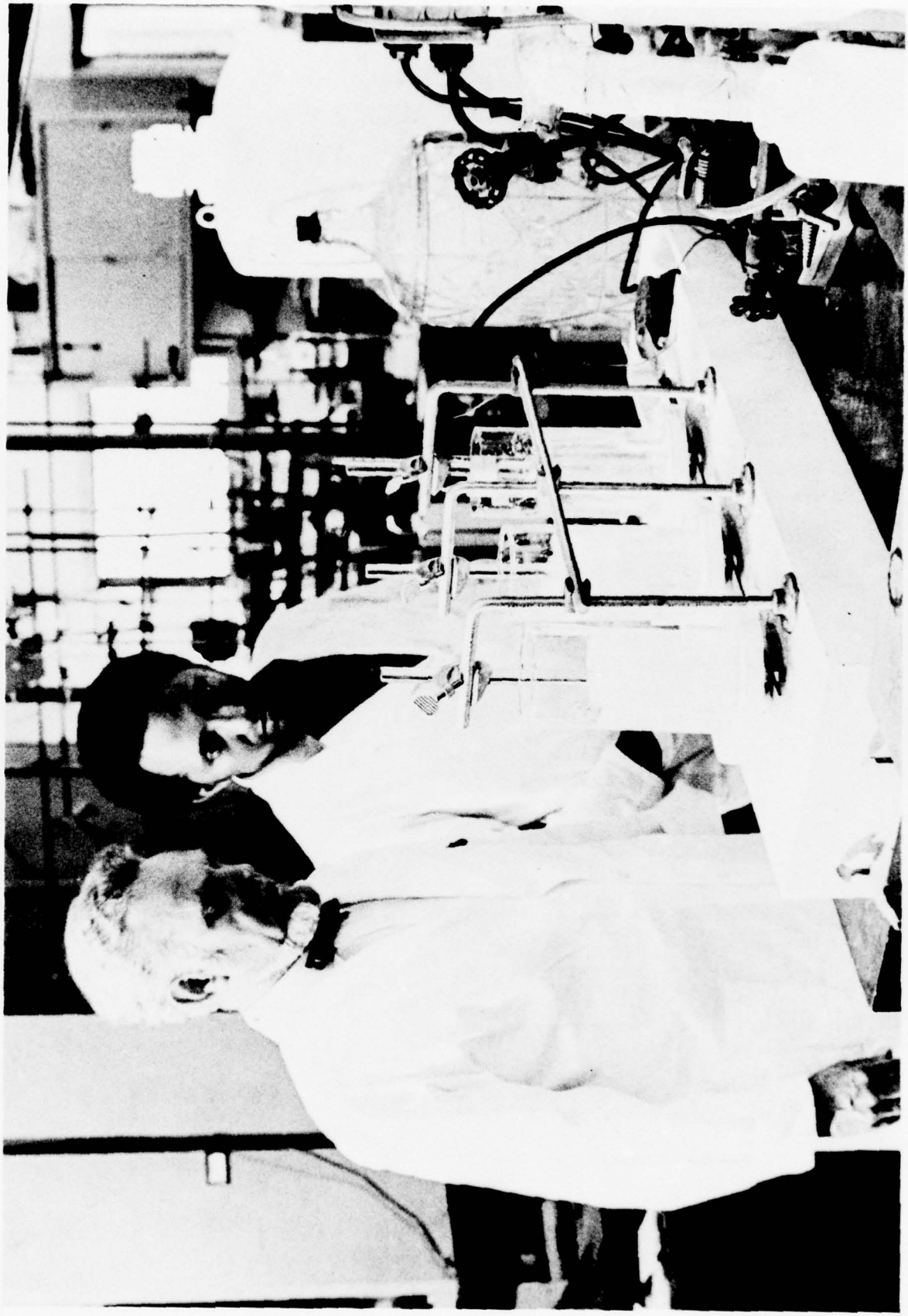


Figure 11. Research on the butanol exptraction process at Stanford Research Institute--
Dr. Sheldon Crane (L) and Dr. Akilu Lemma (R)

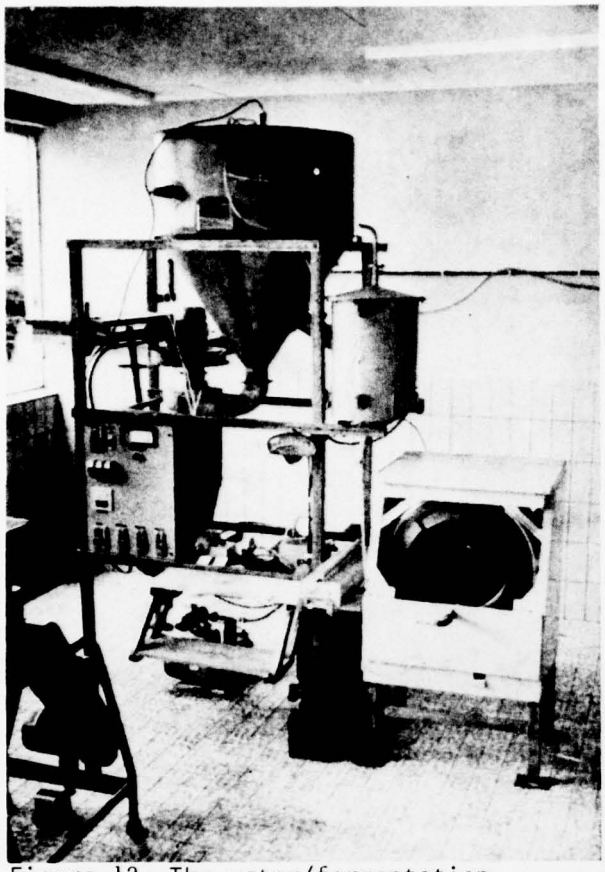


Figure 12. The water/fermentation extraction plant

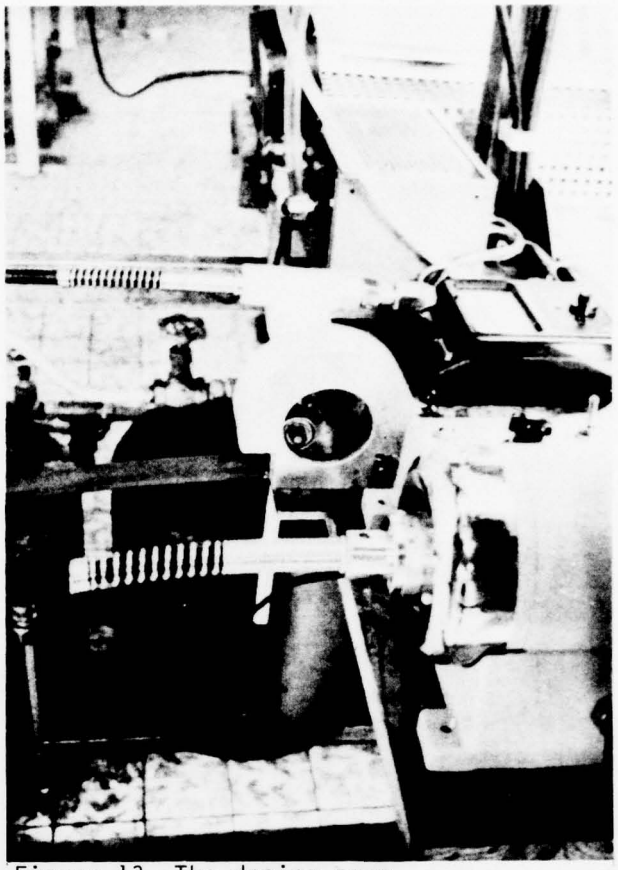


Figure 13. The dosing pump

Figure 14. The dryer

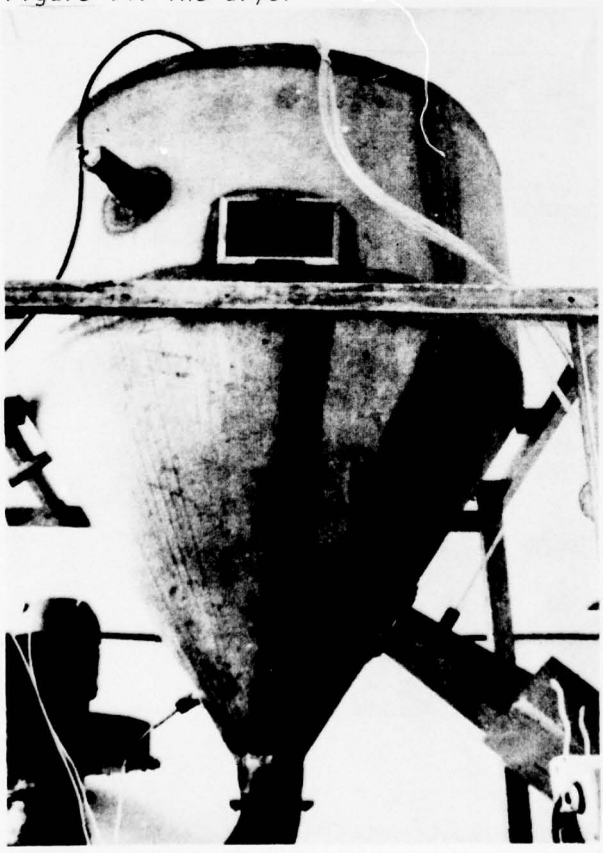
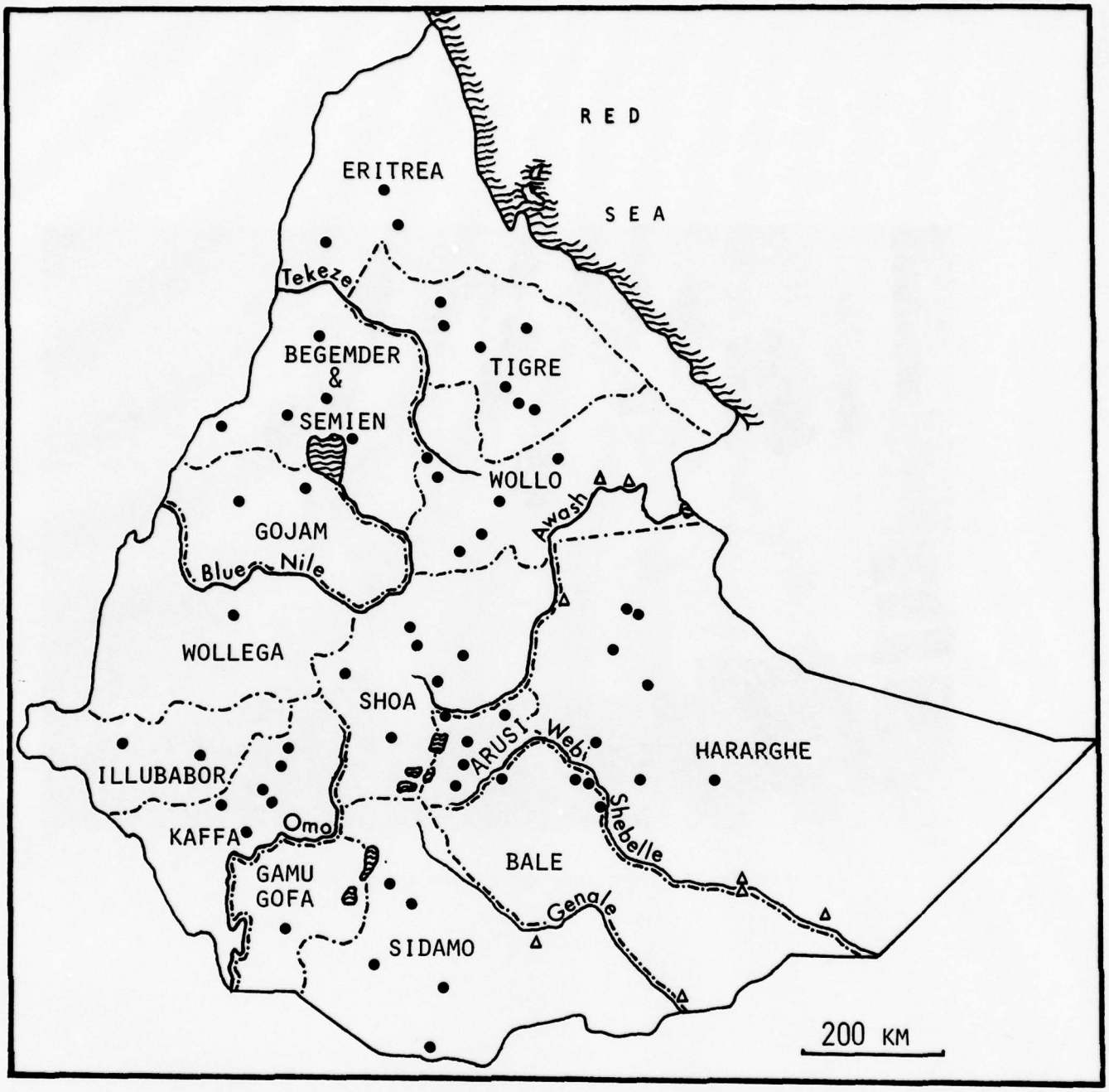


Figure 15. The spray nozzle

Figure 16.
DR. LEGESSE W. YOHANNES, THE INSTITUTE'S AGRONOMIST, STUDYING GERMINATION OF ENDOD UNDER CONTROLLED CONDITIONS

Figure 17.
DR. R.M. PARKHURST EXAMINING ENDOD BERRIES AT THE DEBRE ZEIT EXPERIMENTAL FARM

Figure 18. Institute of Pathobiology staff



MAP DISTRIBUTION OF SCHISTOSOMIASIS IN ETHIOPIA*

● S. mansoni
▲ S. haematobium

Compiled by Institute of Pathobiology
Addis Ababa University
(Aklilu Lemma)

*As per hospital, health center, mission clinic and IPB reports during 1970-1973.

I. REFERENCES ON ENDOD

A. UNPUBLISHED REPORTS

1. Assefa, G. (1976). Progress report: Studies on the insecticidal property of endod: houseflies and black flies (Simulium sp.). Unpublished Research Note No. 16. Institute of Pathobiology.

2. Crane, S.C., Lemma, A. and Heyneman, D. (1973). Properties of Endod (Phytolacca dodecandra): Pilot-scale production and quantitative evaluation of the butanol extract in Ethiopia. Annual Report No. 1 to the Office of Naval Research. Addis Ababa.

3. Getaneh, M. and Lemma, A. (1977). Comparative toxicity studies of 3 molluscicides (Bayluscide, Frescon and Endod) to microflora (Phytoplankton spp.) and microfauna (Zooplankton Crustacea spp. and Arthropoda insects) and toxicity study of endod. Unpublished Research Note No. 4. Institute of Pathobiology.

4. Horton, W.J. (1968). Preliminary report on investigations of molluscicidal components of Phytolacca dodecandra. W.H.O. Molluscicide Information Series. 64(24).

5. Kloos, H. and Lemma, A. (1979). The epidemiology of Schistosoma mansoni infection in Tensae Berhan, Ethiopia. II. Human water contact patterns. Unpublished report. Fresno.

6. Lemma, A. (1972). Studies on the molluscicidal properties of endod (Phytolacca dodecandra). Final Report prepared for the Office of Naval Research. Stanford Research Institute. Menlo Park.

7. Lemma, A. (1976). Ethiopian-Egyptian Collaborative Schistosomiasis Research Project. Unpublished report.

8. Lemma, A. and Heyneman, D. (1971). Studies on the molluscicidal properties of endod: Pilot-scale production and quantitative field evaluation in Ethiopia. Unpublished report. University of California. San Francisco.

9. Lemma, A., Besuneh, T., Tekie, Y. and Yohannes. L.W. (1976). Research Proposal: Agrobotanical studies on endod (Phytolacca dodecandra). Mimeographed. Institute of Pathobiology.

10. Lemma, A. Maxwell, A. and Brody, G. (1972). Antimicrobial and anthelmintic activities of endod (Phytolacca dodecandra). Unpublished Research Note No. 12. Institute of Pathobiology.

11. Lemma, A. and Heyneman, D. (1975). Molluscicidal properties of endod (Phytolacca dodecandra): Pilot scale production of the water extract. Unpublished Research Note No. 11. Institute of Pathobiology.

12. Lemma, A., Flemings, M.B., Assefa, G. and Tesfaye-Yohannes, R.M. (1975). Studies on the larvicidal properties of endod (Phytolacca dodecandra). Unpublished Research Note No. 14. Institute of Pathobiology.

13. Lemma, T. (1978). Medical Plants Chemistry Research. Unpublished Research Note No. 10. Institute of Pathobiology.

14. Lemma, A., Polderman, A.M., DeSole, G., Mazengia, B., Redda, A. and Kloos, H. (1979). The epidemiology of Schistosoma mansoni infection in Tensae Berthan, Ethiopia. I. Prevalence of schistosomiasis. Ethiopian Medical Journal, in press.

15. Lemma, T. (1977). Report of preliminary field trial of a chemical assay of endod. Unpublished Research Note No. 3. Institute of Pathobiology.

16. Lemma, T. and Lemma, A. (1977a.). New approaches to endod (Phytolacca dodecandra) extraction--a comparative study. Unpublished Research Note No. 1. Institute of Pathobiology.

17. Lemma, T. and Lemma, A. (1977b.). Colorimetric determination of endod. Unpublished Research Note No. 2. Institute of Pathobiology.

18. Lemma, T. and Yohannes, L.W. (1977). Selected stage for endod berry harvesting. Unpublished Research Note No. 8. Institute of Pathobiology.

19. Lugt, C.B. (1978). Phytolacca dodecandra (endod) as a means to control schistosomiasis transmitting snails. Unpublished annual report to the Institute of Pathobiology. Unpublished Research Note No. 7. Institute of Pathobiology.

20. Mamo, E. (1977). A short note on toxicity studies of endod (Phytolacca dodecandra) on sheep and dogs. Unpublished Research Note No. 5. Institute of Pathobiology.

21. McEvers, J.C. (1976) Socioeconomic considerations of the Special Programme. Unpublished W.H.O. Document TDR/WP/76.31.

22. Michael, T.G. and Yohannes, L.W. (1977). Preliminary report of the investigation on insect pests of endod (Phytolacca dodecandra). Unpublished Research Note No. 9. Institute of Pathobiology.

23. Parkhurst, R.M. (1977). Endod--Wonder Weed of Africa. Unpublished manuscript. Stanford Research Institue. Menlo Park.

24. Tekie, Y. and Yohannes, L.W. (1977). Progress report and plan of work. Unpublished Research Note No.6. Institute of Pathobiology.

25. Tesfa Yohannes, T.M. (1976). Report on the effect of endod on Culex fatigans and A. gambiae larvae. Unpublished Research Note No. 15. Institute of Pathobiology.

26. United Nations Development Programme (1975). Expanded Programme of Research, Training and Control of Schistosomiasis and other Water-borne Diseases in Ethiopia. Document Eth. 75/038. Addis Ababa.

27. Wossen, C.G. and Lemma, A. (1977). Antibacterial and antiprotozoan properties of endod. Unpublished Research Note No. 13. Institute of Pathobiology.

28. Yasuraoka, K. (1971). Laboratory evaluation of the molluscicidal properties of triterpenoid saponin, a molluscicidal fraction from endod (Phytolacca dodecandra) against Oncomelania nosophora. Unpublished W.H.O. Document WHO/SCHISTO/71.8.

B. PATENTS AND REGISTRATION:

29. Ethiopian Government, Ministry of Interior (1972). Certificate of Registration of Association. Addis Ababa.

30. Jewers, K. and King, T.A. (1974). Improvements relating to molluscicides. The Patent Office, London. Patent no. 1277414.

31. Lemma, A., Parkhurst, R.M. and Skinner, W.A. (1974). Method of producing a molluscicide from endod. United States Patent Office. Patent no. 3,813,383.

32. Parkhurst, R.M. and Stolzenberg, S.J. (1975). Saponin-containing spermatocidal compositions. United States Patent Office. Patent No. 3,886,272.

C. CORRESPONDENCE AND UNPUBLISHED REPORTS ON ASSOCIATED PROJECTS:

33. Letter from R. Malinow to R.M. Parkhurst, July 18, 1978.

34. Malinow, T. Personal communication to G.W. Newell, March, 1978 and letter from G.W. Newell to R.M. Parkhurst, March 7, 1978.

35. Paulini, E. (n.d.). Evaluation of endod extract as molluscicide. Unpublished report. Bela Horizonte, Brazil.

36. Sturrock, R.F. Letter to A. Lemma, 4 August 1972.

37. Upatham, E.S. (1972). The efficacy of endod against St. Lucian B. glabrata. Unpublished report. Castries, St. Lucia.

38. Warley, A.P. Letter to Dr. A. Lemma, 2 February 1972.

D. POPULAR PUBLICATIONS

39. Anonymous (1971). Cleaning out the snail population. Medical World News 3 (Feb. 19).

40. _____(1973). Of snails and parasites and the war on schistosomiasis. World 4(24): 41-42.

41. _____(1974). Birth control substance found in plant material. Intercom (Stanford Research Institute) 219 (Jan.11).

42. _____(1974). Grassroots attack on disease. The Sidney Morning Herald, Nov. 28, p. 24; Nov. 29, p. 14.

43. Muller, M. (1974). Grassroots attack on bilharzia. New Scientist, 4: 14-15 (4 July).

44. _____(1975). Progress report by graduate. In Brief (The Johns Hopkins University, School of Hygiene and Public Health). 11th Alumni Issue: 2-3.

45. Anonymous (1978). Folklore surrounding a common weed used by Ethiopians has led scientist to investigate a totally new means of contraception. Research Resources Reporter 2:(10): 15-16.

E. CONFERENCE REPORTS, REVIEWS, AND RESEARCH PUBLICATIONS

46. Baalawy, S.S. (1972). Laboratory evaluation of the molluscicidal potency of a butanol extract of Phytolacca dodecandra. Bulletin of the World Health Organization 47: 422-425.

47. Beckingham, C.F. and Huntingford, G.W.B. (1954). Some Records of Ethiopia. 1593-1646. London: The University Press, Glasgow.

48. Dalziel, J.M. (1936). The Useful Plants of West Tropical Africa. London: Crown Agents.

49. Flemings, M.B. and Goll, P.H. (1971). Progress report on schistosomiasis (bilharziasis) control in a northern Ethiopian community. Proceedings of the Afro-Asian Symposium on Chemotherapy of Schistosomiasis, Cairo. 665-677.

50. King, T.A., Jewers, K., Richardson, H. and Falshaw, C.P. (1968). Endod saponin, the molluscicide of the fruit of endod (Phytolacca dodecandra). Paper read at the 5th International Symposium of the Chemistry of Natural Products. London, July 1968. Abstract F43.

51. Kloos, H. (1976). Preliminary studies of medicinal plants and plant products in markets of central Ethiopia. Ethnomedizin 4 (1/2): 63-103.

52. Kloos, H. (1977). Schistosomiasis and irrigation in the Awash Valley of Ethiopia. Ph.D. dissertation, University of California, Davis. Published by University Microfilms International, Ann Arbor, Michigan. 284 pp.

53. Kloos, H. and Lemma, A. (1977). Schistosomiasis in irrigation schemes in the Awash Valley, Ethiopia. American Journal of Tropical Medicine and Hygiene 26: 899-908.

54. Lemma, A. (1965a.). A preliminary report on the molluscicidal property of endod (Phytolacca dodecandra). Ethiopian Medical Journal 3: 187-190.

55. Lemma, A. (1965b.). Schistosomiasis in Adwa--a report on an ecological pilot study. Ethiopian Medical Journal 3: 84-92.

56. Lemma, A. (1970a). Present status of endod as a molluscicide for the control of schistosomiasis. Proceedings of the O.A.U. Symposium on Bilharziasis in Africa. Addis Ababa. November 1970.

57. Lemma, A. (1970b.). Laboratory and field evaluation of the molluscicidal properties of Phytolacca dodecandra. Bulletin of the World Health Organization 42: 597-617.

58. Lemma, A. (1971). Present status of endod as a molluscicide for the control of schistosomiasis. Ethiopian Medical Journal 9: 113-118.

59. Lemma, A. (1973). Schistosomiasis: The social challenge of controlling a man-made disease. Impact of Science on Society, (UNESCO) 23: 133-142.

60. Lemma, A. (1973). Will pokeweed extract control the number one parasitic disease? Naval Research Reviews 26:(6): 1-10.

61. Lemma, A. and Duncan, J. (1970). First annual report of the schistosomiasis control project in Adwa, Ethiopia. Paper read at the 2nd International Congress of Parasitology, Washington, D.C., September 1970. Journal of Parasitology 56: 438-439. (Abstract).

62. Lemma, A. and Yau, P. (1974). Studies on the molluscicidal properties of endod (Phytolacca dodecandra). II. Toxicity of various molluscicides to fish and snails. Ethiopian Medical Journal 12: 109-114.

63. Lemma, A. and Yau, P. (1974). Studies on the molluscicidal properties of endod. III. Stability and potency under different environmental conditions. Ethiopian Medical Journal 13: 115-124.

64. Lemma, A. and Ames, B.N. (1975). Screening for mutagenic activity of some molluscicides. Transactions Royal Society of Tropical Medicine and Hygiene 69: 167-168.

65. Lemma, A., Brody, G., Newell, G.W., Parkhurst, R.M. and Skinner, W.A. (1972). Endod (Phytolacca dodecandra), a natural product molluscicide: increased potency with butanol extraction. Journal of Parasitology 58: 104-107.

66. Lemma, A., Goll, P.H., Duncan, J. and Mazengia, B. (1978). Control of schistosomiasis with the use of endod in Adwa, Ethiopia: results of a 5-year study. Proceedings of the International Conference on Schistosomiasis, Cairo: 415-436.

67. Lugt, C.B. (1978). Chemical and quantitative aspects of Phytolacca dodecandra berries as a molluscicide. Ethiopian Medical Journal 16: 140-141 (Abstract).

68. Parkhurst, R.M. (1975). The chemotaxonomy of Phytolacca species. Indian Journal of Chemistry 13: 757-758.

69. Parkhurst, R.M., Thomas, D.W., Skinner, W.A. and Cary, L.W. (1973). Molluscicidal saponins of Phytolacca dodecandra: Oleanoglycotoxin-A. Phytochemistry 12: 1437-1442.

70. Parkhurst, R.M., Thomas, D.W. Skinner, W.A. and Cary, L.W. (1974). Molluscicidal saponins of Phytolacca dodecandra: Lemmatoxin. Canadian Journal of Chemistry 52: 702-705.

71. Parkhurst, R.M., Thomas, D.W. Skinner, W.A. and Cary, L.W. (1973). Molluscicidal saponins of Phytolacca dodecandra: Lemmatoxin-C. Indian Journal of Chemistry 11: 1192-1195.

72. Powell, J.W. and Whalley, W.B. (1969). Triterpenoid saponins from Phytolacca dodecandra. Phytochemistry 8: 2105-2107.

73. Schumacher, E.F. (1973). Small is Beautiful. Economics as if People Mattered. London: Bland & Briggs ltd.

74. Shiff, C.J. (1972). The effects of molluscicides on the microflora and microfauna of aquatic systems, in The Careless Technology. eds. M. Farvar and J.P. Milton, Garden City, N.Y.: 109-115.

75. Spielman, A. and Lemma, A. (1973). Endod extract, a plant-derived molluscicide: Toxicity for mosquitoes. The American Journal of Tropical Medicine and Hygiene 22: 802-804.

76. Stolzenberg, S.J. and Parkhurst, R.M. (1974). Spermicidal actions of extracts and compounds from Phytolacca dodecandra. Contraception 10: 135-143.

77. Stolzenberg, S.J., Parkhurst, R.M. and Reist, E.J. (1975). Blastocidal and contraceptive actions of saponins from Phytolacca dodecandra. Federation Proceedings 34(3).

78. Stolzenberg, S.J., Parkhurst, R.M. and Reist, E.J. (1976). Blastocidal and contraceptive actions by an extract and compounds from endod (Phytolacca dodecandra). Contraception 14: 39-51.

79. Strelcyn, S. (1973). Medecine et plantes d'Ethiopie. II. Naples: Instituto Universario Orientale.

80. Watt, J.M. and Meyer-Brandwijk, M.G. (1962). The medicinal and poisonous plants of eastern and southern Africa. 2nd ed. Edinburgh; E. and S. Livingstone Ltd. (Section on Phytolaccaceae, pp. 837-839).

81. Yohannes, W.L. (1970). Study on phytotoxicity of endod, Phytolacca dodecandra. Proceedings of the O.A.U. Symposium on Schistosomiasis. Niamey, Niger: O.A.U./STRC Publications Bureau.

II. REFERENCES ON SCHISTOSOMIASIS EPIDEMIOLOGY (INCLUDING UNPUBLISHED REPORTS)

82. Arfaa, F. (1975). Studies on schistosomiasis in Somalia. American Journal of Tropical Medicine and Hygiene 24: 280-283.

83. Aram, R.M. (1973a). Report of studies on schistosomiasis in Ethiopia by University of Sussex Ecology Unit, 1970-1973. Unpublished report. 15 pp.

84. Aram, R.M. (1973b). Studies on Biomphalaria pfeifferi in three Ethiopian highland streams. University of Sussex. Unpublished report. 32 pp.

85. Ayad, N. (1956). Bilharziasis survey in British Somaliland, Eritrea, Ethiopia, Somalia, the Sudan and Yemen. Bulletin of the World Health Organization 14: 1-117.

86. Berrie, A.D. (1970). Snail problems in African schistosomiasis. Advances in Parasitology 8: 43-96.

87. Brown, D.S. (1964). The distribution of intermediate hosts of Schistosoma in Ethiopia. Ethiopian Medical Journal 2: 250-259.

88. Brown, D.S. (1965). Freshwater gastropod mollusca from Ethiopia. Bulletin of the British Museum of Natural History (Zoology). 12: 39-94.

89. Brown, D.S. (1976). A tetraploid freshwater snail (Planorbidae: Bulinus) in the Highlands of Kenya. Journal of Natural History 10: 257-267.

90. Brown, D.S. and Burch, J.B. (1967). Distribution of cytologically different populations of the genus Bulinus in Ethiopia. Malacologia 6: 189-198.

91. Brown, D.S. and Lemma, A. (1970). The molluscan fauna of the Awash River, Ethiopia. Annals of Tropical Medicine and Parasitology 64: 533-538.

92. Brown, D.S. and Wright, C.A. (1972). On a polyploid complex of freshwater snails (Planorbidae: Bulinus) in Ethiopia. Journal of Zoology (London) 167: 97-132.

93. Bruijning, C.F.A. (1969). Bilharziasis in irrigation schemes in Ethiopia. Tropical and Geographical Medicine 21: 280-292.

94. Buck, A.A., Spruyt, D.J., Wade, M.K., Deressa, A. and Feyessa, E. (1965). Schistosomiasis in Adwa: A report of an epidemiological pilot study. Ethiopian Medical Journal 3: 93-106.

95. Burch, J.B. (1967). Some species of the genus Bulinus in Ethiopia, possible intermediate hosts of schistosomiasis haematobia. Ethiopian Medical Journal 5: 245-257.

96. Burch, J.B. (1972). Names for two polyploid species of African Bulinus (Basommatophora: Planorbidae). Malacological Review 5: 7-8.

97. Burch, J.B. (1973). Some current research on the genus Bulinus. Malacological Review 6: 43.

98. Chang, W.P. (1961). Report on an epidemiological study on bilharziasis in Gorgora, north shore of Lake Tana. Gondar Health Series 1: 4-7.

99. DeSole, G., Lemma, A. and Mazengia, B. (1978). Preliminary report on schistosomiasis in the Wabi Shebelle Valley. American Journal of Tropical Medicine and Hygiene 27: 928-930.

100. Duncan, J. and Goll, P. (1970). A description of schistosomiasis in Ethiopia with reference to Adwa and HVA Wonji-Shoa Sugar Estate, Proceedings, O.A.U. Symposium on Bilharziasis in Africa. Nov.

101. Duncan, J., Lemma, A., and Mazengia, B. (1970). A comparison of certain stool examination techniques with special reference to their efficacy in surveys for schistosomiasis in the Awash Valley. Ethiopian Medical Journal 8: 17-25.

102. Farooq, M. and Mallah, M.B. (1966). The behavioral pattern of social and religious water contact activities in the Egypt 49 bilharziasis project area. Bulletin of the World Health Organization 35: 377-387.

103. Fuller, E. (1966). A medical survey of the Saysay people in the Blue Nile Gorge. Ethiopian Medical Journal 4: 155-165.

104. Hiatt, R.A. (1976). Morbidity from Schistosoma mansoni infections: an epidemiological study based on quantitative analysis of egg excretion in two highland Ethiopian villages. American Journal of Tropical Medicine and Hygiene 25: 808-817.

105. Hiatt, R.A. and Gebre-Medhin, Menzri (1977). Morbidity from Schistosoma mansoni infections: an epidemiologic study based on quantitative analysis of egg excretion in Ethiopian children. American Journal of Tropical Medicine and Hygiene 26: 473-481.

106. Kloos, H. (1977). Schistosomiasis and Irrigation in the Awash Valley of Ethiopia. Ph.D. dissertation, University of California, Davis, 284 pp.

107. Kloos, H. and Lemma, A. (1974). Bilharziasis in the Awash Valley. II. Molluscan fauna in irrigation farms and agricultural development. Ethiopian Medical Journal 12: 157-173.

108. Kloos, H. and Lemma, A. (1977a). Schistosomiasis in irrigation schemes in the Awash Valley, Ethiopia. American Journal of Tropical Medicine and Hygiene 26: 899-908.

109. Kloos, H. and Lemma, A. (1977b). Bilharziasis in the Awash Valley. III. Epidemiological studies in Nura Era, Abadir, Melka Sadi and Amibara irrigation schemes. Ethiopian Medical Journal 15: 161-168.

110. Kloos, H., Lemma, A. and DeSole, G. (1978). Schistosoma mansoni distribution in Ethiopia: a study in medical geography. Annals of Tropical Medicine and Parasitology 72 (5): 461-470.

111. Kloos, H. and Lemma, A. (1979). The epidemiology of Schistosoma mansoni infection in Tensae Berhan, Ethiopia. II. Human water contact patterns. Unpublished report. Fresno.

112. Kloos, H., Polderman, A.M., DeSole, G. and Lemma, A. (1977). Schistosomiasis haematobium in seminomadic and agricultural Afar in Ethiopia. Tropical and Geographical Medicine 29: 399-406.

113. Kubasta, A.M. (1964). Schistosomiasis mansoni in the Harar Province. Ethiopian Medical Journal 2: 260-271.

114. Lemma, A. (1964). Schistosomiasis in Ethiopia. (Editorial). Ethiopian Medical Journal 3: 1-2.

115. Lemma, A. (1965a.). Schistosomiasis, an emerging problem in Ethiopia. First Annual Meeting, Ethiopian Medical Association, May, Addis Ababa. (Abstract).

116. Lemma, A. (1965b.) Schistosomiasis in Adwa--a report on an ecological pilot study. Ethiopian Medical Journal 3: 84-92.

117. Lemma, A. (1968). Studies on bilharziasis in Wonji - Shoa, and Metahara sugar plantations with recommendations for possible control measures to be taken by the HVA Company. Institute of Pathobiology Internal Publication (30 pp.).

118. Lemma, A. (1969) Bilharziasis in the Awash Valley, I. Epidemiological study with special emphasis on its possible future economic and public health importance. Ethiopian Medical Journal 7: 147-176.

119. Lemma. A., Demisse, M. and Mazengia, B. (1968). Parasitological survey of Addis Ababa and Debre Zeit school children with special emphasis on bilharziasis. Ethiopian Medical Journal 6: 61-71.

120. Lemma, A., DeSole, G., Polderman, A.M., Mazengia, B. and Kloos, H. (1979). The epidemiology of Schistosoma mansoni in Tensae Berhan, Ethiopia. I. Prevalence of schistosomiasis. Ethiopian Medical Journal (in press).

121. Lemma, A. and Duncan, J. (1968). An integrated epidemiological, malacological, parasitological and molluscicidal study of schistosomiasis in Ethiopia. Institute of Pathobiology Internal Publication (36 pp.).

122. Lemma, A., Fuller, G.K. and DeSole, G. (1978). Schistosomiasis in southwestern Ethiopia. Unpublished report, Institute of Pathobiology.

123. Lemma, A., Goll, P., Duncan, J. and Mazengia, B. (1978). Control of schistosomiasis with use of endod in Adwa, Ethiopia: results of a 5-year study. Proceedings of the International Conference on Schistosomiasis (Cairo) 1: 415-436.

124. Lo, C.T. (1970a.). Snail intermediate host of Schistosoma haematobium in Ethiopia. Proceedings of the O.A.U. Symposium on Schistosomiasis, Niamey, Niger. O.A.U./S.T.R.C., Publications Bureau: 85-88.

125. Lo, C.T. (1970b.). Experimental exposure of Bulinus spp. to miracidia of Schistosoma haematobium from Ethiopia. Proceedings of the O.A.U. Symposium on Schistosomiasis, Niamey, Niger. O.A.U./S.T.R.C., Publications Bureau.

126. Lo, C.T. (1972). Compatibility and host-parasite relationships between species of the genus Bulinus (Basommatophora: Planorbidae) and an Egyptian strain of Schistosoma haematobium (Trematoda: Digenea). Malacologia 11: 225-280.

127. Lo, C.T., Flemings, M.B. and Lemma, A. (1973). Schistosomiasis in Harar, Alemaya and Damota Valley. Ethiopian Medical Journal 11: 271-278.

128. Lo, C.T. and Lemma, A. (1975). Studies on Schistosoma bovis in Ethiopia. Annals of Tropical Medicine and Parasitology 69: 375-382.

129. Malek, E.A. (1958). Factors conditioning the habitat of bilharziasis intermediate hosts of the family Planorbidae. Bulletin of the World Health Organization 18: 785-818.

130. Mandahl-Barth, G. (1965). The species of the genus Bulinus, intermediate hosts of Schistosoma. Bulletin of the World Health Organization 33: 33-44.

131. McConnell, E. and Armstrong, J.C. (1976). Intestinal parasitism in fifty communities on the Central Plateau of Ethiopia. Ethiopian Medical Journal 14: 159-168.

132. Pankhurst, (1968). Economic History of Ethiopia. 1800-1935. Addis Ababa: Haile Sellassie I University Press. See pp.48-66; 280-341; 545-579.

133. Polderman, A.M. (1974). Intestinal schistosomiasis north and west of Lake Tana, Ethiopia. Tropical and Geographical Medicine 26: 170-177.

134. Polderman, A.M. (1975). The Transmission of Intestinal Schistosomiasis in Begemder Province, Ethiopia. Ph.D. dissertation, Rijksuniversiteit Leiden (Netherlands), published in Acta Leidensia 42: 1-193.

135. Roundy, R.W. (1975). Ethiopian man-influenced sylvan habitats as human disease foci. Paper presented at the Annual Meeting of the Association of Pacific Coast Geographers, Fresno 66 pp.

136. Russell, H.B. (1958). Final report, Pilot Mobile Health Team, Ethiopia. Unpublished document EM/PHA/62 of the World Health Organization. Geneva. 36 pp.

137. Schaller, K.R. and Kuls, W. (1972). Äthiopien-Ethiopia. Geomedical Monograph 3. New York: Springer Verlag. 159 pp.

138. Southgate, V.R. and Knowles, R.J. (1977). On the intermediate hosts of Schistosoma haematobium from western Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene 71: 82-83.

139. Teklehaimanot, R. and Goll, P. (1978). Investigations into the control of schistosomiasis at the HVA Wonji-Shoa Sugar Estates in Ethiopia. Ethiopian Medical Journal 16: 115-127.

140. Trimingham, J.S. (1952). Islam in Ethiopia. London: Oxford University Press. 299 pp.

141. Wu, S.-K. (1972). Comparative studies on a polyploid series of the African snail genus Bulinus (Basommatophora: Planorbidae). Malacological Review 5: 95-164.

142. Wu, S.-K. (1973). Cross-breeding experiments with the African snail genus Bulinus (Gastropoda: Planorbidae). Malacological Review 6: 203.

143. Wu, S.-K. and Burch, J.B. (1974). Experimental infection on Bulinus sericinus with Schistosoma haematobium. Malacological Review 7: 56.

144. Wu, S.-K. and Burch, J.B. (1975). Bulinus sericinus (Gastropoda: Planorbidae) from Ethiopia. Malacological Review 8: 31-46.

145. Yasuraoka. K. (1973). A survey of the snail vectors of schistosomiasis in Ethiopia. Unpublished Document EM/SCHIS/54, ETHIOPIA 2101/R. 17 pp.

III. ADDITIONAL REFERENCES

146. Flemings, M.B. (1975). Effects of endod on the perispiracular glands of mosquitoes. Unpublished report. C.W. Post College, Long Island University, Brockville, N.Y.

147. Kloos, H., Lemma, A., Kirub, B., Gebre, A., Mazengia, B., Feleke, G., and DeSole, G. (1979). Intestinal parasitism in migrant farm labourers, indigenous farmers and seminomadic pastoralists in irrigation schemes in the Awash Valley, Ethiopia. Ethiopian Medical Journal, in press.

STANFORD
RESEARCH
INSTITUTE

**LIFE SCIENCES
RESEARCH REPORTS**

Volume 3 Number 2
April 1971

A SPECIAL REPORT*

SCHISTOSOMIASIS AND LEISHMANIASIS

"I am working on two projects. One is on the development of a new molluscicide of plant origin for the control of schistosomiasis, a very widespread and important tropical disease. The other project is directed toward the development of a vaccine for the control of leishmaniasis, another widespread tropical disease."

Dr. Aklilu Lemma is a distinguished scientist on sabbatical leave from the Haile Selassie I University, Addis Ababa, where he is Director of the Institute of Pathobiology and Associate Professor of Biology. He received his Doctor of Science degree from Johns Hopkins University, School of Hygiene and Public Health, in 1964. During his tenure at the Haile Selassie I University, he served as Dean of the Faculty of Science from July 1966 to January 1970, when he resigned from the Deanship to go into full-time research and to develop his laboratory. In his capacity as researcher and official of the Haile Selassie I University, he traveled extensively to various parts of Europe, the United States, Canada, South America, and Asia. Among his many professional activities, he has served as Chairman of the Local Organizing Committee for the Pugwash Conference on Science and World Affairs held in Addis Ababa from December 29, 1965 to January 3, 1966; Secretary-General and Chairman of the Local Organizing Committee for the Second International Conference on the Global Impacts of Applied Microbiology held in Addis Ababa from November 6 to 11, 1967; and Vice-Chairman and Chief Organizer of the National Committee for the establishment of the "National Scientific and Technical Research Council of Ethiopia." His honors include various fellowships from the Imperial Ethiopian government, the International Atomic Energy Agency, the World Health Organization, and Johns Hopkins University. He is also a recipient of His Imperial Majesty Haile Selassie I's Gold Medal for achievements in scientific research in Ethiopia. Dr. Lemma has contributed 22 publications in the general area of parasitology and tropical medicine. His major research interests and his reasons for being at SRI are described in the ensuing interview.

*Editor's Note: This is the first Special Report of the Life Sciences Research Reports. We are pleased to have this opportunity to present to our readers the important research of Dr. A. Lemma, Visiting Scientist at Stanford Research Institute. Dr. Lemma also has an appointment as Lecturer at the Stanford University Medical School.

Dr. Otis Would you tell me, Dr. Lemma, about the research you are carrying on at SRI?

Dr. Lemma I am working on two projects. One is on the development of a new molluscicide of plant origin for the control of schistosomiasis, a very widespread and important tropical disease. The other project is directed toward the development of a vaccine for the control of leishmaniasis, another widespread tropical disease.

Dr. Otis Would you describe these diseases and indicate their public health importance in general?

Dr. Lemma Schistosomiasis, or bilharziasis as it is also sometimes called, is a disease transmitted by aquatic snails that breed rapidly in irrigation canals, water reservoirs, lakes, slow-flowing streams, and other such bodies of water. The causative organisms are flukes. Adult male and female pairs of the parasites reside in small blood vessels, usually in the mesentery or the pelvic region of infected individuals. The *Schistosoma* parasite is able to lay large numbers of eggs—a single female parasite is known to lay up to 3,500 eggs per day and has a life span of 20 to 30 years. An infected person may harbor a large number of such females. The great mass of eggs continually laid by such worms, together with the host's reaction to such foreign materials, cause a considerable destruction of the liver or the bladder and ureter, depending on the species of the parasite involved. The parasites that characteristically and preferentially lodge in the mesenteries are *Schistosoma mansoni*, and those that enter the bladder area are *Schistosoma haematobium*; the diseases they cause are known as intestinal schistosomiasis and urinary schistosomiasis, respectively. A third species, *Schistosoma japonicus*, which is mainly found in Asia, also lodges in the intestinal mesenteries.

The capsulated eggs of the parasites are excreted with feces or urine, as the case may be. These eggs hatch when they come in contact with water, and the freely-swimming miracidia enters an appropriate fresh-water snail and develops into a cercaria, the infective form of the parasite. Infected snails continually release millions of these cercariae into streams, canals, or whatever body of water in which the snails may be residing. The cercariae can bore through the skin of people who come into contact with the infected water, enter the blood stream, and finally lodge in their preferred sites as male and female pairs. The schematic life cycle of the parasites is shown in Figure 1.

Schistosomiasis is becoming an increasingly more serious health problem, taking the place of malaria in some countries. According to WHO estimates, more than 200 million people are suffering from the disease, and this number is rapidly increasing. In many of the developing tropical and subtropical countries where problems are many and resources limited, schistosomiasis is spreading more rapidly than it is being controlled and, in some countries, it is causing a major threat to economic development. Agricultural development based on irrigation systems involves the use of canals and water reservoirs, and such bodies of water become excellent new breeding sites for the intermediate host snails of the parasite. Large numbers of infected and uninfected people working in such places inevitably make ideal transmission sites for the rapid spread of the disease. In some endemic areas, shown in Figure 2, it is common to find 100 percent of the people infected with these parasites.

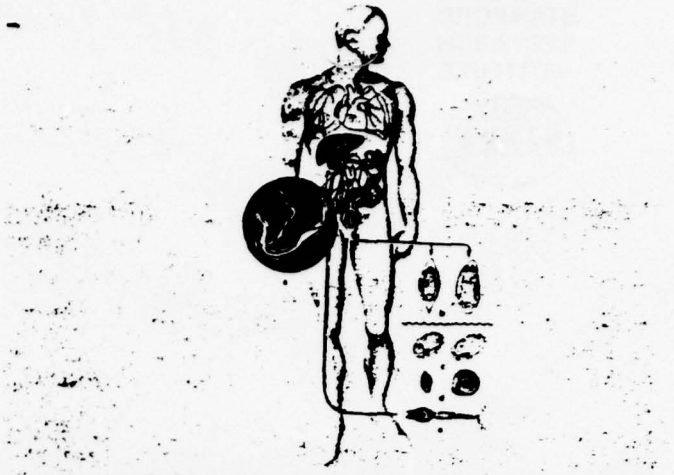


Fig. 1 *Schistosoma*, life-cycle: (a) Pair of worms, (b) Egg of *S. haematobium* and *S. mansoni*, (c) Miracidia, (d) Snail vectors of *S. haematobium (Bulinus)* and *S. mansoni (Biomphalaria)*, (e) Cercaria. (Adapted from Pflanzenschutz-Nachrichten "Bayer" 15/1962, 1.)

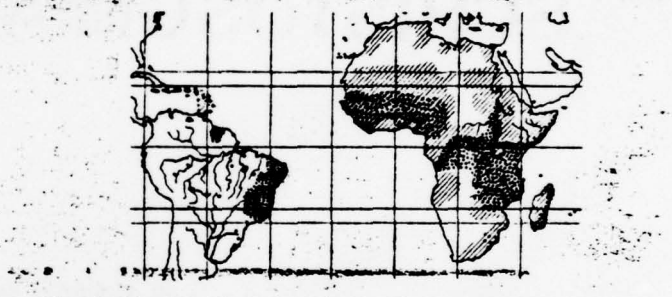


Fig. 2 Map of Africa and environs, South America and the West Indies, showing endemic foci of infection with *Schistosoma mansoni* (dots) and *Schistosoma haematobium* (lines). The solid black areas in the delta region of the Nile River and in northeastern Brazil indicate areas of hyperendemicity. (Modified from Figures 27-13 and 27-18 of Faust, *Animal Agents and Vectors of Human Disease*, Lea & Febiger, Philadelphia. Reproduced by permission of the publisher.)

Agricultural and other water-using projects that begin with good intentions may unfortunately be crippled by schistosomiasis. To cite a few examples, in Aswan Province of Egypt, it has been shown that *S. haematobium* increased from four to fortyfold within three years after introduction of perennial irrigation. The estimated economic loss due to this disease in Egypt alone is about £ 80 million per annum. In Rhodesia, when the incidence of the disease began to reach alarming proportions, the Umshandige Irrigation Scheme had to be

Endod Berries

abandoned in 1949 after ten years of construction, at a total cost of £ three million sterling.

"In view of the promising potential of the Endod plant for controlling schistosomiasis, we are currently studying the possibility of extracting the active molluscicidal principle and determining its chemical structure."

In areas where large agricultural development plans are being implemented, it is imperative that the settlers and laborers be kept healthy. The debilitating effects of schistosomiasis may involve such a heavy loss in manpower (hence in economy) that all large-scale agricultural development plans in tropical countries must take into consideration the importance of this disease. Unfortunately, unlike the case of malaria, there is no "ideal" treatment or control procedure for schistosomiasis. Searches for the "ideal" drug for treatment and chemoprophylaxis, as well as for the "ideal" molluscicide to control the snail population—to interupt the transmission of the disease—are underway in many laboratories in different parts of the world.

The molluscicide we are working on here is a natural product that can be grown in large quantities and can be used either as a crude powder or in a concentrated extract form. The shrub, commonly known in Ethiopia as Endod *(Phytolacca dodecandra)*, produces large quantities of berries that—when dried, ground, and quantitatively suspended in water—may kill snails at as low a concentration as ten parts per million. In view of the promising potential of the Endod plant for controlling schistosomiasis, we are currently studying the possibility of extracting the active molluscicidal principle and determining its chemical structure. We are also studying the mammalian, fish, and plant toxicity of the berries and the extracts to evaluate Endod's safety for large-scale use. Preliminary tests using the crude material have given very encouraging results; mammalian toxicity appears to be comparatively low, and plants do not seem to be affected even at very high concentrations.

The important potential value of such a molluscicide of plant origin is the possibility of getting abundant material for use by rural communities in endemic areas to combat schistosomiasis on a relatively inexpensive, self-help basis.

It is interesting to know that, in addition to its use as a molluscicide, Endod also has cercariacidal, miracidiacidal, and leech-killing properties. The application of Endod in rivers and canals will, therefore, render them temporarily free from *Schistosoma* infection and will also control the leech population. Aquatic leeches attack animals and are economically undesirable pests; to control them, as an offshot from the snail control program, can be very advantageous. We are looking into the possibility of using Endod in an ointment form to be rubbed on the skin to prevent cercarial penetration for chemoprophylactic use.

Dr. Otis What about leishmaniasis?

Dr. Lemma Leishmaniasis is, in general, not so widespread and does not affect as many people as schistosomiasis. It is transmitted by sandflies of the genus *Phlebotomus*, in much the same way as malaria is transmitted by mosquitos. The life cycle of the parasite is shown in Figure 3. The disease is prevalent in the Middle East, Africa, Central and South America, and some parts of Asia, as shown in Figure 4. There are three

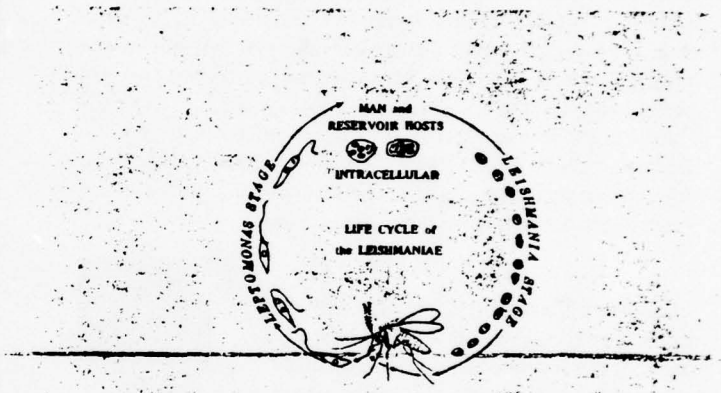


Fig. 3 Diagrammatic representation of the life cycle of species of *Leishmania* which parasitize man. Above, intracellular leishmania stage in mammalian macrophages; below, *Phlebotomus* (sandfly), intermediate host and vector; on right, leishmaniae taken up from infected mammalian host during a blood meal; on left, leptomonad stage in salivary secretions of sandflies, to be introduced into mammalian skin at time of blood meal. (After Faust, *Animal Agents and Vectors of Human Disease*, Lea & Febiger, Philadelphia. Reproduced by permission of the publisher.)

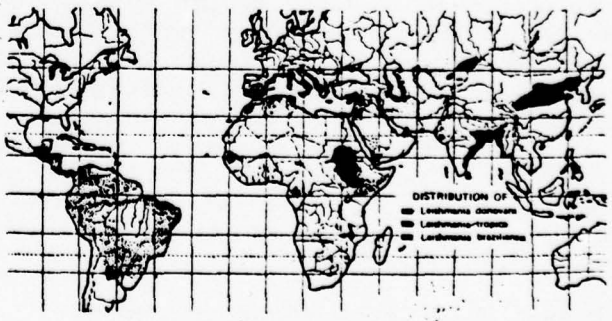


Fig. 4 World distribution of leishmania infections. (After Faust, *Animal Agents and Vectors of Human Disease*, Lea & Febiger, Philadelphia. Reproduced by permission of the publisher.)

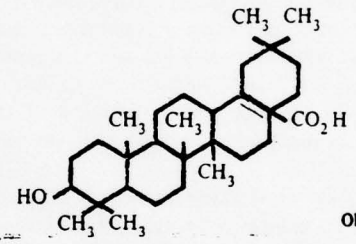
types of the disease: visceral leishmaniasis, or kala-azar, which primarily affects the liver, spleen, and bone-marrow, cutaneous leishmaniasis, or oriental sore, which affects the skin; and mucocutaneous leishmaniasis, or espundia, which affects the mucous membranes. Visceral leishmaniasis is a killer disease and has been known to depopulate vast areas in India and some places in Africa. Cutaneous leishmaniasis is, on the other hand, not so severe a disease; characteristically, it is a self-limiting, ulcerative skin sore that, on healing, leaves the individual with permanent immunity against the disease. However, in some cases, the disease may take a different course in individuals who cannot build up the necessary resistance to it. In some cases, the parasite may disseminate all over the skin of the infected person and cause lepromatous-type lesions that resemble and are often confused with leprosy; in fact, this condition is sometimes referred to as pseudolepromatous (false leprosy) leishmaniasis. Many patients have been misdiagnosed for leprosy and treated for it for several years before it was discovered that they were leishmaniasis cases.

"Cutaneous leishmaniasis is, on the other hand, not so severe a disease; characteristically, it is a self-limiting, ulcerative skin sore that, on healing, leaves the individual with permanent immunity against the disease."

I have been working on the epidemiology of cutaneous leishmaniasis in Ethiopia for the past six years and am now working on the possibility of developing a vaccine for this disease. Since we already know that infection with the organism can result in the development of complete protective immunity to a second attack, and dead organisms (formalin or heat-killed) do not provoke the necessary immunity, I am trying to attenuate the organisms by various means, including irradiation and incorporation of mutogenic chemicals in the media in which the organisms grow. I worked on this project during the past two years in Ethiopia with some financial support from the United Nations International Atomic Energy Agency. I am now continuing the study at SRI using facilities and equipment, such as a Cobalt 60 source, that are not available in Ethiopia. The present work is being supported by the National Science Foundation.

Dr. Otis What is the present status of your studies?

Dr. Lemma Well, I have been here at SRI since June 1970; since then, my colleagues at the Institute and I have made good progress on both projects. I brought about 100 kilograms of Endod berries from Ethiopia, and we are now extracting them with different solvents. We also have established good breeding colonies of bilharzia-transmitting snails for our molluscicidal tests. Dr. Skinner[1] and Mr. Parkhurst[2], the organic chemists working with me, are trying to isolate and characterize the active principle in Endod. We already have some idea as to its structure. The active principle is an oleanolic acid with some as yet undetermined sugar groups that are necessary for making the base molecule water-soluble. An attempt is also being made to obtain oleanolic acid from sources other than Endod with the hope of making synthetic analogs to see if we can get a more potent material than that in the natural product. Parallel with the chemical studies, we are also studying the toxicities of different extracts for fish and mammals. Eventually, we hope to test such purified material to determine its usefulness under field conditions in Ethiopia.



Oleanolic Acid

We are also hoping to develop a chemical assay for determining the concentration of the active principle in water. We have a two-year grant from the Office of Naval Research to support this work.

"Using the guinea pig parasite, *Leishmania enriettii*, as a model, Mr. Cole and I are studying the cellular immunity involved in the infection and the subsequent 'protection' which develops after inoculation of 'normal' and 'attenuated' organisms."

Our leishmaniasis study is also progressing well. Using the guinea pig parasite, *Leishmania enriettii*, as a model, Mr. Cole[3] and I are studying the cellular immunity involved in the infection and subsequent "protection" that develops after inoculation of "normal" and "attenuated" organisms. We are also determining the effects of various physical and chemical mutagenic agents on the viability, morphology, reproduction, and antigenicity of the *Leishmania* parasites. We have already accumulated some useful data, and the findings are encouraging.

---

[1]Dr. W. A. Skinner, Executive Director, Life Sciences Division and Director, Department of Pharmaceutical Chemistry.

[2]Mr. R. M. Parkhurst, Organic Chemist, Department of Pharmaceutical Chemistry.

[3]Mr. L. Cole, Program Manager, Immunobiology Program, Department of Biomedical Research.

Dr. Otis These are very interesting projects. Maybe you could tell me something, Dr. Lemma, about how you first became interested in these particular health problems?

Dr. Lemma My initial interest stemmed from my training. I was trained at the School of Hygiene and Public Health of the Johns Hopkins University. I worked on a *Leishmania* problem for my doctoral thesis, and I have, since then, kept my interest in these organisms. I should perhaps also mention that during the past six years of study on the epidemiology of this disease in Ethiopia, I was continually exposed to people who were suffering from the disease, and I developed greater sympathy for the victims and a greater interest in the disease.

After returning home from the Johns Hopkins University and after looking over some of the important parasitic diseases in Ethiopia, it was evident that schistosomiasis was increasing, and perhaps even posing a potential threat to the economic development of the country. Ethiopia, like many other countries, depends on agricultural products for its national economy. Therefore, there are many agricultural development projects involving irrigation canals. Such canals could greatly contribute to the possible spread of schistosomiasis. It is estimated that, in one of our biggest national plans to develop the Awash Valley into a large agricultural area, the nation could benefit by several hundred million dollars a year. The Awash Valley Authority, an independent government organization, has been established to develop the project, and we are getting assistance from the United Nations and other sources for its development. I was naturally interested to see if schistosomiasis exists in this valley and, if so, to consider what could be done about it. It is now established that the disease does exist there and is rapidly spreading, the government is currently considering various measures for its possible long-range control.

In 1964, the Ethiopian Ministry of Public Health asked me to join some public health officers in examining a village in the northern part of Ethiopia where it was reported that an "epidemic" of schistosomiasis was going on. We went there and, while making an ecological study of the distribution of snails in the streams and identifying transmission sites of the disease, I noticed that in areas where people were washing clothes with a soap-like solution made of the berries of a plant known as Endod, there were more dead snails than in other places, including those areas where people were washing with commercial soap. This happened repeatedly, which gave me the idea that whatever they were using for washing might have some snail-killing properties. This was soon proved in the laboratory, and I have since been studying and using the Endod berries in the control of schistosomiasis. I have published several papers on it and have also been collaborating with various laboratories in different countries, including SRI, on isolating the active principle in the plant and determining its structure and properties.

Dr. Otis So the snail-killing properties of this plant were not known before you discovered them?

Dr. Lemma That is correct. Although the plant has been used by people for various medicinal purposes, its molluscicidal properties were not known before my studies. Now, as a result of my publications, people in different countries are trying it and, I am glad to say, confirming my results.

"I expect to return to my Institute at the Haile Selassie I University in Addis Ababa... and conduct field evaluations of our molluscicide and potential *Leishmania* vaccine."

Dr. Otis What are your plans for the future—that is, for the remainder of the time that you will be here, as well as when you return home?

Dr. Lemma I will continue my work here at SRI until April 1972. I then plan to spend a couple of months at Harvard University to visit and consult with other colleagues working on these parasitic diseases. I also hope to continue my research, during my stay there, on the possible mode of action of Endod against snails, and to investigate the development of "normal" and "attenuated" *Leishmania* organisms in a tissue culture. I expect to return to my Institute at the Haile Selassie I University in Addis Ababa by sometime in July 1972 and conduct field evaluations of our molluscicide and potential *Leishmania* vaccine. I expect to continue collaborating with SRI and hope to supply some field data to our studies.

Dr. Otis Do you expect to come back to the Institute sometime in the future to continue these studies?

Dr. Lemma Well, I cannot predict what is going to happen in the future. I came to SRI with the specific objective of using the extensive resources and experience of the Institute to do a job that I could not do with the limited facilities we have in Ethiopia. I am very grateful to Drs. Skinner and Brody[4] for inviting me to SRI and providing me with adequate space and assistance to carry out my studies. I hope that such a useful arrangement will continue to be available so that I, or some of my colleagues, may have the privilege of using your facilities to answer some important questions that, in some cases, may not be solved otherwise. I also hope to see some of the SRI people avail themselves of the opportunities and facilities in Ethiopia for field work. This would make our collaboration of a mutual and long-lasting benefit.

Dr. Otis Thank you, Dr. Lemma, and best wishes for continued success in your research.

---

[4]Dr. G. Brody, Director, Department of Infectious Diseases, Parasitology, and Toxicology.

World Environment Newsletter

Of Snails and Parasites and the War on Schistosomiasis

No one knows how many people suffer from schistosomiasis. The most frequent estimate is 200 million, but it may well be higher. The reason for the uncertainty is that among populations where a variety of debilitating diseases are prevalent and where protein deficiency and anemia are common, schistosomiasis is difficult to diagnose. It develops almost imperceptibly, with mild symptoms at first. And it rarely kills outright. As one observer put it, "Schistosomiasis brings man to the edge of the grave; then another disease can push him over the edge."

Apart from the humanitarian instinct to relieve suffering, what is of particular concern is, first, that schistosomiasis constitutes a costly and terrible drag on the human energy needed for economic development in poor countries; and second, that the disease is spreading rapidly as a result of the increase throughout the developing world of man-made lakes for power and irrigation systems to increase agricultural production. It is especially year-round irrigation that has caused serious trouble, raising the incidence of schistosomiasis from zero or a negligible level to rates of 75 or even 90 percent of the population in some areas of Egypt, West Africa, the Philippines, China, and other places.

A Strange Life Cycle

Bilharziasis—as the disease is also called—is a pre-eminent example of the relationship between health and environment. It depends on an absence of sanitation and the presence of a particular species of water-borne snail in which the schistosome parasite must find a lodging to complete its life cycle. It is there that the larvae take up residence for about six weeks. Emerging as cercariae, they penetrate the skin of any human (and many animals) with whom they come in contact. Within a few hours the cercariae are in the blood stream, in which they travel for several weeks while maturing. Eventually they are carried to the liver, where they mate and travel on together, lodging finally in the small blood vessels in the wall of the intestines or bladder (depending on the variety of schistosome). Around the fortieth day the production of eggs begins. Some move away to other parts of the body, but others work their way into the bowels or bladder.

What makes the sanitation problem particularly difficult is that the eggs emitted in feces or urine can survive for a month before reaching water. Thus a heavy storm can wash eggs into a stream a considerable distance away. Once in the water, the egg is transformed into a swimming larva known as miracidium, which has at most twenty-four hours to find a snail host or die. Once lodged, another form of reproduction takes place, as each miracidium produces thousands of cercariae to complete (and multiply) the cycle.

Meanwhile the mature schistosomes remain lodged where they were, in the human body, surviving for anywhere from one to thirty-five years. Serious infection is a cumulative process, and the degree of debilitation and illness depends on the length of exposure and the number of schistosomes that the body is harboring.

There is no known cure for schistosomiasis, and methods of treating and controlling it are varied. In what seems to many an endless and discouraging war on the disease, there are three basic approaches, all being pursued, all with serious limitations:

1. Try to kill the snail or make its habitat so uncongenial that it cannot survive, thus breaking the life cycle of the schistosome.

2. Find better treatment for the disease in humans, try to increase man's resistance, or ideally, immunize him.

3. Use education to improve hygiene and promote agricultural practices that will make a less hospitable environment for snails.

There are a wide variety of molluscacides with which to control the snail population, but they are expensive. Experience in Egypt indicates a cost of between $2.50 and $3.00 annually per irrigated acre. In the Philippines it is estimated that the cost of effective use would exceed the entire budget of the Ministry of Health. Moreover, molluscacides must be used indefinitely; and the ecological damage they do, the food chains they may be entering, are not yet adequately known.

Recently attention has turned to biocontrol—an effort to find a predator that will kill the host snail. At least half a dozen techniques are now being investigated, but as yet little has been accomplished outside the laboratory.

Engineering Methods

A third approach to eliminating snails is to make their environment less hospitable, primarily through engineering. The capacity to drain irrigation canals is helpful. Although snails have been known to survive without water for up to eight months—many will manage to burrow deep into stream beds to find moisture—a dry season will substantially reduce the snail population.

Because snails are rarely seen in streams and canals that have average flow velocities of more than about 1.1 feet per second, weed control is of great importance. In the Gezira irrigation scheme in the Sudan, more than $150,000 a year is spent on mechanical weed control. Without such attention the carrying capacity of earthen canals is commonly reduced by one-third to one-half within three to five years, and in one exceptional case the flow dropped by 80 percent.

In the Philippines, where at least half a million persons suffer from schistosomiasis, a five-year plan is under way to clear thousands of square miles

An Ancient Disease

Although profound concern about schistosomiasis is relatively new, the disease is of great antiquity. In 1910 calcified eggs of the schistosoma parasite were found in a mummy dating to some time prior to 1000 B.C.

Washing Rivers Clean

Some years ago, a young Ethiopian parasitologist at Haile Selassie University observed that, along a riverbank where women washed clothes, the schistosome-bearing snail population was negligible in an otherwise infested river (see story this page). Dr. Aklilu Lemma reasoned that the explanation lay in the women's soap, made from the berries of an indigenous plant.

Now Endod, a natural product derived from those berries, is being widely tested as a molluscacide. It is inexpensive, easy to produce, and widely available in many places where schistosomiasis is prevalent. Furthermore, unlike the synthetic chemicals used in most molluscacides, Endod is biodegradable, and its only adverse effect on the environment appears to be that it will kill small fish. Dr. Lemma believes that the most promising approach to schistosomiasis control is a combination of molluscaciding with Endod and biocontrol based on trematode antagonism—essentially finding parasites that will attack snails.

of tropical growth, to change the course of creeks, and to fill lowlands and waterlogged areas. The World Food Program is providing incentive for volunteer labor, which is expected to amount to some 630,000 man-days. Such engineering works must be maintained permanently. This has led to the realization that control of schistosomiasis is inextricably linked with land reclamation and management, improved agricultural practices, rural employment, and water management.

Lining of canals to reduce maintenance costs and improve flows, barriers to prevent the movement of snails, radiation, and electrical charges have all been tried with greater or lesser success. But snails are not confined to streams and canals. One of the worst areas is Lake Volta, the largest artificial lake in the world. It is 250 miles long, and the greater part of its 3000-mile shoreline is ideally suited to harboring snails.

Before the construction of Akosombo Dam, completed in 1966, schistosomiasis was virtually unknown in the area. Now Ewe tribesmen, who have flocked to the lake for its abundant fish, are infected at rates of between 40 and 90 percent depending on locality. Finding ways to kill the snails without killing the fish plus the sheer size of the infected area are major problems.

Rough Treatment

Many people with schistosomiasis would rather suffer the disease than the treatment, which is not pleasant. Of the score or more drugs available, all are in varying degrees toxic to humans, and until very recently, all required extended treatment. Some drugs are given intramuscularly, some intravenously; some are effective against one form of schistosomiasis and not another. They vary widely in price. Thus chemotherapy has had serious limitations.

However, two newcomers to the chemotherapeutic arsenal have engendered some optimism. Metrifonate, effective against two of the three types of the disease, can be given orally and is relatively cheap. The drug of the moment is hycanthone, which can be given in a single, intramuscular injection. Like earlier drugs used, it may cause vomiting and other side effects, and there has been some concern that it may cause hepatitis. Still, it has been administered to more than 300,000 patients, and the evidence suggests that it is safe.

Recent discoveries give moderate encouragement that a means may be found to create resistance to the disease in humans and, conceivably, total immunization. The possibility has seemed so remote that little research support has been given to this most difficult but potentially conclusive approach. Last autumn, however, the Rockefeller Foundation made a $193,000 grant to Brown University for research both in immunization and in drugs that might provide a cure.

Yet only last year, a qualified speaker at a Tulane University symposium on schistosomiasis control could say:

> WHO and AID have now reached the same conclusion that nothing short of environmental sanitation on an unprecedented scale can prevent the continued spread of this disease. A solution is no longer technically feasible through disease-specific microbiological means. The solution appears to be in water protection, water development, waste disposal, surveillance, and health education.

Thus one is brought back again to engineering and to the third objective: altering deeply ingrained habits through education. There is much to be learned besides the advantages of using a latrine. For example, snails may abound in rice paddies. Two decades ago investigations on the island of Leyte showed that with proper plowing and harrowing, and by planting the seedlings in well-spaced rows, the number of snails could be enormously reduced, as much as 95 percent in some instances.

Another discovery of obvious value to bathers and fishermen is that the density of cercariae in the early morning is zero; it reaches its maximum in the early afternoon, then progressively decreases until sundown. The tropical custom of dozing through the hottest hours of the day is now seen to have special merit.

Clearly schistosomiasis is being attacked on a broad front and involves not only doctors and physical scientists but sociologists, anthropologists, engineers, agronomists, and hydrologists as well. Although much has been learned in recent years, there appears to be no breakthrough in sight. A speaker at the Tulane symposium spoke for many when he said, "We begin to wonder whether control of the disease is feasible, whether we are trying for the impossible."

What the Citizen Can Do

Following is a list of publications that can be useful to citizens' groups concerned with environmental responsibility:

A Guide to Citizen Participation in Environmental Action—Regional Plan Association of Southern California, 621 S. Virgil Ave., Los Angeles, Calif. 90005. Price $5.

Community Action for Environmental Quality, Prepared by the Citizens Advisory Committee on Environmental Quality, available from the Government Printing Office, Washington, D. C. 20402. Price $.60.

How to Plan an Environmental Conference—League of Women Voters Education Fund, 1730 M St., NW, Washington, D. C. 30036. Free.

A Citizen's Guide to Clean Air—Conservation Foundation, 1717 Massachusetts Ave., NW, Washington, D. C. 20036. Free.

Clean Water—It's Up to You—Izaak Walton League, 1800 N. Kent St., Arlington, Va. 22209. Free.

Law and Taxation—A Guide for Conservation and Other Non-profit Organizations—Conservation Foundation. Price $1.

Citizen Action Can Get Results—U. S. Environmental Protection Agency, Washington, D. C. 20460. Free.

Environmental Education/Facility Resources—Educational Facilities Laboratories, 477 Madison Ave., New York, N.Y. 10022. Price $2.

What's New in Urban Parking?

Four experts in urban parking problems made the following predictions to the editors of *Nation's Cities*:

- Facilities for fringe parking on the edge of central business districts with connecting shuttle buses or elevated people movers will increase.
- The single-purpose municipal parking garage will decline. Air rights for combined commercial and residential uses over off-street parking facilities will increase.
- There will be more off-street parking facilities in residential areas.
- All-day parking permits on a weekly, monthly, or yearly basis will grow.
- New techniques for the construction of parking garages will be employed, with emphasis on readily portable precast concrete modules.
- Methods for cutting parking personnel costs will be pursued. For example: automated garage gates, computerized billing, and monthly parking permits used interchangeably in different garages.

SCHOOL OF PUBLIC HEALTH AND TROPICAL MEDICINE

AUSTRALIAN DEPARTMENT OF HEALTH AND THE UNIVERSITY OF SYDNEY

TELEPHONE
660 4555

BUILDING A27

THE UNIVERSITY OF SYDNEY

NEW SOUTH WALES 2006

17th January, 1975.

Dr. Aklilu Lemma,
C/- Dr. Harry Hoogstraal,
NAMRU 3,
CAIRO. EGYPT.

Dear Aklilu,

I was very interested to come across your name in our "funny pages" recently and thought you would like to have the attached copies. This is obviously a much better way of presenting scientific discovery than in the traditional stuffy journals!

I trust that you are well and that we shall meet again before long.

Yours sincerely,

With kind regards.

B. McMillan
Associate Professor in Medical Parasitology

Reprinted from the Sidney Morning Herald, Nov. 28, 1974, p.24 and Nov. 29, 1974, p.16, by permission.

Grassroots attack on bilharzia

New Scientist 4 July 1974
vol. 63

An African researcher has made encouraging progress in using a pesticide from an indigenous plant to combat bilharzia—the parasitic disease that afflicts 250 million people in the Third World

Mike Muller
is a freelance journalist specialising in Third World affairs

Ethiopia is not a country from which we expect to hear good news. But over the past five years an experiment has been going on there which has made encouraging progress in the fight against a disease that ravages three continents. The village of Adwa in northern Ethiopia is already remembered in African history as the place where the 19th century Italian colonisers were halted. Now it is the setting for a new initiative against a hardier and more insidious enemy—the bilharzia parasite.

Bilharzia ranks with malaria as one of the most widespread and serious parasitic diseases in the world, afflicting an estimated 250 million people in Asia, Africa, Latin America, and the Caribbean. It is transmitted through contact with contaminated water. So, unlike malaria with its more democratic vector, bilharzia is invariably a problem only among poorer communities in the developing countries. It is a debilitating disease rather than a fatal one. It can take years for tissue damage caused by the parasite to manifest itself in the form of internal bleeding and malfunctions of the bladder, liver, and intestines. The disease saps energy and shortens the life span but it does not contribute obviously to mortality statistics.

Perhaps for these reasons it has not been the focus of any major health offensive—outside of mainland China. Treatment is possible, although expensive and pointless in communities where reinfection is almost inevitable. In isolated irrigation schemes the disease has been limited by eradicating the parasite's intermediate host with commercial pesticides. But these solutions are not practicable for most poor rural communities.

It is in this context that the work of Dr Aklilu Lemma of the Haile Selassie University in Addis Ababa is important. Dr Lemma has just completed the field work of a five year pilot bilharzia control project in Adwa. Such control programmes have been carried out before but in this one there is a vital new factor. The key to it is a pesticide produced from a locally occurring plant. The availability—and low cost in foreign currency—of the plant extract may make bilharzia control practicable on a community scale in Ethiopia and probably other countries as well.

Life cycle

The parasite responsible for bilharzia has a life cycle which is dependent on an intermediate host—a common species of water snail—for transmission to the final host. Eggs from the mature parasite are continually excreted by infected persons or animals. If they reach water they hatch into an intermediate form which seeks out a snail host. In the snail, after further transformations, the parasite begins to release numerous "cercariae", the form which affects man. These cercariae can penetrate skin, so any contact with infected water is enough to transmit the disease.

Bilharzia can be controlled by eradicating the snails or by keeping humans (and their waste) away from water bodies that could be infected. Treatment in itself is of little value for even if all human carriers were temporarily cured, the parasite population could be maintained by infected animals. Even if all affected communities were provided with

safe water, washing facilities and toilets, farmers and fishermen would still be vulnerable and children would still swim in rivers. So in practice, snail eradication has to be the focal point of any control programme.

Dr Lemma's present work on bilharzia control started almost by accident. While conducting a survey of the snail population along a stream in northern Ethiopia, he found a large number of dead snails just downstream from a village washing place. Upstream and further downstream there was an abundant population of live snails. Investigation revealed that the "soap" used by the villagers for washing clothes—ground berries of a plant called endod—had molluscicidal as well as detergent properties.

Further investigation showed that Sun-dried ground endod kills snails at a dilution of 15-30 ppm and that it has a very low toxicity to mammals and plants. The endod bush (Phytolacca dodecandra) is popular with villagers in northern Ethiopia as a hedge and the berries can be bought locally.

In 1969, Dr Lemma began a field trial at Adwa. The idea was to control the snail population in the two streams that cross the village by regular application of endod and to check the effectiveness of these efforts by observing, among other factors, the incidence of infection amongst local inhabitants, particularly young children. A preliminary survey in 1971 showed that the incidence had already dropped dramatically—from 50 to 15 per cent—and the final results are expected to show an even further decline. The large decline in the rate of infection among one to five year-olds has been particularly encouraging as this group became vulnerable to infection after the control programme began.

While the field programme was progressing, Dr Lemma worked for a while at the Stanford Research Institute in California to elucidate the structure and functions of the active ingredient in the endod berry, to examine its properties, and to develop a simple extraction technique to concentrate it. He established that a butanol extract comprising 20 per cent of the berry weight is effective at dilutions of two to three ppm, which is in a comparable range to available commercial products.

Other avenues of research are still open. Dr Lemma wants to see villagers encouraged to use endod as soap. But with an eye to social trends he is also investigating the possibility of formulating a detergent cum molluscicide from the endod. In Adwa and elsewhere villagers have begun to take cardboard cartons of synthetic detergent to the streams with them. There is also a need to develop improved strains of the plant and to overcome if possible the loss of potency that was observed when the plant was cultivated in East Africa.

Perhaps the most exciting aspect of the Adwa project is its simplicity. Endod berries are bought in the local market. They are ground, a few hundred kilos at a time, in the mill usually used by the villagers for grinding chili peppers. The endod is applied along stream banks with watering cans every three to eight weeks. The control programme for a community of about 20 000 involves only three people full time and much of their work is part of on-going research.

The simplicity of the project contrasts dramatically with the procedures necessary when the more expensive synthetic molluscicides are used. The consequence of this was highlighted by a Rhodesian health worker who wrote, "due to the more or less stringent requirements in dispensing molluscicides the application generally rests with specially trained personnel. Snail control can therefore be carried out with little or no cooperation from those people who are being protected."

The irony is that community involvement is essential if any bilharzia control programme is to be successful. Here Lemma believes that his current work can make an impact. "Community involvement through health education, active participation by the people involved and the systematic application of locally grown and processed endod should become routine. These activities by individuals, families, and villages when developed become a truly self-help form of health control."

Lesson from Lake Victoria

Experience with pilot control programmes elsewhere in Africa has demonstrated the importance of involving local communities in their own protection. Around Lake Victoria in Tanzania a control programme reduced the incidence of bilharzia from 60 to 20 per cent inside pilot areas. But there has been no follow-up—partly because it would take about one third of the country's annual health budget to maintain the programme in the Lake Victoria area alone. What has been discouraging is that stream banks which were cleared in urban areas to destroy snail habitats have been overgrown again—because no-one explained the benefits of keeping the banks clear.

Also in Tanzania, another experiment has demonstrated in economic terms the benefits to be derived from successful control of the disease. A programme of snail control was initiated on a large sugar estate near Mount Kilimanjaro and at the same time estate workers were screened and treated if infected. The expenditure on controls has been more than outweighed by the increased productivity of healthy workers. And researchers at the Tropical Pesticides Research Institute calculated that the overall saving was over £20 000 per year.

The benefits of controlling bilharzia amongst rural communities just emerging from a subsistence economy cannot be as easily demonstrated. But Aklilu Lemma's approach to the control of bilharzia, which derives so much from local resources, is one which can itself catalyse further development. Many of the problems of the Third World will be solved only when the social climate encourages people to tackle them themselves. In the long run, grass roots involvement will achieve far more than solutions imposed by outside experts.

In Brief (The Johns Hopkins University, School of Hygiene & Public Health), 11. Issue.

Schistosomiasis Progress Reported By Graduate

Reprinted by permission.

An alumnus of the School has found that the vector responsible for schistosomiasis can be controlled by a pesticide made from the berries of a plant which flourishes in his native Ethiopia.

Dr. Aklilu Lemma, ScD '64, Director of the Institute of Pathobiology at Haille Selassie I University, has developed a simple yet successful method of controlling the transmission of schistosomiasis by using dried and ground berries of the endod plant *(Phytolacca dodecandra)*, which are used by villagers in Ethiopia as a detergent for washing clothes at streamside.

"In 1964, while making an ecological study to determine the distribution of schistosome-transmitting snails in a small stream in northern Ethiopia, I observed large numbers of dead snails at spots immediately downstream from where local people had done laundry using endod," according to Dr. Lemma. "Areas further upstream and downstream from the laundry site were abundant with live snails."

Like a detective following a chain of clues Dr. Lemma found after further investigation that pulverized endod berries spread along the stream banks near the village killed snails without significant toxic effects upon mammals and plants.

In 1965, WHO estimated that 200 million people in Africa, the Middle East, Asia, and parts of the Americas were affected by schistosomiasis, also called bilharzia. The number has probably risen considerably since that time, Dr. Lemma feels.

One of the most important effects of the disease is a substantial loss of energy. Because of the slow process of tissue damage and the chronic nature of schistosomiasis, the extent of the abnormality imposed on infected individuals is often not fully realized until irreversible destruction to the liver and other tissues has occurred. Generally schistosomiasis shortens the lifespan without killing outright.

The wide availability and low cost of the endod plant, which grows in Africa, South America and Asia, means that schistosomiasis control can become practicable on a community scale in Ethiopia and probably in other countries.

Eggs from the mature parasite responsible for the disease are continually excreted by infected animals and humans. If these eggs reach water they hatch into an intermediate form which seeks out a common species of fresh-water snail as a host. After further transformation in the snail, the parasite begins to release numerous cercariae, the form which affects man.

It is widely felt that the most practical method of controlling schistosomiasis is by eradicating the snails, since without them the parasite would not develop to the stage which threatens man.

In 1969 Dr. Lemma began a field trial in the village of Adwa, where 70% of the total population and 50% of children aged 1-5 were infected with *Schistosoma mansoni*. One of the three species of schistosome affecting man, this causes intestinal schistosomiasis, affecting the liver and organs of the lower pelvic cavity.

Dr. Lemma hoped to control the snail population in the two streams that cross Adwa by regular application of endod. The Adwa project is simple in operation. Endod berries are bought in the local market, sun-dried, then ground in the mill villagers use for grinding chili peppers.

The pulverized endod, suspended in an aqueous solution, is applied along the stream banks with watering cans every three to eight weeks. Only three full-time people are needed in the control program for a community of 17,000.

Results of a preliminary study in 1971 reveal that the incidence among children 1-5 had already dropped from 50% to 15%, while incidence among children in a nearby untreated control village had increased by 10%. The final evaluation of the program is expected to show even more striking results.

An alumnus quite by accident discovered that the berries of the endod plant can be used to control schistosomiasis.

1976

RECOMMENDATIONS OF THE SUBCOMMITTEE ON MOLLUSCICIDE CONTROL OF VECTOR SNAILS AND CONTROL PROJECTS

The subcommittee met on the 22 and 23rd of October 1975 and agreed on the following recommendations:-

1. The subcommittee reviewed the situation with regard to snail control with molluscicide and noted that molluscicide is one of the effective measures for controlling schistosomiasis. Their use should continue in conjunction with other recognized measures.
2. For effective use of molluscicides it is recommended that it should be based on appropriate, prior studies on snail biology, water management, irrigation practices and weed clearance and covered drains.
3. Evaluation of the toxicity and pathogenicity of molluscicides in man, domestic animals, crops and wild life should continue. Further studies on the effects of molluscicides on biota should be directed to permit long term assessment of any cumulative effects. The possible development of snail resistance to molluscicides should also be investigated.
4. Focal transmission control was discussed and agreed that it could be used whenever it reduces transmission and saves cost of molluscicide.
5. Research on novel molluscicides and new formulation of available molluscicides should be encouraged, including slow release formulations with regard to effectiveness and toxicity.
6. In view of the increasing cost of available synthetic molluscicides alternative means of controlling snails with the use of locally produceable natural product molluscicides should be encouraged. Although the potencies of some plant molluscicides may not be competitive with some of the synthetic molluscicides, their use in the control of schistosomiasis in rural areas on a self-help basis is recommended. Endod (Phytolacca dodecandra), the plant which has been most extensively investigated, offers good possibilities for use in schistosomiasis control and further studies on it are recommended.
7. Further attention should be paid to cost benefit effectiveness of molluscicide programs.
8. The great need for trained personnel makes it highly necessary that international and national centers be organized for the purpose of training on molluscicide control.
9. On account of the seriousness of schistosomiasis as a public health problem in many countries affecting a considerable part of the world's population, it is recommended that a specific international and national adequate funds and program for combatting schistosomiasis be created to assist countries where the disease is prevalent.

Institute of Pathobiology (Council of Water-Borne Disease)

There is a need to initiate a study to determine the epidemiological and socioeconomic effects of water-borne diseases in the framework of irrigated agricultural projects (river basin development). An amount of US$300,000 is being earmarked from 1977 to 1979 pending a concrete proposal for the National University in cooperation with other relevant agencies like NWRCEO/MPH/AVA, etc.

Further Actions Required

The project should be jointly developed by the Institute, National Water Resource Commission, Ministry of Public Health and Awash Valley Authority to ensure effective environmental control.

The Ethiop

Vol. XXX — No. [illegible]
Annual Subscription $ 6.50
Price Per Copy 10 Cents.

The Press Stimulates change

MORNING
Addis Ababa — Sunday, Jan

Something For The Kemetcha'

Self-Help To Control Bilharzia

In the northern town of Adwa where bilharziasis was affecting a large number of the population, a systematic attempt was made to control the disease on a community self-help basis primarily with the use of Endod, the Ethiopian soapberry plant. The very successful results obtained from the model study in Adwa can now be used in other areas where this disease is a problem.

British and Ethiopian Scientists under the able leadership of Dr. Aklilu Lemma started the Adwa project six years ago. In an interview held at the Institute of Pathobiology of which he is the head, Dr. Aklilu explained that the study started with a thorough pre-control epidemiological base-line data collection to determine the prevalence and intensity of the disease in the different age groups, sex, religion, and location of the people in Adwa. Ecological and snail distribution studies in the two main rivers which cross the town, showed the specific disease transmission sites. The different parts of the river where people wash their clothes, collect water, deficate and pollute the water, cross the river by wading through it and where children swim and entertain themselves, were delineated by a series of behavioural studies conducted by the team.

The population of Adwa was then given a series of health education program through lectures, demonstration, and film shows about the life-cycle, hazards, and possible means of the control of bilharziasis. Such programs were conducted the presence of senior government officia religious leaders, local chiefs, and elder people and students of the town.

Dr. Aklilou Lemma and Dr. Shell Crane testing different extracts of Endod at Stanford Research Institute in California.

Bilharziasis affects over 200 million pe ple in the world and it is a debilitati disease. It is very widely distributed the continent of Africa where it affe over 50 million people. In Ethiopia, it particularly prevalent in the Tigre a Hiar provinces and it is present in pr tically every province of the country. I velopment areas such as the Awash V ley the Wabi Shebeli valley, the Blue N valey are known to be infected and it likly that the spread of the disease thee areas will increase considerably irigation schemes for large scale agricu tual programs are implemented. In Egy ard the Sudan where the disease is redy well established, it is causing a co

(*Contd. on page 7 col. 3*)

NEWSPAPER
ary 5. 1975 — (Tahsas 27, 1967)
118247, 118248, 110829.
Editorial office: 112212, 111629.
P.O. Box 1074.

Self-Help to Co

(*Contd. from page* 1 *col.* 3)

siderable economic loss and health hazard.

The life-cycle of the parasite involves the passing of eggs of the mature worm, which resides in small blood vessels, with the stool or urine of the affected individuals. A single parasite has the capacity to lay many thousands of eggs per day continuously for many years. An infected person may have many of such worms. The mass of eggs produced by such worms alongwith the blood destruction activities of the worms causes the bilharzia disease.

When the eggs of the parasites passed with urine or stool reach water, they hatch and the microscopic intermediate larva seeks and infects appropriate species of aquatic snails. It multiplies and transforms in the tissues of the snail in large numbers and the infective larva is then released in millions in the surrounding bodies of water. The infective larvae which is called "cercariae" are microscopic and have the special ability to swim freely in the water, seek appropriate host, penetrate through the skin during contact with water, and thus infect the host. Repeated exposure to infected water results with more parasites in the affected individual. Treating such individuals does not help because they can go back to the infected water and get fresh parasites. Teaching people not to deficate or urinate near water has not been very successful. The only practical way of controlling the disease is, therefore, controlling the snail population which is an essential intermediate host in much the same way as mosquitoes are for the control of malaria.

There are many chemicals which are used for killing snails in Bilharzia control efforts. However, all of such chemicals are very expensive and they also have different disadvantages for continued use in bilharzia affected areas. The discovery by Dr. Aklilu Lemma about ten years ago that Endod, the Ethiopian soapberry plant, is a potent snail killer has brought much hope and opened a new approach to the control of the disease on a com-

In a five year long continuous and comprehensive bilharzia control program launched in Adwa, using very simple techniques and locally available manpower under the supervision of the Institute of Pathobiology staff, it was possible to reduce the prevalence of the disease in children between the ages of 1-5 from 50% before control to only 7% after control. The overall prevalence of the disease in the 20,000 inhabitants of Adwa dropped from 64% to 30%. Dr. Aklilu and his team explained in the 4th International Congress of Parasitology held in Munich last August, that such an 85% reduction in the transmission of the disease was achieved by very simple means at an average total cost of only 20 Eth. cents per head per year. Because of the simplicity of the techniques involved ways are now being investigated by which this scheme may fit in with the Development through co-operation campaign (the Zemetcha).

While the Adwa bilharzia control program was going on, more work was progressing on the studies on Endod. During a two years research and sabbatical leave in the United States, Dr. Aklilu Lemma with a group of prominent chemists and biologists at the Stanford Research Institute (SRI) and the University of California, was able to develop a patented procedure for the extraction of the active principal in the Endod berries. A series of studies on the biological and chemical properties of the extract was also extensively studied and published in various international journals. SRI chemists under the leadership of Dr. Aklilu Lemma, Dr. Skinner, executive director of the Life Sciences Division and a distinguished organic chemist Drs. R.M. Parkhurst and W.A. Thomas, both Senior organic chemists, were able to isolate and identify the specific active ingredient in the Endod berries.

The active principle was called *Lemmatoxin*, named after the Ethiopian Scientist, Dr. Aklilu Lemma, who dis-

ntrol — Bilharzia

According to Dr. Aklilu L[illegible], the ultimate aim in the Endod studies is to develop, in the simplest way possible, a local material and local know-how for the control of bilharziasis on a community-self-help basis.

Selected variety of the Endod seeds can be supplied to farmers to grow. A trailer mountable extractor could go around the villages once a year or so to extract large quantities of the berries on the spot. Such an extract could be prepared in different formulations including brickets of different hardness that could be dropped in water and allowed to dissolve slowly, thus releasing the toxin to kill snails and mosquito larvae over a long period of time. Studies on the ecological hazard of such an application are now under investigation, but from the five years work done in Adwa it appears that Endod does not have any apparent adverse effect on the ecology.

A preventative for schistosomiasis

Schistosomiasis is one of the major parasitic diseases of the world, inflicting 200 million people in Africa, the Middle East and Asia. Although there are some treatments for the disease, they are not altogether satisfactory, and even worse, they are not available to the majority of people who need them. What is really needed is a preventative for schistosomiasis, specifically, a means of killing the freshwater snails that serve as a host for the parasites.

Such a preventative may have been found by Aklilu Lemma of Haille Selassie I University in Ethiopia. It is dried and ground berries of the endod plant, which are used by villagers in Ethiopia as a detergent for washing clothes at streamside.

In 1964 Lemma was studying the schistosome-transmitting snails in a small stream in northern Ethiopia when he observed large numbers of dead snails at spots downstream from where local people had done laundry using endod. Lemma then found that pulverized endod berries spread along stream banks could kill snails without hurting other animals and plants. In 1969 he began a field trial in the village of Adwa, where 70 percent of the total population and 50 percent of young children had schistosomiasis. Endod berries were applied to stream banks with watering cans every three to eight weeks.

Preliminary results from the study show that schistosomiasis among the children has dropped from 50 percent to 15 percent.

Reprinted from Science News, vol. 107 (25), p. 402

Research Resources Reporter
October 1978

Information Exchange

Research Accomplishments

Folklore surrounding a common weed used by Ethiopians as soap has led scientists to investigate a totally new means of contraception.

Known as *endod*, the plant contains a chemical compound that stops development of the fertilized ovum. It was first applied in research in Africa by an American-trained Ethiopian physician, Dr. Aklilu Lemma, as a preventive measure against bilharzia, a debilitating, snail-borne, parasitic disease, also called schistosomiasis.

Drs. Robert Parkhurst and Elmer Reist of Stanford Research Institute (SRI) and their colleagues, intrigued by the possibilities of the chemical, conducted further studies and are currently using instruments at the Stanford Magnetic Resonance Laboratory to examine the structures of *endod's* active agent, as well as similar compounds from other plants.

"We're still on the frontier. So far, nothing really practical has come out of it," Dr. Parkhurst says. "But we have found, purified and are studying three compounds from *endod* and other plants from outside Africa that show antifertility activity in animals."

Through experiments conducted at the Stanford Magnetic Resonance Laboratory, the scientists know the basic structure of the agents. Each is composed of either two or three tightly bound sugar and triterpene molecules. The scientists are now studying the position of linkage and are producing similar molecules from other sugars to determine whether these are equally active or whether specific sugars are necessary.

"Once we know what we've got," Dr. Reist says, "we might be able to make analogs. From the NMR studies, we'll be able to describe the structure of natural agents, make new compounds based on this information, and check their structure. Then we'll see how the biological activity of the new compounds compares with the natural products."

Currently, the extracts have proven very effective as antifertility drugs, but the means of application is impractical for human use, according to Dr. Parkhurst. Each test subject either a rat or mouse, receives an injection into the uterus, so that the drug will directly affect fertilized ova. Through some unknown process, the compounds force fertilized eggs to stop cell division and disengage from the uterine lining if they have become implanted there. The first compound of this type was isolated from *endod* berries.

From ancient times, natives of Ethiopia have used the berries to make soap. While studying the incidence of bilharzia in the late 1960s, Dr. Lemma observed that there were no live snails, which are hosts for the parasite, for several miles downstream from where women washed clothing with *endod* berries. Within a few days, Dr. Lemma proved that the juice of the berries was highly poisonous to the parasite-carrying snails and he spread the word. "Crush the berries and throw them into the stream."

The campaign was a success and Dr. Lemma soon traveled to Stanford Research Institute to conduct more studies. There he met Dr. Parkhurst and the two men discussed the potential use of *endod*.

"The bilharzia parasites look like sperm, and on a hunch I wondered if *endod* extract would make a good spermicide," Dr. Parkhurst remem-

Left:
Dr. Aklilu Lemma examines a snail which carries the parasite responsible for bilharzia. While conducting research in Ethiopia, Dr. Lemma observed that a native plant called endod *effectively kills the parasite's snail host. Subsequent studies of* endod *extracts led scientists to discover their various properties including anti-fertility activity.*

Below:
Fork-tailed larva (400 microns long) of the schistosome parasite, which causes bilharzia.

bers. "So in the testing, we found it was effective, in microgram amounts, against sperm and eggs."

In order to fertilize the egg, sperm cells, when released into the female reproductive tract, must swim through the long fallopian tubes. In the presence of even a trace of *endod*, they are unable to complete the passage. "Then the question was: what would happen if we placed this right in the uterus? Would it keep a fertilized egg from developing?" Dr. Parkhurst recalls. Tests conducted on rats showed that as late as six days after fertilization, the egg was destroyed by *endod* extract. "Now we have to establish a family of compounds to see which ones are going to have the best activity and minimal side effects," Dr. Parkhurst explains.

So, *endod* has progressed in some ten years from ancient folk medicine to modern biochemistry. The properties of extracts from this and other plants are still very much a mystery, drawing the interest of scientists in widely different fields. Besides its contraceptive effects, researchers are investigating an active agent in *endod* which may be used as an antidote for overdosage of certain drugs and for certain poisons, since it induces immediate vomiting without lasting effects. Indeed, the scientists have learned that a strong tea made from *endod* berries is used by Ethiopians as an antidote to poison.

Other researchers have proven that extracts from the weed are lethal to the fungus that causes athlete's foot. A laboratory team at Harvard found that larvae of the mosquito that carries malaria are ten times more sensitive to *endod* extracts than is the water snail. The larvae cannot develop into adults in the presence of even a trace of *endod*, according to Dr. Parkhurst.

"Many peripheral studies have come out of the original one made by Dr. Lemma in Ethiopia," says Dr. Parkhurst. "We are dealing with a very broad spectrum of possible uses."

Research into endod extracts and similar compounds is primarily supported by the National Institute of Child Health and Human Development. The Stanford Magnetic Resonance Laboratory is supported by the NIH Division of Research Resources, and the National Science Foundation.

Chemical and quantitative aspects of Phytolacca dodecandra berries as a mollusicicide: Charles B Lugt, Institute of Pathobiology, Addis Ababa University.

The shrub, *Phytolacca dodecandra*, is used in Ethiopia as a substitute for soap and is known for its molluscicidal potency. The plant occurs in many varieties with respect to morphology, saponin content of the berries and molluscicidal potency of them. In order to select an appropriate variety for berry production as a means to control bilharziasis transmitting snails, we have to take into account these differences. From the chemical point of view of this plant, as far as the berries are concerned, the active molluscicidal principles are triterpenoid glycosides, to which at C3 three or more sugars are attached. On TLC more than eight different components can be observed, of which some show strong molluscicidal activity, while others show no action at all. Because of their foaming capacity, these glycosides are also called saponins. One of the main characteristics of saponins is that they are able to haemolyse bloodcells. This study shows that the haemolytic capacity of the berry extracts parallels their molluscicidal potency. As a routine part of the work, the dry berry output per raceme (inflorescence) of various types was measured. Together with these figures and with those of their molluscicidal potency, the ratio between the two was determined. It showed that the unripe stage of the berries is the most suitable time to harvest the fruits although there are some interesting exceptions to this rule.

Reprinted with permission from the Ethiopian Medical Journal, 16: 40-41.

Phytochemistry, 1969, Vol. 8, pp. 2105 to 2107. Pergamon Press. Printed in England.

SHORT COMMUNICATION

TRITERPENOID SAPONINS FROM *PHYTOLACCA DODECANDRA*

J. W. POWELL and W. B. WHALLEY

The School of Pharmacy, The University, London W.C.1

(*Received* 27 *March* 1969)

Abstract—Two aglycones from the saponins of the molluscicidal fruit of *Phytolacca dodecandra* have been identified as oleanolic acid and bayogenin.

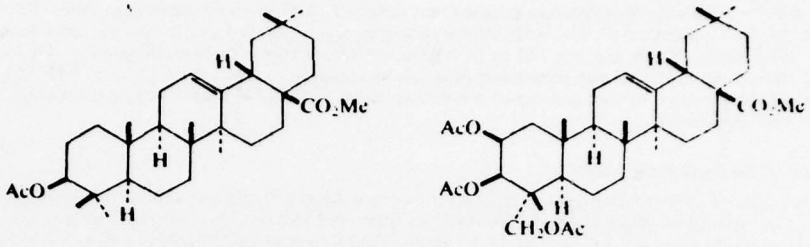
THE FRUITS of *Phytolacca dodecandra* (Phytolaccaceae) (known in Ethiopia as "Endod") have been extensively used as a poison in Africa[1,2] and are currently receiving considerable attention as a potential molluscicide for the control of bilharzia.[1,2] It has been suggested[1] that the toxic principle which is present in various parts of the plant is a steroidal saponin. Through the courtesy of Dr. B. A. Hems of Glaxo Research Ltd., we have been able to examine a quantity of the fruits and have carried out a preliminary investigation of the saponins.

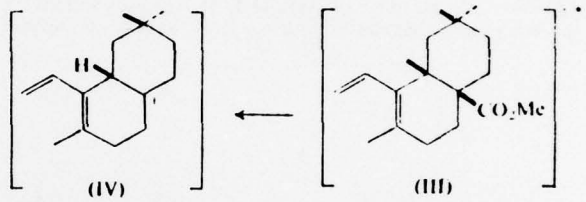
The dried, crushed fruits were defatted with petroleum ether and then extracted with methanol to furnish a water-soluble gum having the general properties of a saponin. This extract was tested against young adult snails of a Puerto Rican strain of *Australerbis glabratus* with the following reproducible results: (*a*) 1:7500 parts in water, all snails killed in 4 days; (*b*) 1:3250 parts in water, all snails killed after 48 hr.

Hydrolysis of the crude saponin gave a high melting product which was devoid of ester groups but which contained hydroxyl and carboxyl groups (i.r. spectrum) and which was acetylated and then methylated. Separation of the mixed product by chromatography furnished methyl *O*-acetyl oleanolate (I) and methyl tri-*O*-acetylbayogenin[3] (II), thereby indicating the presence of the oleanolic acid and bayogenin moieties as aglycones in the original saponins. The mass spectrum of (II) showed a base peak at *m/e* 203 (accurate mass measurement) corresponding to the ion (IV) produced by way of the retro-Diels-Alder product (III). The NMR spectrum of methyl tri-*O*-acetylbayogenin showed, *inter alia*, signals at τ 4·6 (multiplet, 2 protons, H-3 and H-12), 5·05 (doublet, $J = 4$ c/s, 1 proton, H-3), 6·2 (close AB system, 2 protons, $-CH_2OCOCH_3$), 6·35 (singlet, 3 protons, $-COOCH_3$), 7·9 (singlet, 6 protons, $2\times -OCOCH_3$), 8·0 (singlet, 3 protons, $-OCOCH_3$), 8·8, 8·9, 8·95, 9·2 (singlets, 3 protons each, $>C.CH_3$) and 9·1 (singlet, 6 protons, $2\times C.CH_3$). The coupling constant between H-2 and H-3 of 4 c/s is in agreement with the assigned stereochemistry of the acetoxyl groups as 2β, 3β. The chemical shift of the close-coupled AB system attributed

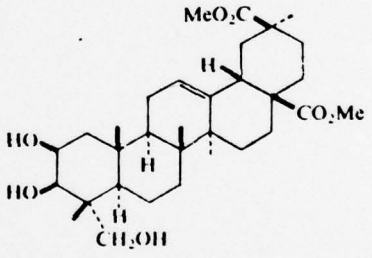
[1] J. M. WATT and M. BREYER, *Medicinal and Poisonous Plants of Southern and Eastern Africa*, Brandwijk (1962).
[2] C. WEISS, JR., *Scient. Res.* 64–74 (1967).
[3] R. A. EADE, J. J. H. SIMES and B. STEVENSON, *J. Australian Chem.* 900 (1963).

to the protons on C-23 is within the range given by Gaudemer *et al.*[4] for an equatorial $-CH_2OCOCH_3$ attached to C-4. Thus the NMR and mass spectral data are fully in accord with the structure and stereochemistry previously assigned[3] to bayogenin.





$C_{15}H_{23}$ = 203 (100%) $C_{17}H_{26}O_2$ = 262 (71%)



(V)

The close structural relationship between bayogenin and phytolaccagenin[5] (V) from *P. americana* L. ("pokeroot") is in accord with the close botanical relationship between the two plant species.

At this stage of our work we had become aware of the more extensive investigation upon this project being carried out by the Tropical Products Institute, who have also reported the isolation of oleanolic acid.[6] We have thus discontinued this investigation.

[4] A. GAUDEMER, J. POLONSKY and E. WENKERT, *Bull. Soc. Chim. Fr.* 407 (1964).
[5] G. H. STOUT, B. M. MALOFSKY and V. F. STOUT, *J. Am. Chem. Soc.* **86**, 957 (1964).
[6] T. A. KING, K. JEWERS, H. RICHARDSON and C. P. FALSHAW, Abstracts of 5th International Symposium on the Chemistry of Natural Products, F43, London (1968).

EXPERIMENTAL

The NMR spectra were determined in deuterio-chloroform solution on a Varian A 60-A spectrometer.

Extraction of Dried Fruit from Phytolacca dodecandra

The dried fruit (610 g) was coarsely crushed and defatted (Soxhlet) with petroleum ether (b.p. 60–80°) during 8 hr. Subsequent extraction with methanol during 24 hr yielded crude saponin as a brown gum (310 g). Hydrolysis of this saponin (72 g) in boiling methanol (600 ml) containing conc. HCl (150 ml) occurred during 90 min, with the separation of a semi-crystalline product (11·6 g), m.p. 345–350°. After collection of the product, further heating of the filtrate under reflux (2 hr) furnished an additional quantity (2 g) of a similar material.

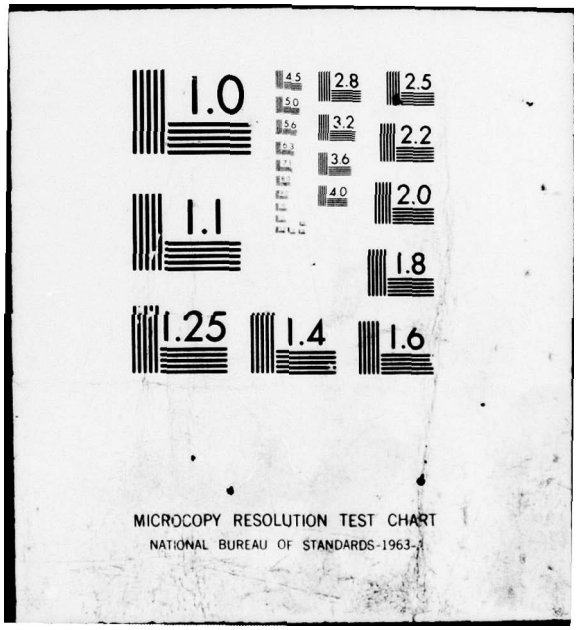
Separation of the Mixed Aglycones

Crude aglycone (2·7 g) dissolved in pyridine (20 ml) and Ac_2O (50 ml) was kept at 20° during 48 hr. On isolation the mixture of crude acetates, dissolved in methanol (50 ml), was treated with ethereal CH_2N_2 [from nitrosomethylurea (15 g)], during 18 hr at 0°. A solution of the product in benzene (10 ml) was chromatographed on silica gel. Elution with ethyl acetate/benzene (4%) gave methyl *O*-acetyloleanolate (0·6 g) which formed prisms, m.p. 216–222°, from alcohol; further elution with ethyl acetate/benzene (10–20 per cent) yielded methyl tri-*O*-acetylbayogenin in prisms (0·5 g), m.p. 195–203°, from methanol. Both specimens were identical (m.p., mixed m.p., i.r., NMR and mass-spectra) with authentic specimens.

Acknowledgements—We are indebted to Dr. D. L. H. Robinson and Mr. D. F. Moore of Glaxo Laboratories Ltd. for the biological testing and to Professor R. A. Eade for the specimen of bayogenin.

AD-A080 030 CALIFORNIA UNIV SAN FRANCISCO F/G 6/5
STUDIES ON MOLLUSCICIDAL AND OTHER PROPERTIES OF THE ENDOD PLAN--ETC(U)
JUN 79 A LEMMA, D HEYNEMAN, H KLOOS N00014-76-C-0218
UNCLASSIFIED NL

2 OF 6
AD
A080030


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

Phytochemistry, 1973, Vol. 12, pp. 1437 to 1442. Pergamon Press. Printed in England.

MOLLUSCICIDAL SAPONINS OF *PHYTOLACCA DODECANRA*: OLEANOGLYCOTOXIN-*A*

ROBERT M. PARKHURST, DAVID W. THOMAS and WILFRED A. SKINNER
Life Sciences Division, Stanford Research Institute, Menlo Park, CA 94025, U.S.A.

and

LEWIS W. CARY
Varian Associates, Palo Alto, CA 94306, U.S.A.

(*Received* 15 *September* 1972. *Accepted* 28 *December* 1972)

Key Word Index—*Phytolacca dodecandra*; Phytolaccaceae; oleanoglycotoxin-*A*; molluscicide; oleanolic acid; glycoside.

Abstract—The structure of one of the major molluscicidal saponins of the fruit of *Phytolacca dodecandra* has been elucidated as 3-[2,4-di-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl]-olean-12-ene-28-oic acid. The combined use of 300 Mc. PMR, MS and GC–MS led to this structural assignment.

INTRODUCTION

THE SHRUB *Phytolacca dodecandra*, commonly known in Ethiopia as Endod, produces large quantities of berries that, when dried, ground and suspended in water, may kill snails at as low a concentration as 10 ppm.[1–3] In view of the potential of the Endod plant for controlling schistosomiasis, a disease transmitted by aquatic snails, there have been several attempts to elucidate the structure of the active principle. Horton[4] speculated that the active material may be a glycoside or glucuronide of oleanolic acid. King *et al.*[5] and Powell and Whalley[6] found four different sugars, oleanolic acid, and bayogenin in the hydrolysis products of the crude active fraction. The work described here concerns the isolation and structure determination of one of the major biologically active saponins; future papers will be concerned with the structures of other components of the complex mixture.

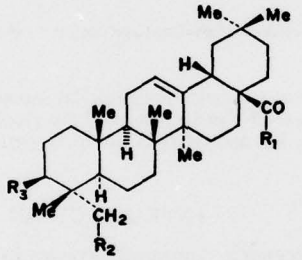
RESULTS

For isolation of the saponins, the dried finely ground Endod berries were defatted with light petroleum, then extracted with warm water. Partition with butanol provided a biologically active light tan powder, representing 20–25% of the initial ground berries.[7] This

[1] LEMMA, A. (1965) *Ethiop. Med. J.* **3**, 187.
[2] LEMMA, A. (1970) *Bull. World Health Organ.* **42**, 597.
[3] LEMMA, A. and DUNCAN, J. (1970) *J. Parisatol.* **56**(4), 213.
[4] HORTON, W. J. (1968) *World Health Organ. Molluscicide Information Series*, No. 24, V.
[5] KING, T. A., JERVERS, K., RICHARDSON, H. and FALSHAW, C. P. (1968) Abstract of 5th International Symposium on the Chemistry of Natural Products, F. 43, London.
[6] POWELL, J. W. and WHALLEY, W. B. (1969) *Phytochemistry* **8**, 2105.
[7] LEMMA, A., BRODY, G., NEWELL, G. W., PARKHURST, R. M. and SKINNER, W. A. (1972) *J. Parasitology* **58**, 104–107.

material was acetylated and examined by TLC, which showed a complex mixture of over ten distinct components, three of these appearing to be major. One of the major components, representing about 18% of the crude saponin acetates, was isolated by repetitive chromatography in sufficient quantity for further study. Deacetylation of this material yielded a biologically active substance (ED_{90}, 3 ppm, 24 hr, *Biomphalaria glabrata*) which was named oleanoglycotoxin-*A*. Reacetylation gave material identical with the originally isolated acetate.

Acid hydrolysis of oleanoglycotoxin-*A* gave a water-insoluble solid with the same R_f as oleanolic acid (I), which had previously been established as the major sapogenin of the Endod saponins.[4-6] Further examination of its permethyl derivative by GC–MS demonstrated that the major component (86%) had a retention time and MS identical with those of authentic methyl *O*-methyloleanolate (II). A lesser component (11%) gave a MS identical with that of authentic methyl di-*O*-methylhederagenin (III). This would indicate that oleanoglycotoxin-*A*, in spite of the chromatographic homogeneity of its acetate, contains a minor component derived from hederagenin (IV). The aqueous phase of the acid hydrolysis of oleanoglycotoxin-*A* contained only glucose, unambiguously identified by GLC of its trimethylsilyl derivative.[8]



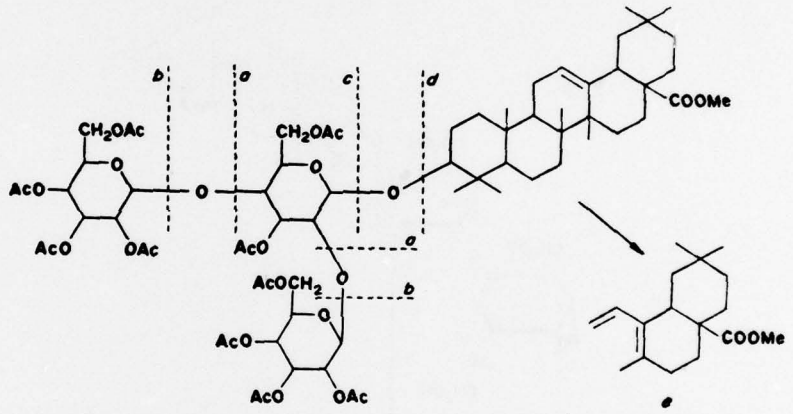
(I) $R_1 = R_3 = OH$; $R_2 = H$
(II) $R_1 = R_3 = OMe$; $R_2 = H$
(III) $R_1 = R_2 = R_3 = OMe$
(IV) $R_1 = R_2 = R_3 = OH$

The first indication of the total composition of oleanoglycotoxin-*A* was given by the NMR spectrum of its peracetyl derivative. In particular, the presence of ten acetate methyls (ppm, 1·73 (3 × Me); 1·76; 1·80 (2 × Me); 1·91; 1·93; 2·06 and 2·10) indicated 3 mol of glucose present to 1 mol of oleanolic acid, whose seven methyl groups are clearly apparent at 0·87, 0·92, 0·96, 1·00, 1·05, 1·19 and 1·24 ppm. Because of its low abundance and structural similarity to oleanolic acid, the hederagenin component of oleanoglycotoxin-*A* is not expected to make an observable contribution to the NMR spectrum.

MS not only confirms this conclusion, but gave additional structural information. Peracetyl oleanoglycotoxin-*A* was methylated with diazomethane, yielding a derivative which gave a MS with a group of peaks at *m/e* 1374, 1375 and 1376. The peracetate methyl ester of a triglucoside of oleanolic acid required a MW of 1376. Evidently, in this spectrum *m/e* 1374 and 1375 are due to pronounced losses of H_2 and H, respectively, from the molecular ion. This behavior has been observed also for permethyl and peracetyl oleanoglycotoxin-*A*, and numerous other related saponins, including a synthetic peracetyl monoglucoside of oleanolic acid.

[8] SWEELEY, C. C., BENTLEY, R., MAKITA, M. and WELLS, W. W. (1963) *J. Am. Chem. Soc.* **85**, 2497.

The MW (1376) of the major component could be confirmed by further examination of this MS. Loss of a terminal peracetyl glucose unit (mass 347) (Scheme I) is expected to give rise to an intense 'M minus 347' peak, observed here as fragment *a* at *m/e* 1029. This peracetyl glucose is itself observed without the glucosidic oxygen as ion *b* at *m/e* 331. That all three glucose units are contained within one single saccharide moiety, as opposed to being attached independently at different points to the oleanolic acid unit, is demonstrated by the appearance of a peracetyl triglucose unit in the MS at *m/e* 907 (*c*). This saccharide is clearly attached at the hydroxyl of oleanolic acid; MS loss of the trisaccharide unit with the linking oxygen gives one of the most intense peaks of the spectrum at *m/e* 453 (*d*). The appearance of fragment *e* at *m/e* 262, originating by a mechanism typical of Δ^{12}-unsaturated triterpenes, confirms that the carboxyl is present as a methyl ester, ruling out the possibility of the saccharide being bound to the carboxyl group.



SCHEME 1. MS FRAGMENTATION OF PERACETYL OLEANOGLYCOTOXIN-*A* METHYL ESTER.

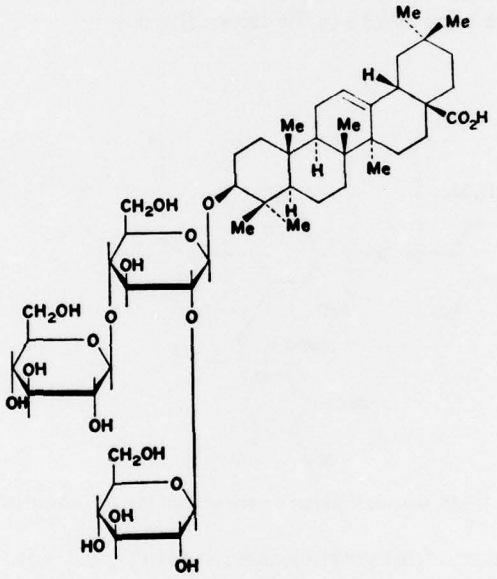
A mass spectrum of peracetyl oleanoglycotoxin-*A* (i.e. not treated with diazomethane) was similar to that of the methyl derivative, except that all fragments which contain the carboxyl group appeared 14 m.u. lower. That is, fragments *M*, *a*, *d* and *e* appeared at *m/e* 1362, 1015, 439 and 248, respectively. Those fragments which had lost the carboxyl function (i.e. M − COOMe, *d* − HCOOMe and *e* − COOMe) remained unchanged. Thus, the methyl ester of the derivative illustrated in Scheme 1 was introduced by treatment with diazomethane, and oleanoglycotoxin-*A* therefore contains a free carboxylic acid.

All the MS data thus far discussed referred to derivatives of the major component of oleanoglycotoxin-*A*, i.e. a triglucoside of oleanolic acid. Also visible in the MS are peaks which may be attributed to a triglucoside of hederagenin, the minor component already mentioned. MS fragments containing the sapogenin portion of the molecule appear to a minor extent shifted 58 units toward higher mass; e.g. note in particular the pair of peaks for fragments *a* at *m/e* 1029 and 1087. This is indicative of the additional *O*-acetyl group in the hederagenin moiety.

In order to determine the structure of the trisaccharide present, oleanoglycotoxin-*A* was permethylated and then hydrolyzed to give a mixture of partially methylated glucose derivatives. The mixture thus obtained was pertrimethylsilylated and analyzed by GC–MS. The resulting gas chromatogram, revealed the presence of two pairs of glucose derivatives,

each pair representing an equilibrium of α- and β-anomers of a single derivative. A comparison of the MS of these sugars with those previously reported[9] for trimethylsilylated 2,3,4,6-tetra-*O*-methylglucopyranose and trimethylsilylated 3,6-di-*O*-methylglucopyranose established their identity. Oleanoglycotoxin-*A* thus contains 2 mol of 1-substituted glucose to 1 mol of 1,2,4-trisubstituted glucose, bound to each other in a single saccharide unit. Only one structure is possible (excluding assignment of anomeric configurations) for the major component of oleanoglycotoxin-*A*, namely that indicated by formula V.

The anomeric assignments presented here are derived from a more detailed analysis of the NMR spectrum of the acetate. Three 1-proton doublets at 4·23, 4·35 and 4·82 ppm, each with coupling *J* 8 Hz, are due to the C-1 protons of the three glucose units. The coupling constants imply a β-pyranoside in each case, since an α-pyranoside or either of the anomeric furanosides would give splittings of 4·2 Hz or less.[10]



(V)

The remainder of the saccharide signals in the NMR spectrum may be classified in three groups: (a) all C-H adjacent to an acetate appear higher than 5·0 ppm; (b) C-H_2 adjacent to an acetate (e.g. all C-6 protons) appear somewhat upfield, from 3·9 to 4·6 ppm; (c) C-H adjacent to –OR functions appear even further upfield, from 3·2 to 3·9 ppm. Included within the last group are the C-5 protons at 3·26 and 3·42 ppm (two and one protons, respectively), and the protons adjacent to the saccharide linkage positions, i.e. C-2 and C-4 of the central glucose at 3·82 ppm at 3·54 ppm, respectively.

These assignments were verified by spin decoupling experiments. Thus, irradiation of the C-5 protons at 3·26 and 3·42 ppm caused partial collapse of the signals assigned to the C-6

[9] PETERSON, G. and SAMUELSON, O. (1968) *Svensk Papperstid* 71 (20), 731.
[10] CAPON, B. and THACKER, D. (1964) *Proc. Chem. Soc.* 369.

protons, and irradiation of a C-3 proton (i.e. at 5·4 ppm) caused simultaneous collapse of the central glucose C-2 and C-4 signals to doublets.

Thus, the major constituent of oleanoglycotoxin-*A* is 3-[2,4-di-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl]-olean-12-ene-28-oic acid (V). The minor hederagenin-containing constituent probably has a similar structure, although small amounts of trimethylglucoses identified in the acid hydrolysis product of permethyl oleanoglycotoxin-*A* leaves open the possibility that hederagenin and/or a small part of the total oleanolic acid may be associated with a linear trisaccharide.

EXPERIMENTAL

All GLC analyses were carried out with a 3 m × 3·2 mm column of 1% SE-30 on 100–120 mesh Gas Chrom Q, at a flow rate of 23 ml/min. Temperatures required were 280° for the methylated sapogenins, 180° for the pertrimethylsilylated glucose. MNR spectra were determined with a Varian HR 300 nuclear magnetic resonance spectrometer employing a superconducting magnet at 70·5 kG. The sample was examined in a 5-mm sample tube at 35°. Mass spectra were determined with an LKB model 9000 combination gas chromatograph–mass spectrometer.

Extraction of crude saponins. The dried fruit of *Phytolacca dodecandra* (Endod) was ground to a fine powder and defatted with light petrol. The defatted material was extracted with warm H_2O–BuOH. Evaporation of the BuOH gave a brown gum which upon trituration with Et_2O solidified to a tan powder representing 20–25% of the wt of the dried fruit.[7]

Acetylation of crude saponins. 20 g with Ac_2O–pyridine gave a brown gummy product which was dissolved in tetrahydrofuran, passed through a short Florisil column, and again evaporated *in vacuo* giving 19·55 g of almost colorless amorphous saponin acetate mixture.

Isolation of oleanoglycotoxin-A acetate. 2 g of the crude saponin acetates was chromatographed on a column (18 × 3 cm, 50 g Mallinckrodt SilicAR-CC7, 100–200 mesh) using $CHCl_3$–Et_2O gradient elution and following the progress of the fractions with TLC on SilicAR-7GF plates. One fraction, 348 mg, eluted with 5% Et_2O in $CHCl_3$. was further purified by repetitive chromatography on thick plates (SilicAR-7GF, $CHCl_3$–Et_2O, 1:1) finally giving 41·6 mg of the acetate as a colorless glassy solid, R_f 38–46 (SilicAR-7GH, Et_2O), $[\alpha]^{23}_D$ +17·37 ± 4·35° (c = 0·576, $CHCl_3$). (Found: C, 59·4; H, 7·19. Calc. for $C_{68}H_{98}O_{28}$: C, 59·89; H, 7·24%.) NMR spectrum, see text; MS, *m/e* 203, 248, 331, 393, 438, 439, 907, 1015, 1073, 1314, 1316, 1360, 1372, 1374, 1418. Comparison of the area of the TLC spots of the pure and crude materials allowed an estimate of the weight percent of oleanoglycotoxin-*A* acetate in the crude mixed saponin acetates to be 18%.

Deacetylation of oleanoglycotoxin-A acetate. The acetate (2·3 mg) was treated with an excess of MeOH–conc. NH_4OH (1:1) at 50° for 12 hr, then evaporated. Extraction with *n*-BuOH, followed by evaporation, gave the biologically active saponin as a colorless powder.

Methylation of oleanoglycotoxin-A acetate. The acetate (0·6 mg) in 10 ml of MeOH was methylated with excess CH_2N_2 in Et_2O. TLC indicated only a single product was formed. MS, *m/e* 203, 262, 331, 393, 452, 453, 907, 1029, 1087, 1316, 1317, 1360, 1374, 1375, 1376.

Acid hydrolysis. Oleanoglycotoxin-*A* (1·3 mg) was heated on the steam bath for 24 hr in a sealed tube containing 0·2 ml of 1 N HCl. The solid precipitate was centrifuged to one end, the liquid decanted to the other end and the tube cut in half. The solution was lyophilized and the residue was gas chromatographed as its pertrimethylsilyl derivative,[8] prepared by dissolving in HMDS–TMCS–pyridine. Only glucose was found to be present. The solid precipitate, crystd from EtOH–H_2O, was identical with an authentic sample of oleanolic acid on TLC in two solvents, R_f 70 (SiGF–EtAc); R_f 90 (SiGF acetone), m.p. 306–307·5°, m.m.p., no depression. (Found: C, 78·46; H, 10·54. Calc. for $C_{30}H_{48}O_3$: C, 78·90; H, 10·59%.) This material was permethylated[11,12] with MeI and NaH in $HCONMe_2$. Examination of the product by GC–MS revealed the presence of 86% methyl *O*-methyloleanolate, 11% of methyl di-*O*-methylhederagenin, and 3% of two unidentified compounds.

Permethylation. Oleanoglycotoxin-*A* (9·8 mg) was methylated twice with 50 mg NaH and 0·1 ml Me I in 0·2 ml dry $HCONMe_2$[11,12] and the product was purified by TLC. MS, *m/e* 187, 203, 219, 262, 391, 395, 407, 423, 453, 509, 861, 1094.

Acid hydrolysis of permethyl oleanoglycotoxin-A. The material (3·3 mg), 5 ml dioxane (distilled from sodium

Acid hydrolysis of permethyl oleanoglycotoxin-A. The material (3·3 mg), 5 ml dioxane (distilled from sodium), 4 ml of dist. H_2O and 1 ml of conc. HCl were refluxed for 15 hr. The solution was evaporated *in vacuo*, one drop of pyridine added and the sides washed down with Et_2O. Evaporation gave a yellow gum, which was derivatized by dissolving in 100 μl HMDS–TMCS–pyridine. GC–MS showed α- and β-anomers

[11] Brimacombe, J. S., Jones, B. D., Stacey, M. and Willard, J. J. (1966) *Carbohyd. Res.* **2**, 167.
[12] Thomas, D. W. (1969) *FEBS Letters* **5**, 53.

of trimethylsilylated-2,3,4,6-tetra-*O*-methylglucopyranose and trimethylsilylated-3,6-di-*O*-methylgluco pyranose.

Acknowledgements—The authors are indebted to Professor Aklilu Lemma for his interest and samples of *Phytolacca dodecandra*, to Mr. Percy Yau for biological testing, and to Professor C. Djerassi and Dr. J. R. Dias (Stanford University) for providing a sample of authentic hederagenin. Thanks are expressed also to Dr. B. C. Das and Mr. P. Varenne (Gif-sur-Yvette, France) for determining the complete MS of oleanoglycotoxin-*A* acetate. This work was supported by the Office of Naval Research, contract NOOO 14–71–C–0123, Stanford Research Institute Research and Development funds, and General Research Support Grant RO–5522 from the National Institutes of Health.

Reprinted from the *Indian Journal of Chemistry*, Vol. 11, No. 11, November 1973, pp. 1192-1195

Molluscicidal Saponins of *Phytolacca dodecandra*: Lemmatoxin-C

R. M. Parkhurst, David W. Thomas & W. A. Skinner
Life Sciences Division, Stanford Research Institute
Menlo Park, California 94025
and
Lewis W. Cary
Varian Associates, Palo Alto, California

Received 14 *September* 1973; *accepted* 1 *October* 1973

Lemmatoxin-C, one of the molluscicidal saponin components of the fruit of *phytolacca dodecandra* (Endod), has been found to be a mixture of two closely related oleanolic acid derivatives, each with a linear trisaccharide at the 3-position of oleanolic acid and rhamnose as the end sugar.

IN continuation of our work on molluscicidal saponins of *Phytolacca dodecandra*[1], we have isolated a chromatographically homogeneous fraction, lemmatoxin-C, from the complex mixture of crude saponin acetates. Although lemmatoxin-C has only half the biological activity of lemmatoxin, it still remains interesting from the standpoint of structure-activity relationship. Interesting also is the fact that the synthetic β-glucoside of oleanolic acid has only one-tenth the molluscicidal properties of lemmatoxin-C.

Lemmatoxin-C represents approximately 17% of the crude saponin acetates. Although lemmatoxin-C acetate could not be crystallized, as was the case with oleanoglycotoxin-A and lemmatoxin, its chromatographic homogeneity prompted us to start our analytical study. The NMR spectra gave the first indication that this material was not a single compound. Although the presence of oleanolic acid and three sugar groups was relatively apparent as in the case of oleanoglycotoxin-A and lemmatoxin, there were variations in the sugar region of the spectrum that could only be due to multiple components, i.e. less than one-proton peaks. This immediately complicated the interpretation of the already complex NMR spectrum.

Gas chromatography of the per-trimethylsilylated sugars from total acid hydrolysis of lemmatoxin-C confirmed the multiple component theory in that rhamnose, glucose and galactose were liberated in the ratio of 1·0: 4·0: 0·6 moles respectively.

Mass spectral examination of lemmatoxin-C acetate, however, gave a fairly clear picture of the structures involved (structures I and II are presented as an aid to the interpretation). In the molecular ion region, peaks at 1316, 1317 and 1318, which are 58 mass units lower than the corresponding mass peaks for oleanolglycotoxin-A and lemmatoxin confirmed that all components contained the rhamnose unit. A peak at 273 (*a*) further indicated that the rhamnose unit was the end sugar in all cases since no appreciable peaks appeared at 331, which would correspond to a terminal glucose or galactose. Peaks at 1272 ($M-HCO_2H$) in the spectrum of the acetate and at 1258 ($M-HCO_2CH_3$) in the acetate methyl ester spectrum indicates that the carboxylic acid group is free in lemmatoxin-C.

A rather intense peak at 561 (*c*), the rhamnose and the hexose to which it is attached, is indicative of a linear saccharide moiety. This peak is relatively minor in the mass spectra of the branched species, oleanoglycotoxin-A and lemmatoxin. Another peak at 849 (*e*) represents a trisaccharide unit, and peaks at 453 (*f*) and 262 (*g*) represent the sapogenin counterpart with its Δ^{18} fragmentation product. Peaks at 741 (*d*) and 1029 (*b*), representing oleanolic acid with one and two hexose units respectively, again reinforce our speculations concerning the linear nature of lemmatoxin-C.

We can, at this point, say something about the mixture with which we are dealing. If all galactose units occur with a glucose unit, 70% of lemmatoxin-C must be rhamnose-glucose-glucose oleanolic acid and 30% must be the corresponding galactose-containing species. If all the galactose occur with another galactose unit, 85% of lemmatoxin-C must be rhamnose-glucose-glucose oleanolic acid and 15% must be the corresponding galactose-containing species.

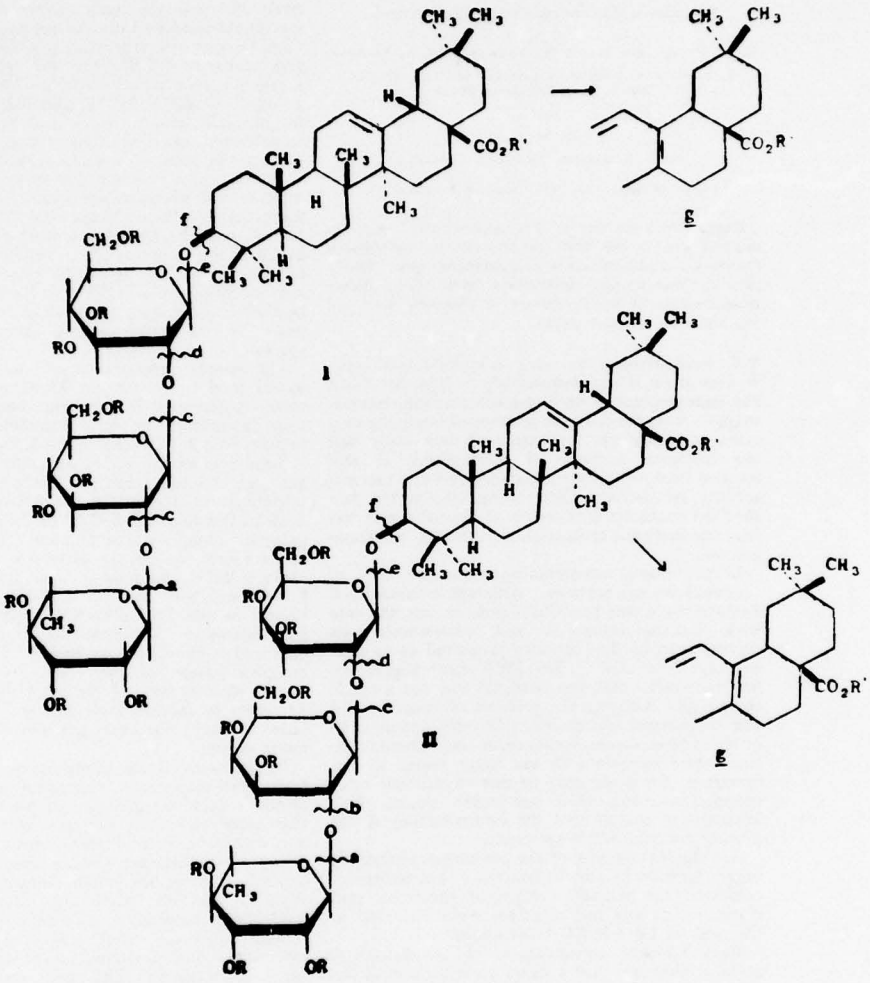
The general appearance of the NMR spectra would tend to favour the 70: 30 mixture in that several peaks seem to represent about $\frac{1}{4}$ of a proton upon integration (i.e. most downfield doublets and triplets near δ 3·75 ppm in the C_6D_6 spectra).

Total acid hydrolysis of permethyl lemmatoxin-C and gas chromatography of the products as their trimethylsilyl derivatives yield large amounts of 3,4,6-tri-O-methyl-1,2-di-O-(trimethylsilyl)glucose and galactose along with other minor products. From this evidence, we can speculate that the linear sugar chain is largely 1-2 linked. Two anomeric protons, δ 4·44 and 4·47 ppm, $J = 8$ Hz ($CDCl_3$), show clearly in the 220 MHz NMR spectra and show β-configuration. The remaining anomeric proton is obscured in the C_6-proton region. The particularly complex nature of the NMR spectra precludes certain identification of the other anomeric proton. Attempts to spin-decouple in the C_2-proton region failed to clarify the exact position of the third anomeric proton.

In the cases of oleanolglycotoxin-A and lemmatoxin, the sugar unit connected directly to the oleanolic acid was glucose. If we could speculate that some biogenetic pathway acts in this plant in a way common to all these derivatives, we might also presume that the glucose remains next to the oleanolic acid in the minor component of lemmatoxin-C. However, partial hydrolysis experiments would be required for a more definitive answer.

Some additional insight can be gained from the molar rotations of lemmatoxin-C and its relevant sugar components. The two anomeric methyl glycosides of peracetyl L-rhamnose (rhamnose occurs almost exclusively in nature as the L-form) and the α- and β-anomers of the corresponding galactose and glucose derivatives[2] have molar rotations as shown in Table 1. The algebraic addition of all possible combinations of the anomeric forms of these three sugars and all possible combinations of two glucose anomers with the two rhamnose anomers results in a total positive molar rotation except for the α-rhamnose-β-glucose-β-glucose combination and the α-rhamnose-β-galactose-β-glucose combination, which result in total molar rotations of −295° and −280·3° respectively. This is in fair agreement with the molar rotation of −219° required for the trisaccharide moiety of lemmatoxin-C, although this does not completely eliminate

INDIAN J. CHEM., VOL. 11, NOVEMBER 1973



1.	R = OH	R' = H	Lemmatoxin-C
2.	R = Ac	R' = H	Lemmatoxin-C acetate
3.	R = Ac	R' = CH_3	Lemmatoxin-C acetate methyl ester
4.	R = CH_3	R' = CH_3	Permethyl Lemmatoxin-C

	Lemmatoxin-C R = Ac R' = H		Lemmatoxin-C R = Ac R' = H
M	1318	d	741
a	273	e	849
b	1029	f	453
c	561	g	282

TABLE 1 — MOLAR ROTATIONS OF LEMMATOXIN-C AND ITS RELEVANT SUGAR COMPONENTS

	$[\alpha]_D$ (deg.)	M_D (deg.)
Lemmatoxin-C acetate	+11·87	+156·0
Oleanolic acid	+83·0	+375·0
Sugar moiety required	—	−219·0
α-Methylrhamnoside acetate	−53·7	−163·4
β-Methylrhamnoside acetate	+45·7	+139·1
α-Methylgalactoside acetate	+133·0	+482·0
β-Methylgalactoside acetate	−14·0	−50·7
α-Methylglucoside acetate	+130·5	+472·8
β-Methylglucoside acetate	−18·2	−65·9
α-Rhamnoside + β-glucoside + β-glucoside (methyl acetate)	—	−295·0
α-Rhamnoside + β-galactoside + β-glucoside (methyl acetate)	—	−280·3

the possibility of the presence of some additional α-linkages in the minor component.

It is now possible to propose tentative structures for the major component of lemmatoxin-C as 3-O-(α-L-rhamnopyranosyl-2′-O-β-D-glucopyranosyl-2″-O-β-D-glucopyranosyl)olean-12-ene-28-oic acid (at least 70%) and the remaining component as the galactose containing species (structures I and II respectively).

Mass spectra were determined with either a CEC model 21-110B mass spectrometer or an LKB model 9000 combination gas chromatograph-mass spectrometer. Quantitative gas chromatograms were obtained with a Varian aerograph model 550-B instrument with flame ionization detector. All analyses were carried out with a 10′×$\frac{1}{8}$″ column of 1% SE-30 on 100-120 mesh Gas Chrom Q, at 200°C and a flow rate of 23 ml/min.

Separation of lemmatoxin-C acetate — Crude saponin acetates (2 g) was chromatographed on a column (18×3 cm, 50 g, Mallinckrodt silic-AR-CC7, 100-200 mesh), using $CHCl_3$-ether gradient elution and following the progress of the fractionation via TLC on silic-AR-7GF plates. A fraction, 1015 mg, eluted with 1% ether in $CHCl_3$, was further purified by repetitive chromatography on thick plates [silic-AR-7GF; $CHCl_3$, $CHCl_3$-ether (1:1), and ether], finally giving 35·2 mg of lemmatoxin-C acetate as a colourless glassy solid, R_f = 0·62-0·68 (silic-AR-7GF; ether), $[\alpha]_D^{25}$ = +11·87° (*c* = 1·87, $CHCl_3$).

Lemmatoxin-C acetate methyl ester — Lemmatoxin-C acetate was methylated with excess diazomethane in methanol-ether mixture as previously reported[8].

Deacetylation of lemmatoxin-C acetate — Lemmatoxin-C acetate (9 mg) was dissolved in 3 ml of methanol and 1 ml of conc. ammonium hydroxide and maintained at 50° for 24 hr. The solution was evaporated in a slow stream of nitrogen and extracted with *n*-butanol. Evaporation of the butanol gave lemmatoxin-C as a colourless, amorphous solid.

Total hydrolysis of lemmatoxin-C — Lemmatoxin-C (1 mg) was dissolved in 2 ml of dioxane-2*N* HCl (1:1) and sealed in a glass tube. The sealed tube was placed in a steam bath for 17 hr and finally evaporated to dryness in a slow stream of nitrogen. Oleanolic acid, which crystallized out, was removed by centrifugation and its identity was established via TLC. The sugars were per-trimethylsilylated in pyridine for gas chromatographic analysis as previously described[8].

Total hydrolysis of permethyl lemmatoxin-C — Lemmatoxin-C (8 mg) was permethylated by the usual procedure and purified from incomplete methylation products via TLC (silic-AR-7GF; $CHCl_3$-ether, 1:1). The permethyl lemmatoxin-C was subjected to total acid hydrolysis in dioxane-2*N* HCl (1:1) as described previously[8].

The hydrolysis products were per-trimethylsilylated in pyridine for combined gas chromatography-mass spectral analysis.

The authors are indebted to Dr Akiliu Lemma for his interest and samples of *P. dodecandra* and to Percy Yau for biological testing. This work was supported by the Office of Naval Research, contract NOOO 14-71-C-0123, Stanford Research Institute Research and Development funds, and General Research Support Grant RO-5522 from the National Institutes of Health.

References

1. LEMMA, AKLILU, BRODY, GERALD, NEWELL, GORDON W., PARKHURST, R. M. & SKINNER, W. A., *J. Parasitology*, **58** (1972), 104-7.
2. PARKHURST, R. M., THOMAS, DAVID W., SKINNER, W. A. & CARY, LEWIS W., *Phytochem.*, **12** (1973), 1437.
3. *Polarimetry, saccharimetry and sugars*, edited by Frederick J. Bates and associates (US Government Printing Office, Wash.), 1942.

Molluscicidal Saponins of *Phytolacca dodecandra*: Lemmatoxin

R. M. PARKHURST, DAVID W. THOMAS, AND W. A. SKINNER
Life Sciences Division, Stanford Research Institute, Menlo Park, California
AND
LEWIS W. CARY
Varian Associates, Palo Alto, California

Received October 27, 1972[1]

Lemmatoxin, one of the molluscicidal saponin components of the fruit of *Phytolacca dodecandra* (endod), has been found to be a derivative of oleanolic acid substituted in the 3 position with a branched trisaccharide containing two glucose units and one galactose unit. A structure is proposed.

On trouve qu'une des composantes des saponines molluscicides du fruit du *Phytolacca dodecandra* (endol), la lemmatoxine, est un dérivé substitué en position-3 de l'acide oléanolique par un trisaccharide ramifié contenant deux unités de glucose et une unité de galactose. Une structure est proposée.
[Traduit par le journal]

Can. J. Chem., **52**, 702 (1974)

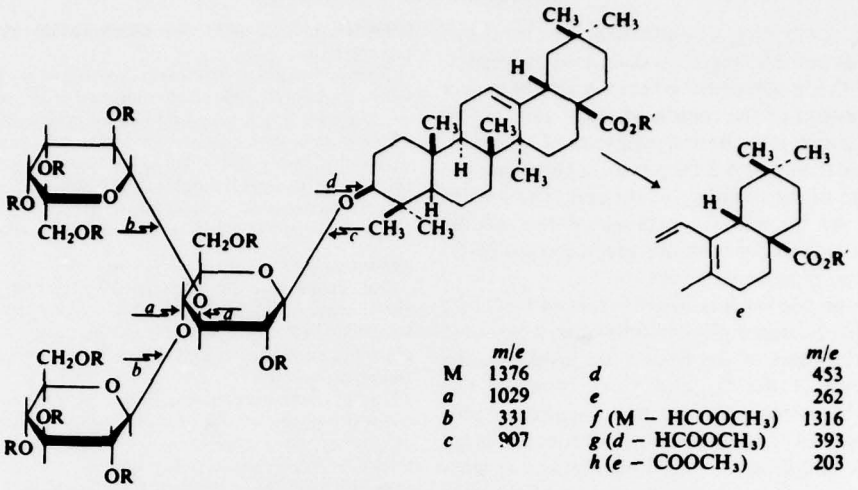
Since the reported isolation and structure determination of the major biologically active saponin of endod, oleanoglycotoxin-A (1), further work has led to the isolation of another saponin. Although present in lesser amount in the crude saponin mixture, this component seems to be about twice as biologically active (LD_{90}, 1.5 p.p.m., 24 h, *Biomphalaria glabrata*) as the major component. The discovery of other biological activity of the saponin not related to its molluscicidal properties is also under investigation. We have designated this material lemmatoxin (R = OH; R′ = H) and present here evidence for our assignment of structure.

During the chromatographic isolation of oleanoglycotoxin-A as its acetyl derivative from the crude saponin acetates from endod, several less polar components were recovered as mixtures. Repeated chromatography of one of these mixtures on silica gel thick layer gave lemmatoxin acetate (R = Ac; R′ = H) as a noncrystalline, glassy material, which seemed to be homogeneous by virtue of its movement on thin-layer silica gel plates as a single spot in several solvent systems. Lemmatoxin acetate could be deacetylated to give a biologically active saponin and reacetylated (1) to give back lemmatoxin acetate identical to that of the originally isolated material. Comparison of the size of the spots of this material and the size of the corresponding spot in the t.l.c. of the crude saponin acetate mixture allowed us to estimate its approximate concentration in the crude material at 16%.

Acid hydrolysis of lemmatoxin gave oleanolic acid as in the case of oleanoglycotoxin-A and also glucose and galactose, in the ratio of 2:1, respectively, which were identified as their pertrimethylsilyl derivatives by gas chromatography. Gas chromatography of the total acid hydrolysis products of permethyl lemmatoxin (R = R′ = CH_3) as their pertrimethylsilyl derivatives showed 1 mol of 2,6-di-*O*-methyl-1,3,4-tri-*O*-(trimethylsilyl)-D-glucose, 1 mol of 2,3,4,6-tetra-*O*-methyl-1-*O*-trimethylsilyl-D-galactose, and 1 mol of 2,3,4,6-tetra-*O*-methyl-1-*O*-trimethylsilyl-D-glucose, which were identified by comparison of their mass spectra with those in the literature (2), indicating that a galactose and glucose unit are attached to a central glucose unit in the 3 and 4 positions, assuming the central glucose to be in its pyranose form.

Acid hydrolysis of lemmatoxin under mild conditions gave glucose and galactose in almost equal amounts, as identified by gas chromatography of their pertrimethylsilyl derivatives, and another saponin along with free oleanolic acid in almost equal amounts. The formation of free oleanolic acid would be accompanied by the release of twice as much glucose as galactose and we would expect that the new saponin formed would retain glucose in preference to galactose. The hydrolytic loss of sugars in the 3 position in preference to the 4 position would tend to indicate that the galactose is located at position 3 (3). The new saponin

[1]Revision received September 17, 1973.



	m/e		m/e
M	1376	*d*	453
a	1029	*e*	262
b	331	*f* (M − $HCOOCH_3$)	1316
c	907	*g* (*d* − $HCOOCH_3$)	393
		h (*e* − $COOCH_3$)	203

FIG. 1. Interpretation of the mass spectrum of lemmatoxin acetate methyl ester (R = Ac; R′ = CH_3). The evidence for galactose and glucose being attached at positions 3 and 4, respectively, rather than 4 and 3, respectively, is not complete (see Text).

formed by this partial hydrolysis was not distinguishable from an authentic sample of oleanolic acid β-cellobioside by any of our thin-layer chromatographic systems when compared as either the peracetyl or permethyl derivative, further supporting our indication that galactose is located at the 3 position. Attempts to investigate the total hydrolysis products of the permethyl derivative of the new saponin formed by partial hydrolysis of lemmatoxin via gas chromatography – mass spectroscopy were inconclusive however, because the expected peaks for 2,3,4,6-tetra-*O*-methyl-1-*O*-trimethylsilyl-α- and β-D-glucose and 2,3,6-tri-*O*-methyl-1,4-di-*O*-trimethylsilyl-α- and β-D-glucose were very small due to the limited amount of sample available and the relatively high background of column bleed in the area of these peaks. This leaves still some doubt as to the position of attachment of the glucose and galactose units.

Mass spectra of lemmatoxin acetate and of its methyl ester (R = Ac; R′ = CH_3) gave added structural information. The molecular weight (1376) of the methyl ester could be seen by examination of this mass spectrum (Fig. 1).

That all three sugar units are contained within one single saccharide moiety is confirmed by the appearance of a peracetyl trihexose unit in the mass spectrum at *m/e* 907 (*c*). The appearance of fragment (*e*) at *m/e* 262, originating by a mechanism typical of Δ^{12}-unsaturated triterpenes, confirms that the carboxyl is present as a methyl ester, ruling out the possibility of the saccharide being bound to the carboxyl group.

A mass spectrum of peracetyl lemmatoxin was similar to the methylated compound with the exception that all fragments containing the carboxyl function appeared 14 mass units lower. Thus, the methyl ester of the derivative illustrated in Fig. 1 was introduced by treatment with diazomethane, and lemmatoxin therefore contains a free carboxylic acid.

The n.m.r. spectra of lemmatoxin acetate were taken in $CDCl_3$, C_6D_6:$CDCl_3$ (1:1), and two mixtures of these solvents. Two one-proton doublets δ ($CDCl_3$) 4.66, 4.70 p.p.m., (J = 8 Hz) each of which lean downfield and may be associated with the anomeric protons of two of the sugar units in the β configuration. The third anomeric proton is less well defined and partially covered by the C_6 protons, but can be detected via solvent shift as a doublet δ ($CDCl_3$) 4.37 p.p.m., (J = 8 Hz) indicating the β configuration.

Further upfield in the $CDCl_3$ spectra there are two multiplets δ 3.59 and 3.70 p.p.m., representing one and two protons, respectively. The fact that they lean downfield and their width suggest that these are three C_5 protons. The C_5 proton of galactose is only very weakly coupled to the C_4 proton and appears almost as a triplet (4). Spin decoupling at these multiplets causes the expected collapse of the minor splitting in the C_6 proton

quartets, confirming our previous assumptions. The triplet at δ 3.95 p.p.m. is collapsed by irradiation at 293 Hz downfield where we would expect the C_2 proton of the middle glucose unit; therefore this triplet must be assigned to the C_3 proton. The other triplet at δ 3.86 p.p.m. is therefore the C_4 proton of the middle glucose unit. The upfield shift of the C_3 and C_4 protons of the middle glucose is consistent with our proposed 3,4-di-*O*-substituted glucose structure.

Although the far less likely furanoid structure for the disubstituted glucose unit cannot be ruled out on the basis of the hydrolysis products, the appearance of the C_4 and C_5 protons of the central glucose unit in the n.m.r. suggests a splitting pattern of a pyranose form as does the large anomeric coupling (5). The low intensity of mass fragments resulting from the fission of the side chain from a five-membered furanose ring to give the planar oxinium ion also suggests the pyranose over the furanose form (6).

Since the anomeric configuration was clearly established by n.m.r., the unlikely possibility of L sugars can be ruled out by the optical rotation on the basis of Klyne's rule (7).

	$[\alpha]_D$ (deg)	M_D (deg)
Lemmatoxin acetate	+7.4±0.05	+101
Oleanolic acid	+83	+378
Sugar moiety required		−278
Methyl β-cellobioside acetate	−26	−169
Methyl β-D-galactoside acetate	−14	−51
Methyl β-cellobioside + methyl β-D-galactoside (acetates)		−218

The above data suggest that lemmatoxin is 3-*O*-[4′-*O*-(β-D-glucopyranosyl)-3′-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]olean-12-ene-28-oic acid (Fig.1).

Experimental

Mass spectra were determined with either a CEC Model 21-110B mass spectrometer or an LKB Model 9000 combination gas chromatograph – mass spectrometer. Quantitative gas chromatograms were obtained with a Varian-Aerograph Model 550-B instrument with flame ionization detector. Analyses were carried out with a 10 ft × 1/8 in column of 1% SE-30 on 100–120 mesh gas chrom Q at 170° and He flow of 20 ml/min for the TMS derivatives of unmethylated sugars. Analyses of TMS derivatives of methylated sugars were chromatographed similarly except that temperature was programmed and mass spectral comparison was used for identification rather than retention time.

Nuclear magnetic resonance spectra were determined with a Varian HR 300 nuclear magnetic resonance spectrometer employing a superconducting magnet at 70.5 kG. The sample was examined in a 5 mm sample tube at 35°.

All thin-layer and thick-layer chromatography was carried out using Mallinckrodt SilicAR-7GF plates and solvent systems *A*, chloroform; *B*, chloroform–ether (1:1); *C*, ether; *D*, ethyl acetate or; *E*, acetone.

Separation of Lemmatoxin Acetate

The crude saponin acetates from endod. 2 g, were chromatographed on a column (18 × 3 cm, 50 g, Malinckrodt SilicAR-CC7, 100–200 mesh) using chloroform–ether gradient elution and following the progress of the fractionation with t.l.c. A fraction, 1050 mg, eluted with 1% ether in chloroform was further purified by repetitive chromatography on thick plates (*B*) finally giving 20.9 mg of lemmatoxin acetate as a colorless glassy solid, R_f = 0.50–0.54 (*C*); $[\alpha]_D^{23}$ + 7.4 ± 0.05 (*c*, 1.26 chloroform); m.s. *m/e* 1362 (M^+), 1316, 1015, 907, 439, 393, 331, 248, 203; n.m.r. ($CDCl_3$) δ 0.72, 0.74 (CH_3), 0.89 ($3CH_3$), 0.92, 1.22 (CH_3), 1.95, 1.98, 2.00, 2.02 (CH_3CO), 2.08 ($3CH_3CO$), 2.09, 2.10, 2.13 (CH_3CO), 2.81 (C_{18}—H, m), 2.98 (C_3—H, m), 3.59 (C_5—H, m), 3.70 (C_5—2H, m), 3.86 (C_4—H, t), 3.95 (C_3—H, t) 4.05–4.50 (C_6—$3CH_2$, overlapping quartets), 4.37, 4.66, 4.70 (anomeric doublets, *J* = 8 Hz), 4.9–5.4 (complex series), 5.27 p.p.m. C_{12}—vinyl H, t).

Lemmatoxin Acetate Methyl Ester

Lemmatoxin acetate, 2 mg, was methylated with excess diazomethane in methanol–ether solution at room temperature overnight. Thin-layer chromatography showed the formation of a single new product, the methyl ester R_f = 0.62–0.67 (*C*); m.s. *m/e* 1376 (M^+), 1316, 1029, 907, 453, 393, 331, 262, 203.

Deacetylation of Lemmatoxin Acetate

Lemmatoxin acetate, 6.6 mg, was dissolved in 3 ml of methanol and 1 ml of concentrated ammonium hydroxide and maintained at 50° for 24 h. The solution was evaporated in a slow stream of nitrogen and extracted with 1-butanol. Evaporation of the butanol gave lemmatoxin as a colorless solid. This material could be reacetylated with excess acetic anhydride – pyridine at room temperature overnight to give back lemmatoxin acetate, R_f = 0.50–0.54 (*C*).

Total Hydrolysis of Lemmatoxin

Lemmatoxin, 1 mg, was dissolved in 2 ml of dioxane – 2 *N* HCl (1:1) and sealed in a glass tube. The sealed tube was placed in a steam bath for 15 h. The solid precipitate was centrifuged to one end; the liquid was decanted to the other end and the tube was cut in half. The precipitate was identified as oleanolic acid by comparison with an authentic sample via t.l.c. in two solvents, R_f = 0.70 (*D*); R_f = 0.90 (*E*). A drop of pyridine was added to the liquid portion before it was evaporated to dryness in a slow stream of nitrogen. The sugars remaining were pertrimethylsilylated in pyridine for gas chromatographic analysis. The retention times were compared with authentic samples of glucose and galactose treated similarly. The area of the peaks representing glucose (α-anomer, retention time 5.8 min; β-anomer, retention time 9.3 min)

and galactose (α-anomer, retention time 5.2 min; β-anomer, retention time 6.3 min) were in the ratio of 2 to 1.

Partial Hydrolysis of Lemmatoxin

Lemmatoxin, 1 mg, was dissolved in 4 ml of dioxane – 2% HCl (1:1) and heated on the steam bath for 3 h in a sealed tube. The solution was evaporated to a small volume in a slow stream of nitrogen; water and 1-butanol were added to separate the saponin from the free sugars. The butanol layer was evaporated and the solid remaining was acetylated and subjected to t.l.c. The t.l.c. showed only a trace of lemmatoxin acetate and about equal amounts of a new saponin acetate and oleanolic acid acetate by comparison of the areas of the spots. The saponin acetate was recovered and chromatographed in several solvent systems, R_f = 0.07–0.10 (*A*), R_f = 0.42–0.54 (*B*), R_f = 0.75–0.82 (*C*), R_f = 0.94—0.99 (*D*). The same values were obtained for authentic oleanolic acid β-cellobioside heptaacetate run on the same plate. The new saponin acetate was again recovered deacetylated and permethylated as described (8), R_f = 0.20–0.30 (*A*). The same R_f value was obtained for the standard compound treated similarly. The permethylated (8) new saponin was hydrolyzed in dioxane – 2 *N* HCl (1:1) as described for total hydrolysis above and the permethylated sugars pertrimethylsilylated for GC/MS.

Total Hydrolysis of Permethyl Lemmatoxin

Lemmatoxin, 1 mg, was permethylated and hydrolized in dioxane – 2 *N* HCl (1:1) as described above. The hydrolysis products were pertrimethylsilylated in pyridine and subjected to gas chromatography which showed 2,3,4,6-tetra-*O*-methyl-1-*O*-trimethylsilyl glucose, 2,3,4,6-tetra-*O*-methyl-1-*O*-trimethylsilyl galactose, and 2,6-di-*O*-methyl-1,3,4-tri-*O*-(trimethylsilyl) glucose in the ratio of 1:1:1. Peaks were doublets representing the two anomeric forms in each case. Mass spectrometry was used for identification and the area under the respective peaks used to determine the quantity.

The authors are indebted to Professor Aklilu Lemma for his interest and samples and to Percy Yau for biological testing. This work was supported by the Office of Naval Research Contract N00014-71-C-0123, Stanford Research Institute Research and Development funds, and General Research Support Grant RO-5522 from the National Institute of Health.

1. R. M. Parkhurst, D. W. Thomas, W. A. Skinner, and L. W. Cary. Phytochemistry, **12**, 1437 (1973).
2. G. Petersson and O. Samuelson. Svensk Papperstid. **71**, 731 (1968).
3. Z. Wojciechowski, A. Jelonkiewicz-Konador, M. Tomaszewski, J. Jankowski, and Z. Kasprzyk. Phytochemistry, **10**, 1121 (1971).
4. C. V. Holland, D. Horton, and M. J. Miller. J. Org. Chem. **32**, 3077 (1967).
5. B. Capon and D. Thacker. Proc. Chem. Soc. 369 (1964).
6. N. K. Kochetkov and O. S. Chizhov. *In* Advances in carbohydrate chemistry. Vol. 21. *Edited by* M. L. Wolfrom. Academic Press, New York and London. 1966. p. 62.
7. W. Klyne. Biochem. J. **47**, xli (1950).
8. D. W. Thomas. FEBS Lett. **5**, 53 (1969).

Reprinted from the *Indian Journal of Chemistry*, Vol. 13, No. 7, July 1975, pp. 757-758

The Chemotaxonomy of *Phytolacca* Species

R. M. PARKHURST

Stanford Research Institute, Menlo Park
California 94025, USA

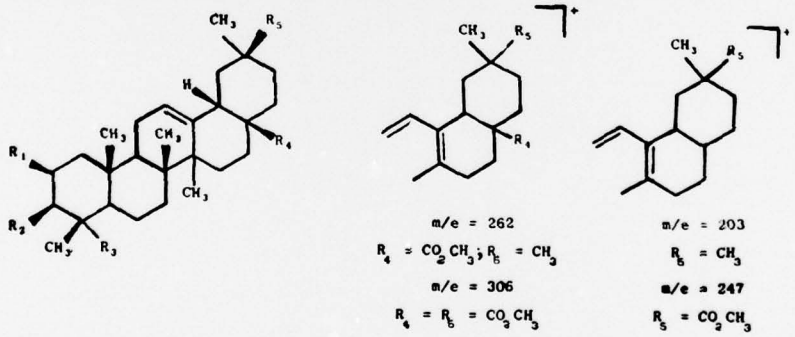
Received 13 *December* 1974; *accepted* 20 *January* 1975

The aglycones of *P. dodecandra* consist of oleanolic acid (66·2%), hederogenin (8·9%), 2-hydroxyoleanolic acid (6·5%) and bayogenin (14·9%), all 28-carboxy-oleanenes while *P. americana, P. dioica, P. octandra, P. rivinoides* and *P. esculenta* seem to contain 28,30-dicarboxy and/or carbomethoxy oleanenes.

THE potent molluscicidal activity and high yield of crude saponins (25% dry weight) from the berries of *Phytolacca dodecandra* (Endod) attracted the attention of Lemma[1] nearly ten years ago. Since then, these saponins have been found to be fungicidal[2], emetic[3], larvacidal for the mosquito[4], and a potent spermicide[5]. Continued interest[6,7] in the biological activity of this plant has uncovered female antifertility activity[8] and prompted a more careful look at its general chemistry.

Powell and Whalley[9] unequivocally identified oleanolic acid (A) and bay genin (D) as the principle aglycones of this plant but their study was not quantitative. The discovery of a trisaccharide of hederogenin (C) as a minor impurity in a chromatographically homogeneous sample of oleanoglycotoxin-A[10], one of the molluscicidal saponins, was the first indication of the presence of other aglycones. It was of interest to determine the composition of the aglycones of the crude butanol extract upon which much of the preliminary biological data is based[11,12].

The aglycones were obtained by precipitation from the mild acid hydrolysis of the crude saponins in 42·1% yield. This would indicate an average of about four sugar units per aglycone in the saponin mixture. Permethylation[10] of the crude aglycones and gas chromatography showed the presence of five peaks with retention times of 4·85, 5·75, 5·95, 7·07 and 9·10 min.* The first peak, 3-O-methyl-oleanolic acid methyl ester from oleanolic acid, previously shown to be the major aglycone, represented 66·2% of the mixture as calculated from the area under the GC curve. The second and third peaks, permethyl hederogenin and permethyl 2-hydroxyoleanolic acid (B)[13], represented 8·9% and 6·5% respectively and their identity confirmed by comparison of retention time and mass spectra with authentic samples. The fourth peak (14·9%) had a mass spectrum consistent with that reported previously for derivatives of bayogenin[9]. The fifth chromatographic peak represented only 3·5% and mass spectra through different sections of this peak showed that it consisted of more than one compound. Although no molecular ions were observed, all spectra from this peak showed prominent *m/e* 203 and 262 from the retro-Diels-Alder fragmentation of a monocarboxylic acid methyl ester and lack of fragments 247 and 306 which would be expected from a dicarboxylic acid methyl ester function (Chart 1).



	R_1	R_2	R_3	R_4	R_5
A : oleanolic acid	H	OH	CH_3	CO_2H	CH_3
B : 2-hydroxyoleanolic acid	OH	OH	CH_3	CO_2H	CH_3
C : hederogenin	H	OH	CH_2OH	CO_2H	CH_3
D : bayogenin	OH	OH	CH_2OH	CO_2H	CH_3
E : phytolaccagenin	OH	OH	CH_2OH	CO_2H	CO_2CH_3
F : 2-deoxyphytolaccagenin	H	OH	CH_2OH	CO_2H	CO_2CH_3
G : serjanic acid	H	OH	CH_3	CO_2H	CO_2CH_3
H : jaligonic acid	OH	OH	CH_2OH	CO_2H	CO_2H

Chart 1 — Aglycones of *Phytolacca* species

*GC analysis carried out with a 3 m×3·2 mm glass column of 1% SE-30 on 100-120 mesh Gas Chrom Q; He, 30 ml/min, 230°C. Mass spectra determined with LKB model 9000 combination GC/MS.

Phytochemical work on the genus *Phytolacca* has been far from complete but of the species that have been investigated, *P. dodecandra* is the exception to the apparent trend of isolation of dicarboxylic acids and/or esters in the aglycone fractions. *P. americana* (Pokeweed) contains phytolaccagenin (E) and 2-deoxyphytolaccagenin (F)[14]. Work in these laboratories indicates that they are present in a ratio of ~3:1 and no monocarboxylic compounds could be detected†. Crude saponins from the South American giant species, *P. dioica* (Ombú tree) were found to constitute only 1% of the dry weight of the berries. GC analysis† of the permethylated aglycones showed clearly the absence of oleanolic acid in any detectable quantities and a remarkable resemblance to the GC of *P. americana* both quantitatively and qualitatively for both major peaks and most minor peaks. *P. octandra*[15] and *P. rivinoides*[16] contain serjanic acid (G) and *P. esculenta*[17] contains jaligonic acid (H). All of these aglycones (E-H) are 28,30-carboxy and/or carbomethoxy derivatives of the oleanene structure. Further chemical work on other members of the genus will be necessary before the total lack of dicarboxylic triterpenes in *P. dodecandra* casts doubt on its botanical assignment.

The author wishes to express his appreciation to Dr J. R. Dias (Stanford University) for the authentic sample of hederogenin, Prof. J. J. H. Simes (University of New South Wales) for the authentic sample of 2-hydroxyoleanolic acid and Prof. Bruce K. Cassels (Universidad Tecnica del Estado) for samples of *Phytolacca dioica*. Thanks are also expressed to Dr D. W. Thomas and Mrs Hanna Chandler (SRI) for the mass spectra.

†GC analysis carried out with a 6 ft × 1/8 in. glass column of 1% SE-30 on 60-80 mesh Gas Chrom Q; He, 45 ml/min. Programmed 100° to 300°C at 32°C/min on Hewlett-Packard 5700A instrument with FID.

References

1. Lemma, A., *Ethiopian Med. J.*, **3** (1965), 187.
2. Maxwell, A. (private communication).
3. Newell, G. W. (private communication).
4. Spielman, A. & Lemma, A., *Am. J. Trop. Med. Hyg.*, **22** (5) (1973), 802.
5. Stolzenberg, S. J. & Parkhurst, R. M., *Contraception*, **10** (1974), 135.
6. Parkhurst, R. M., Thomas, D. W., Skinner, W. A. & Cary, L. W., *Can. J. Chem.*, **52** (5) (1974), 702.
7. Parkhurst, R. M., Thomas, D. W., Skinner, W. A. & Cary, L. W., *Indian J. Chem.*, **11** (1973), 1192.
8. Stolzenberg, S. J., Parkhurst, R. M. & Reist, E. J., *Fed. Proc.*, **34** (1975), 339.
9. Powell, J. W. & Whalley, W. B., *Phytochemistry*, **8** (1969), 2105.
10. Parkhurst, R. M., Thomas, D. W., Skinner, W. A. & Cary, L. W., *Phytochemistry*, **12** (1973), 1437.
11. Lemma, A., Brody, G., Newell, G. W., Parkhurst, R. M. & Skinner, W. A., *J. Paraditology*, **58** (1972), 104.
12. Baalawy, S. S., *Bull. W.H.O.*, **47**(3) (1972), 422.
13. Bannon, C. D., Eade, R. A., James, P., McKenzie, G. P & Simes, J. J. H., *Aust. J. Chem.*, **26** (1973), 629.
14. Johnson, A., Ph.D. thesis, University of Rhode Island, 1973.
15. Howard, H. T. C., *Phytochemistry*, **12** (1973), 2307.
16. Gonzalez, A. G., Breton, F., Castaneda, J. P., Fraga, B. M. & Morales, A., *Quimica*, **68** (1972), 1057.
17. Woo, W. S., *Lloydia*, **36** (3) (1973), 326.

የኢትዮጵያ ንጉሠ መንግሥት ሚኒስቴር
የሕዝብ አስተዳደር ፕሊስ ክፍል
MINISTRY OF INTERIOR
PUBLIC SECURITY DEPT.

ፖ.ሳ.ቁ. 35

ቁጥር፡

አ.አ.

ስን፡ IDE ን.ም.

የፖ.ሣ.ቁ. | 105
P O. Box |

የስልክ ቁ. | 10855
Tel. No. | 10856

Certificate of Registration
of Association

Application for the registration of the ETHIOPIAN SCIENCE FOUNDATION having been received at the Office of Associations at Addis Abeba this second day of October, 1972 and found to be in accordance with the Civil Code of Ethiopia and LEGAL NOTICE No. 321 of 1966, said application and the Memorandum of Association submitted in conjunction therewith are hereby approved as in conformity with all applicable requirements, and said association is hefeby registered to have and exercise the purposes, rights, powers and duties provided in the Memorandum of Association thereof and by law. This Certificate, bearing the registration number Seventy-nine.. which shall henceforth be used as the identifying number of the association as required by law, shall be valid for unlimited period.

Done at Addis Ababa this 9th day of
October , 1972

By .....
Solomon Tadik
Min. of State
Registrar of Association

የኢትዮጵያ ፡ ንጉሠ ፡ ነገሥት ፡ መንግሥት ፡
ባገር ፡ ግዛት ፡ ሚኒስቴር ፡
የሕገብ ፡ ወጥታ ፡ ጥበታ ፡ ዋና ፡ መሥሪያ ፡ ቤት ፡
የማኅበሮች ፡ መመዝገቢያ ፡ ጽ/ ቤት ።

ማኅበር፡ለመመዝገብ፡የሚሰጥ፡የምስክር፡ወረቀት።

የ ኢትዮጵያ ፡ የሕዝብ ጥናት ፡ ማዳበሪያ ፡ ድርጅት

እንዲመዘገብ ፡ በ መስከረም ፲፬ ፡ ቀን ፡ ፲፱፻፻፲፭ ፡ ዓ፡ ም፡ ለዚህ ፡ መሥሪያ ፡ ቤት ፡ ቀርቦ ፡ ስለነበር ፡ በፍትሐ ፡ ብሔር ፡ ሕግና ፡ ስለማኅበሮች ፡ ምዝገባ ፡ ደንብ ፡ የሕግ ፡ ክፍል ፡ ማስታወቂያ ፡ ቁጥር ፡ ፫፻፳፩/፸፱ ፡ መሠረት ፡ እንደተፈለገው ፡ መሆኑ ፡ ስለተረጋገጠ ፡ የማኅበሩ ፡ መቋቋምና ፡ ስለዚሁ ፡ ጉዳይ ፡ የቀረበው ፡ የማኅበሩ ፡ መመሥረቻ ፡ ደንብ ፡ ተገቢ ፡ የሚያደርገውን ፡ ተፈላጊዎች ፡ ሁሉ ፡ ፈጽሞ ፡ የተገኘ ፡ በመሆኑ ፡ በሕግና ፡ በመመሥረቻው ፡ ደንብ ፡ የተሰጠውን ፡ ዓላማ ፤ መብት ፤ ሥልጣንና ፡ ግዴታ ፡ እንዲኖረው ፡ ተፈቅዷል ።

ይህም ፡ ማኅበር ፡ በዛሬው ፡ ቀን ፡ ለ ኑሕተመሸኑ ፡ ዘመን ፡ ተመዝግቧል ። የመዝገቡም ፡ ተራ ፡ ቁጥር ፡ ሺ፡ ዘጠኝ ፡ ነው ። ይህ ፡ ቁጥር ፡ ለወደፊቱ ፡ በሕግ ፡ እንደተፈለገው ፡ የማኅበሩ ፡ መለያ ፡ ቁጥር ፡ ሆኖ ፡ ይሰራበታል ።

አዲስ ፡ አበባ ፡ መስከረም ፲፱ ፡ ቀን ፡ ፲፱፻፻፲፭ ፡ ዓ፡ ም፡

PATENT SPECIFICATION (11) 1277417

1277417

NO DRAWINGS

(21) Application No. 29760/68 (22) Filed 21 June 1968

(23) Complete Specification filed 18 June 1969

(45) Complete Specification published 14 June 1972

(51) International Classification A01N 9/08 9/28

(52) Index at acceptance

C2C 3A7V1A4 3A7V1E2 3A7V1H 3A7V1P 3A7V3A4 3A7V3E2 3A7V3G1 3A7V3J3 3A7V3P

A5E 1A3G 1A5B2 1C15B2 1C15B3 1C15F3 1C2J 1C2L 1C7G 1C7N

(72) Inventors KENNETH JEWERS and TERENCE ALBERT KING

(54) IMPROVEMENTS RELATING TO MOLLUSCICIDES

(71) We, NATIONAL RESEARCH DEVELOPMENT CORPORATION, a British Corporation, established by Statute of Kingsgate House, 66—74 Victoria Street, London S.W.1 do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to molluscicides.

Molluscs have been found to be responsible for the spread of many human and animal infections particularly in tropical regions, especially bilharzia and fascioliasis. These are major diseases in Africa, Asia and South America, the causative agent being certain species of nematodes of the genus *Schistosoma*. It has been found that in the life cycle of *Schistosoma*, water-snails act as a host at the laval stage in the development of the worm and the disease can be transmitted to humans who come into contact with the water in which infected water-snails have been living. A measure of control of bilharzia can be achieved by an attack on the snails which act as a vector in the transmission of the disease, and hence there is a need for materials capable of controlling the growth of the species of snail that can act as host to the disease causing organisms.

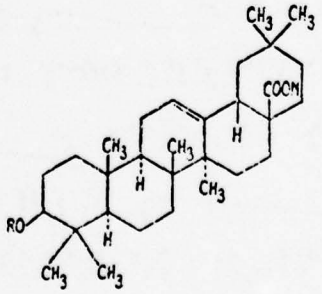
Molluscicidal agents to be used effectively in the control of the snail vectors should desirably have a high level of toxicity towards the snail species, coupled with low mamalian toxicity and high stability, since it is usually necessary to apply the molluscicidal agents to very large volumes of water, e.g. in rivers, canals, lakes, etc., to ensure that toxic levels of the molluscicide are maintained for prolonged periods of time in the regions where the snail vectors are found.

It has now been found that in the plant *Phytolacca dodecandra*, also known as Endod, there exist one or more compounds which are molluscicidally inactive in their naturally occurring form but which can be converted to the active form when extracted from the plant and treated as hereinafter described

According to the present invention a process for the production of a molluscidal agent comprises extracting *Phytolacca dodecandra* with an alcoholic solvent and subecting the material so extracted to alkaline hydrolysis.

It has been found that the berries of *Phytolacca dodecandra* represent the most valuable source of the molluscicidal material, and extraction will normally be from the berries alone which are preferably first dried and ground. The molluscicidal agents also occur in the leaves and/or other parts of the plant, and these may also be used as a source of the molluscicide. However, the cost of processing is increased by the use of these lower yielding materials and removal of too much of the plant tissue may reduce the crop of berries from subsequent harvests.

The molluscicidal agent produced by the process defined above contains a mixture of triterpenoid saponins having the formula:—



I

where R is a glycoside chain containing two to four sugar units, and M is hydrogen. Hydrolytic degradation of the side chains gives rise to 2 to 4 sugar units, predominantly 2 units of D-glucose, smaller amounts of arabinose, rhamnose and xylose also being

SPECIFICATION AMENDED - SEE ATTACHED SLIP

identified. The major product is therefore oleanolic acid 3-di-D-glucoside.

The molluscicidal agent may be used in the form of an alkali metal salt e.g. potassium or sodium, instead of the free carboxylic acid and the present invention includes the molluscicidal agent extracted from endod by out process in free carboxylic form and in alkali metal salt form. The invention also includes the alkali metal salts of formula I above where R represents a di-D-glucoside radical and M represents an alkali metal.

We have shown that when an alcoholic solvent is used in the extraction of the saponin fraction from endod, an inactive material is isolated in which the carboxy group is present as a sugar ester. Alkaline hydrolysis of this material has afforded active saponins which contain a free carboxy group. The importance of the free COOH group is also shown by the fact that the methyl ester is also inactive. Alkaline hydrolysis leaves the glycosidic side chain attached to the 3β-oxygen of oleanolic acid unaffected. Comparative tests have indicated that it is possible to recover approximately twice as much molluscicidal saponin from endod by alcoholic extraction than by aqueous extraction.

The extraction is preferably carried out on defatted berries which are then exhaustively extracted with a hot alcoholic solvent, conveniently refluxing methanol or ethanol, which may contain small amounts of water, perhaps 5 to 15% by weight. Most of the extractable saponin is extracted within about 12 hours, following which, the extracts, conveniently in the same alcoholic solvent, may be made alkaline, for example by the addition of an alkali metal hydroxide such as sodium or potassium hydroxide, to give an alcoholic alkaline solution which can be refluxed for several hours to ensure that the ester group is converted to the carboxy group. Alternatively, the alcoholic extract of the berries may be evaporated to remove alcohol and the residue subjected to alkaline hydrolysis in aqueous medium. In the latter case it is possible to dispense with the initial defatting step and to remove the fat layer when the residue of the alcohol extract is dispersed in the aqueous alkali.

The saponin may be recovered from the alcoholic alkaline solution in the free acid form by neutralisation, to about pH 6 to 7, conveniently by the addition of hydrochloric acid, either as a gas or as a methanolic solution.

The water solubility of the compounds of this invention is very important because of formulation problems. Many molluscicidal agents are only effectively molluscicidal when they are actually dissolved in the water in which the mollusc lives, and it is frequently found that apparently water soluble materials, which are formulated as concentrates in organic solvents, precipitate when added to large volumes of water, so that it is not possible to take full advantage of their molluscicidal activity. The compounds of the present invention, which are glycosides of oleanolic acid, are readily dispersed in water so that it is possible to formulate the material in aqueous solvents as well as in organic solvents, the compositions of both types are further embodiments of this invention. Such compositions may contain the usual surface active agents, together with inert diluents, e.g. water or chlorinated hydrocarbon solvents, depending on the particular manner in which the composition is intended to be used in the control of the snail vector. This control is achieved by introducing the molluscicidal agent into the water where molluscs occur and such a method of control forms still a further feature of the invention. Thus, for example, a composition containing at least 100 parts per million (p.p.m.) of active material may be supplied as a drip feed to natural waters at such a rate as to achieve a concentration of at least 5, preferably at least 10 p.p.m. therein.

The following Examples are given to illustrate the process of the invention:—

EXAMPLE 1

Samples of berries from *Phytolacca dodecandra* is dried and ground to a fine powder. The powdered berries are then defatted by extraction for 24 hours with petroleum ether and 50 g. loaded into a Soxhlet extractor and continuously extracted overnight with 500 ml. refluxing methanol. After 12 hours extraction, the methanol solution is cooled and sufficient methanolic potassium hydroxide added to give a strongly alkaline solution e.g. 5N. The alkaline solution is refluxed for 3 hours and then allowed allowed to cool. Sufficient methanolic hydrochloric acid is then added to neutralise the solution to about pH 6 to 7. The neutralised solution is filtered to remove precipitated inorganic material and then concentrated down to small bulk and added to diethyl ether. The endod saponin precipitates out and is separated, washed and dried to give about 11 g. of saponin as an amorphous yellow solid. Aqueous extraction of a similar quantity of material yields about 7.5 g. saponin. The endod saponin is identified as a compound of Formula I.

EXAMPLE 2

The procedure of Example 1 is followed up to the end of the Soxhlet extraction with methanol. The methanolic solution is then evaporated to dryness and a portion (10 g.) of the saponin is refluxed with Normal aqueous potassium hydroxide (1 litre) for 4 hours. The product is cooled and then acidified with concentrated hydrochloric acid (85 ml.). The

product is then freeze-dried or spray-dried, to give 61.8 g. of a product containing KCl. The saponin may be removed, if desired, by extraction with alcohol to give a compound of Formula I in substantially the same yield as is obtained in Example 1.

EXAMPLE 3

The *Phytolacca dodecandra* berries (50 g.) are ground and extracted with methanol or 95% ethanol for 12 hours. The extract is then evaporated to dryness and the product (13.4 g.) is dispersed in water (1 litre). A fatty layer appears which is then separated either mechanically or by extraction with a fat solvent e.g. ether. The defatted aqueous product is then refluxed with potassium hydroxide (50 g.) or equivalent sodium hydroxide for 4 hours and worked up as described in Example 1, to give 69 g. of a mixture of the saponin and sodium or potassium chloride from which the saponin is subsequently extracted.

The glycoside of oleanolic acid described above has been found to have a useful level of toxicity towards species of mollusc responsible for the transmission of human and animal disease. They are toxic towards species of *Biomphalaria* and *Bulinus*, which are known to be carriers of *Schistosoma*, and to species of *Lymneae*, which act as host to *Fasciola* organisms causing liver fluke infection in cattle and sheep. The products are also toxic to leaches.

The toxicity of the product of Example 1 to *Biomphalaria glabrata* has been determined in a test at pH 6.3 in aqueous solution. In this test, groups of 10 snails are exposed to the molluscicide at varying concentrations for 24 hours and are then transferred into ordinary water for a further 24 hour period. A mortality count is taken at the end of the second 24 hour period and an LC_{50} figure, the concentration necessary to kill 50% of the sample, is calculated. An LC_{50} value of 1 to 2 parts per million is found at pH 6.3, at pH 7.4 LC_{50} is about 5 parts per million. The LD_{50} value for this compound, when administered orally to mice, is >500 mg./kg. body weight, indicating low mammalian toxicity by this route.

One further and particularly important advantage of the compounds of the present invention as compared with molluscicides available hitherto is the fact that they are extremely stable. For example, they are stable to boiling water. Furthermore, they are especially stable on storage and may be kept in transpatent glass bottles for up to 12 months without significant loss of activity. Existing molluscicides on the other hand are very unstable compounds and require to be protected from light and they have a comparatively short shelf life.

WHAT WE CLAIM IS:—

1. Process for the production of a molluscicidal agent which comprises extracting *Phytolacca dodecandra* with an alcoholic solvent and subjecting the material extracted to alkaline hydrolysis.

2. Process according to claim 1, in which the berries of the plant are used.

3. Process according to claim 2, in which the berries are defatted prior to extraction.

4. Process according to claim 1, 2 or 3, in which the hydrolysis is conducted in alcoholic medium.

5. Process according to claim 1, 2 or 3, in which the alcoholic solvent is removed after extraction and the residue is hydrolysed in aqueous alkaline solution.

6. Process according to any one of claims 1 to 5, in which the molluscicidal agent is recovered in solid form after acidification of the hydrolysate.

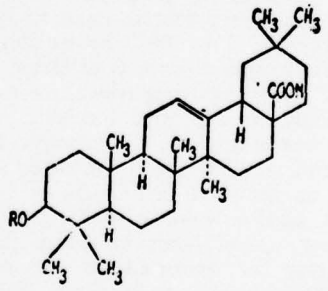
7. Process according to claim 1, substantially as described in Example 1.

8. Process according to claim 1 substantially as described in Example 2 or 3.

9. A molluscicidal agent whenever produced by a process according to any one of the preceding claims.

10. A molluscicidal agent in the form of an alkali metal salt obtained by a process according to any one of claims 1—5 or 7—8.

11. A saponin salt of the formula



where R represents a di-D-glucoside radical and M represents an alkali metal.

12. A salt according to claim 10 or 11 in which the alkali metal is potassium.

13. A salt according to claim 10 or 11 in which the alkali metal is sodium.

14. A molluscicidal composition comprising, as molluscicide, a saponin salt according to any one of claims 11—13 together with an inert carrier or diluent.

15. A molluscicidal composition comprising a molluscicidal agent according to claim 9 or 10 and an inert carrier or diluent.

16. A composition according to claim 14 or 15 in which the concentration of the molluscicidal agent is at least 100 p.p.m.

17. A composition according to any one of

claims 14—16 substantially free from tissue components of *Phytolacca dodecandra*.

18. A method for the molluscicidal treatment of water which comprises adding a molluscicidal agent according to any one of claims 9—17 to the water in an amount sufficient to produce therein a concentration of at least 5 p.p.m. of molluscicide.

J. A. KEMP & CO.,
Chartered Patent Agents,
14 South Square,
Gray's Inn, London W.C.1.

Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1972.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.

SPECIFICATION NO 1277417

By a direction given under Section 17(1) of the Patents Act 1949 this application proceeded in the name of THE SOLICITOR FOR THE AFFAIRS OF HER MAJESTY'S TREASURY, a British subject, of 3 Central Buildings, Matthew Parker Street, London, SW1.

THE PATENT OFFICE

R 14256/3

United States Patent Office

3,813,383
Patented May 28, 1974

3,813,383

METHOD OF PRODUCING A MOLLUSCICIDE FROM ENDOD

Akilu Lemma, Addis Ababa, Robert M. Parkhurst, Menlo Park, and Wilfred A. Skinner, Jr., Portola Valley, Calif., assignors to Ethiopian Science Foundation, Addis Ababa, Ethiopia

No Drawing. Filed Jan. 19, 1973, Ser. No. 325,037
Int. Cl. C07g 17/00; A01n 9/08
U.S. Cl. 260—236.5 5 Claims

ABSTRACT OF THE DISCLOSURE

A molluscicide is produced from Endod by removing the fatty constituents of Endod by extraction with a fat solvent, soaking the defatted Endod in warm water, extracting the soaked Endod with a solvent which is substantially insoluble or only slightly soluble in water and evaporating at least a portion of the solvent.

The invention herein described was made in the course of or under a contract or subcontract thereunder with the Department of the Navy.

BACKGROUND OF THE INVENTION

Molluscs have been found to be responsible for the spread of many human and animal infections particularly in tropical regions, especially bilharzia and fascioliasis. These are major diseases in Africa, Asia and South America, the causative agent being certain species of nematodes of the genus Schistosoma. It has been found that in the life cycle of Schistosoma, water-snails act as a host at the larval stage in the development of the worm and the disease can be transmitted to humans who come into contact with the water in which infected water-snails have been living. A measure of control of bilharzia can be achieved by an attack on the snails which act as a vector in the transmission of the disease, and hence there is a need for materials capable of controlling the growth of the species of snail that can act as host to the disease causing organisms.

Molluscicidal agents to be used effectively in the control of the snail vectors should desirably have a high level of toxicity towards the snail species, coupled with low mammalian toxicity and high stability, since it is usually necessary to apply the molluscicidal agents to very large volumes of water, e.g. in rivers, canals, lakes, etc., to ensure that toxic levels of the molluscicide are maintained for prolonged periods of time in the regions where the small vectors are found.

A molluscicide extracted from Endod has been used heretofore to kill the host water-snails and thus prevent the spread of these diseases, particularly Schistosomiasis. Preference may be had to British Pat. 1,277,417 published June 14, 1972 (a corresponding United States application was filed). The extract of the British patent is produced by extracting defatted Endod berries with a hot alcoholic solvent and then subjecting the alcoholic extract to alkaline hydrolysis. The alcohol specifically disclosed is methanol.

Since molluscicides are necessarily used in large bodies of water, potency generally has a marked effect on costs. The method of this invention provides a molluscicide with slightly more or equal potency than the molluscicide produced by the methanol process of British Pat. 1,277,417 without the use of continuously refluxing methanol and extended alkaline hydrolysis which are both time consuming and more costly. In addition, it is not significantly affected by variation in environmental factors such as pH, ambient temperature, ultraviolet light from the sun, and the presence of different concentrations of organic matter. The method of the invention can be used inexpensively in tropical areas where Endod is found and the need for a cheap and safe molluscicide is great.

In the method disclosed in the British patent defatted Endod berries are treated with methanol. It was found that n-butanol cannot be substituted for methanol in the process disclosed in British Pat. 1,277,417 to produce an active molluscicide. Unexpectedly, it has now been found that n-butanol can be used to produce a highly desirable molluscicide when it is used after the Endod has been defatted and soaked in water.

SUMMARY OF THE INVENTION

Defatted Endod is extracted with water. The water extract is then extracted with a slightly water soluble monoatomic aliphatic alcohol in which water is slightly soluble. The alcohol is of the formula $C_nH_{2n+1}OH$, when n is a number from 4 to 8, advantageously from 4 to 6, for example, 3-pentanol, 1-hexanol, 2-hexanol, 1-heptanol and preferably 1-butanol (n-butanol). Advantageously the mutual partial solubility of the alcohol in water and the water in the alcohol will not exceed 9 grams of solute in 100 ml. of solvent at 20° C. Advantageously, a portion or all of the alcohol is evaporated.

DETAILED DESCRIPTION OF THE INVENTION

It is well known to defat Endod berries by treating the berries with a fat solvent to remove the fatty constituents. A wide range of fat solvents may be employed, for example, petroleum ether or an alkyl oxide such as diethyl ether. The defatting is advantageously carried out on dried Endod berries which preferably have been ground to a powder.

The defatted Endod is extracted with water. The water advantageously is at a temperature in the range of from 20° C. to 100° C. It is preferred to carry out the extraction for at least 12 hours. The alcohol set forth above in the summary of the invention is then added to the water extract and remaining solids and mixed therewith. The alcohol extract is then recovered by, for example, introducing the water-alcohol-solids into a percolation column. The recovered alcohol extract can be mixed directly with water as a molluscicide or the alcohol can be evaporated to provide a solid molluscicide for subsequent mixing with the water to be treated. If desired, before adding the alcohol, the solids can be separated out of the water extract as by centrifuging and the alcohol added to the remaining aqueous liquid. A still purer product is achieved if the material remaining after evaporating the alcohol is washed with diethyl ether.

The invention will be further clarified by reference to the following examples:

EXAMPLE I

Endod berries, collected from wild plants in Ethiopia, are sun-dried for 3 days, ground to a fine powder (100–200 mesh) and used as stock material. 25 kg. of ground berries are extracted with 80 liters of petroleum ether by percolation to remove fatty materials. Evaporation *in vacuo* of the petroleum ether gives about 200 g. of a green wax, which is inactive against snails. The defatted ground berries are extracted twice with 200 liters of warm water at 70° C. each time the mixture is allowed to stand overnight. Solids are removed by centrifugation to yield 400 liters of a clear brown solution. The inactive solid residue, half the weight of the original material, is discarded. The remaining aqueous solution is then extracted twice with 200 liters of n-butanol, which yields 5 kg. upon evaporation. After washing with diethyl ether, the product is a light tan powder. When *Biomphalaria glabrata* are exposed for 24 hours to the butanol extract at 26° C. and pH of 7.4, the LC_{90} (lethal concentration to kill 90% of the snails) of the extract is 3.0 parts per million (p.p.m.);

the corresponding value for the crude material is 22.3 p.p.m.

EXAMPLE II

Ten 25-gram samples of ground Endod berries are extracted with petroleum ether to remove inert fatty materials. These 25-gram samples are mixed with water in the ratios of 25:12, 25:20, 25:25, 25:30, 25:35, 25:40, 25:45, 25:50, 25:55 and 25:60 and each is allowed to stand overnight. Each mixture is placed in a glass percolation column (3 cm. in diameter) with a glass wool plug at the bottom. One hundred ml. of n-butanol are added to the top of each column and allowed to pass through the mixture. Evaporation of the n-butanol extract gives solid residues which range from 4.38 gm. for the first and 9.78 gm. for the last. All of the solid residues recovered from the n-butanol extractions are toxic to snails, although only the material (8.31 gm.) recovered from the 25:35 mixture is as toxic as the material obtained in Example I.

Dry Endod powder is percolated with n-butanol alone (without water) giving inactive material.

It is to be understood that the foregoing examples are by way of illustration of the method of the invention and are not intended to be limiting.

What is claimed is:

1. The method of producing a molluscicide comprising: extracting defatted Endod with water, and extracting the thus formed extract with a mono functional aliphatic alcohol having a low solubility in water, said alcohol having the formula $C_nH_{2n+1}OH$ when n is a number from 4 to 8.

2. The method of claim 1 in which the alcohol is evaporated after extraction.

3. The method of claim 1 in which the water is at a temperature in the range of from 20° to 100° C.

4. The method of claim 1 in which the alcohol is n-butanol.

5. The method of claim 4 in which the water is at a temperature in the range of from 20° to 100° C.

References Cited

FOREIGN PATENTS

1,277,417 6/1972 Great Britain ..... 260—236.5

OTHER REFERENCES

C. A. 72:19,094s (1970), Powell et al.

Phytochemistry 8:2105–2107. (1969), Powell et al.

HENRY R. JILES, Primary Examiner

S. D. WINTERS, Assistant Examiner

U.S. Cl. X.R.

424—195

United States Patent [19]

Parkhurst et al.

[11] **3,886,272**

[45] **May 27, 1975**

[54] **SAPONIN-CONTAINING SPERMATOCIDAL COMPOSITIONS**

[75] Inventors: **Robert M. Parkhurst**, Redwood City; **Sidney J. Stolzenberg**, Palo Alto, both of Calif.

[73] Assignee: **Stanford Research Institute**, Menlo Park, Calif.

[22] Filed: **Apr. 5, 1974**

[21] Appl. No.: **458,164**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 384,101, July 30, 1973, abandoned.

[52] **U.S. Cl.** **424/180**; 260/210

[51] **Int. Cl.²** **A01N 9/00**

[58] **Field of Search** 260/210; 424/180

[56] **References Cited**

UNITED STATES PATENTS

3,442,911 5/1969 Baxendale 424/180

Primary Examiner—Elbert L. Roberts
Attorney, Agent, or Firm—Donovan J. De Witt

[57] **ABSTRACT**

Saponins of the type found in Phytolacca dodecandra and Calendula officinalis are found to have utility as spermatocides and as antiblastocyst and abortion agents, they manifesting such activity either when employed in extract form or in the form of compounds having the structure of those found in said extracts. They are of low mammalian toxicity and have evidenced no significant topical irritation on being introduced into the vaginal cavity of the test animals or when applied to abraded or unabraded rabbit skin. These saponins are totally efffective even at low concentration in inducing substantially instantaneous inactivation of both human and animal sperm, and upon interuterine administration they are also capable of destroying blastocysts both before as well as after implantation.

15 Claims, No Drawings

1

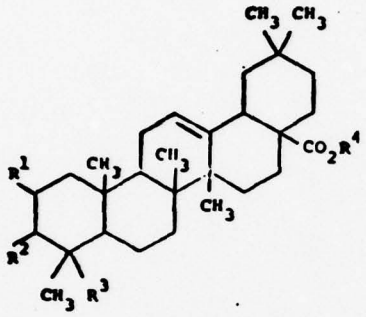
SAPONIN-CONTAINING SPERMATOCIDAL COMPOSITIONS

RELATED APPLICATION

This application is a continuation-in-part of application Ser. No. 384,101 filed July 30, 1973 and now abandoned.

SUMMARY OF INVENTION

Saponins of the type found in *Phytolacca dodecandra* (endod berries) and *Calendula officinalis* (marigold plants) have utility as spermatocides, antiblastocysts and abortion agents. These saponins are made up of compounds having the following structure:



2

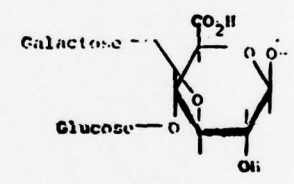
wherein R^1 is hydrogen or hydroxyl, R^2 is a saccharide unit made up of from two to five sugar moieties selected from the group consisting of glucose, xylose, arabinose, galactose and rhamnose, R^3 is methyl or hydroxymethyl and R^4 is hydrogen or a glucose sugar moiety.

The saponins found in P. dodecandra are saccharide derivatives of oleanolic acid, bayogenin, hederogenin or 2-hydroxyoleanolic acid. These latter compounds all possess the general structure shown above (I), the particular nature of the several R^1, R^3 and R^4 groups present in each of the said compounds being shown in Table I below. In turn, the saponins of C. officinalis are saccharide derivatives of oleanolic acid except that with some of these saponins the (R^4) hydrogen group of oleanolic acid is replaced by a glucose moiety such as in structures II and IV and VIII.

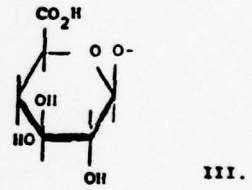
Table I

	R^1	R^3	R^4
Oleanolic acid	H	CH_3	H
Bayogenin	OH	CH_2OH	H
Hederogenin	H	CH_2OH	H
2-hydroxyoleanolic acid	OH	CH_3	H

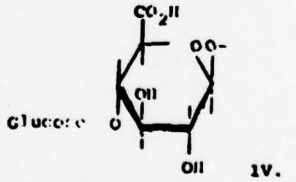
The (R^2) saccharide group present on the saponins derived from C. officinalis takes one or another of the following forms:



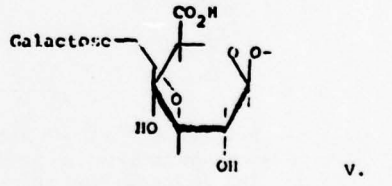
II.



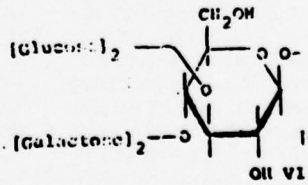
III.



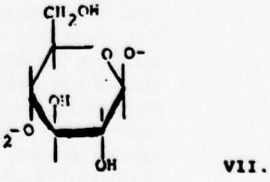
IV.



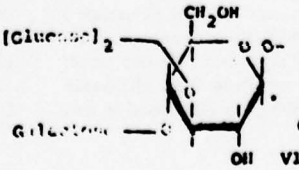
V.



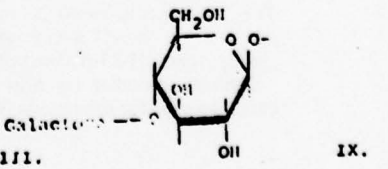
VI.



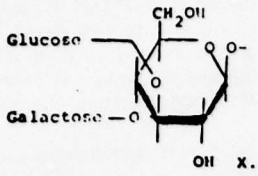
VII.



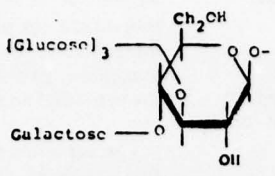
VIII.



IX.

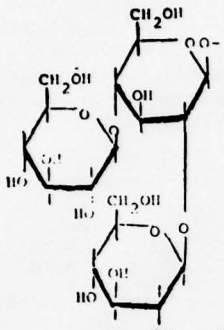


X.

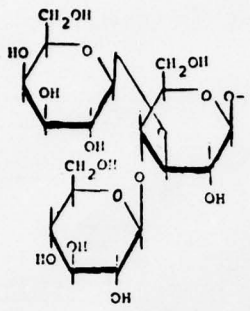


XI.

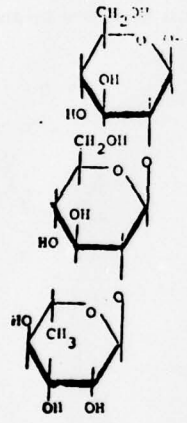
The (R^2) saccharide group present on the saponins derived from P. dodecandra takes one or another of the following forms:



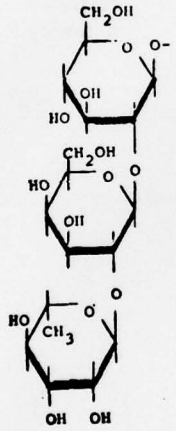
XII.



XIII.



XIV.



XV.

In the structures shown above (II – XV), the unattached valences represent hydrogen atoms.

The saccharide units illustrated by formulae XII, XIII, XIV and XV are variously associated with one or another of the compounds oleanolic acid, bayogenin, hederogenin and 2-hydroxy oleanolic acid. Thus, one fraction recovered by chromatographic separation of a butanol extract of endod berries, which is described in Example 2 below, is made up of approximately 86 percent of a saponin in which oleanolic acid is associated with the saccharide of structure XII, with 11 percent representing hederogenin associated with this same saccharide unit. The first of these compounds can be designated as 3-0-[2,4-di-0-(β-D-glucopyranosyl)-β-D-glucopyranosyl]-olean-12-ene-28-oic acid. It is also designated, hereinafter, as oleanoglycotoxin-A. This work is published in Phytochemistry 12 1437 (1973). The hederogenin-based compound can be designated as 3-0-[2,4-di-0-(β-D-glucopyranosyl)-β-D-glucopyranosyl]-23-hydroxyolean-12-ene-28-oic acid.

Similarly, another fraction resulting from the same chromatographic separation, which is described in Example 3, consists of at least about 98 percent of a saponin in which oleanolic acid is associated with the saccharide of structure XIII. This compound may be termed 3-0-[4'-0-(β-D-glucopyranosyl)-3'-0-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-olean-12-ene-28-oic acid. It is also designated as oleanoglycotoxin-B or as lemmatoxin, see Canadian J. Chem. 52 702 (1974).

Still another fraction recovered from the same chromatographic separation procedure, which is described in Example 4, is made up of a mixture of saponins in which oleanolic acid is associated with the saccharide of structure XIV, (70 percent), together with that in which oleanolic acid is associated with the saccharide of structure XV. These compounds can be termed 3-0-{2'-0-[2''-0-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranosyl} olean-12-ene-28-oic acid and 3-0-[2'-0-[2''-0-(α-L-rhamnopyranosyl-β-D-galactopyranosyl]-β-D-glucopyranosyl] olean-12-ene-28-oic acid, respectively. The mixture is also designated as oleanoglycotoxin-C or as lemmatoxin-C, see Indian J. Chem. (1974) in press.

For a discussion of the particular saponins recovered by solvent extraction from the C. officinalis, reference

is made to the articles by Zofia Kasprzyk and Zdzislaw Wojciechowski, "The Structure of Triterpenic Glycosides from the Flowers of Calendula Officinalis L.," Phytochemistry, Vol. 6, pp. 69–75 (1967), and, "The Structure of Glycosides of Oleanolic Acid Isolated from the Roots of Calendula Officinalis," Phytochemistry, Vol. 10, pp. 1121–1124 (1971) (Pergamon Press, printed in England).

Saponin- containing extracts useful in inactivating sperm or inducing abortion in man or animals can be obtained from either endod berries or or marigold plants (using one or more of the flower, stem, leaf or root portions thereof) by conventional extraction procedures. For example, dried raw material may first be ground and then treated with fat solvent such as petroleum ether or diethyl ether to remove fatty constituents. The defatted material is then extracted one or more times with warm water. Following removal of solids by centrifugation there may be obtained a clear, brown solution of the desired saponin materials. This aqueous solution may then be extracted one or more times with an appropriate alcohol such as 3-pentanol, 1-hexanol, 2-nexanol or 1-heptanol, and preferably with 1-butanol (n-butanol). Upon evaporation of the solvent and further washing with ether, the desired product is obtained as a light tan powder (in the case of endod berries) or as a somewhat darker brown powder as obtained either from marigold flowers or from whole marigold plants. A modified endod procedure of this character is described by Aklilu Lemma, Gerald Brody, Gordon W. Newell, R. M. Parkhurst and W. A. Skinner, "Studies of the Molluscicidal Properties of Endod (Phytolacca Dodecandra): I. Increased Potency with Butanol Extraction," The Journal of Parasitology, Vol. 58, No. 1, pp. 104–107, (February 1973).

DESCRIPTION OF PREFERRED EMBODIMENTS

The following examples are variously illustrative of the preparation of saponin fractions and of the manner in which the said fractions may be employed to effect the methods of the present invention:

EXAMPLE 1

Preparation of Aqueous, Freeze-dried, and n-Butanol Extracts of Endod Berries; Preparation of n-Butanol Extract of Whole Marigold Plants; Preparation of n-Butanol Extracts of Marigold Flowers.

Endod berries, collected from wild plants in Ethiopia, were sun-dried for 2 to 3 days, ground to a fine powder, and used as stock material. To obtain 5 kg of this extract, 24 kg of ground berries were extracted with 80 liters of light petroleum ether by percolation. Evaporation in vacuo of the petroleum ether gave about 200 g of a green wax. The defatted material was extracted twice with 200 liters of warm water; the mixture being allowed to stand overnight with each extraction. Solids were removed by centrifugation to yield 400 liters of a clear, brown aqueous solution of the extracted saponins. The inactive solid residue (about half of the weight of the original material) was discarded. An aliquot of this clear, brown water extract was freeze-dried in a vacuum and the resulting brown solid was used in certain of the biological tests described hereafter. The remaining aqueous solution was then extracted twice with 200 liters of n-butanol to obtain 5 kg of the saponin material upon evaporation of the alcohol. This product upon trituration with ethyl ether solidified to a tan powder representing the desired end product.

Using the same general procedures as described in the foregoing paragraph, n-butanol extracts were similarly prepared from whole, flowering, marigold plants, including the root portions thereof as well as from marigold flowers. In each case the marigold materials were thoroughly dried and then ground before being subjected to the defatting and saponin-extraction procedures. The dried saponin-containing powders obtained from these preparations assessed essentially the same characteristics as those obtained from endod berries except that while the endod spaonin powder had a light tan color that of the marigold material had a somewhat darker brown coloration.

EXAMPLE 2

Chromatographic Separation of Endod Saponin Powder

20 g of the endod saponin powder prepared in Example 1 from the butanol extract were admixed with acetic anhydride and pyridine to give a brown gummy product which was dissolved in tetrahydrofuran, passed through a short Florisil column, and evaporated in vacuo giving 19.55 g of an almost colorless, amorphous saponin acetate mixture. Isolation of acetate. As the next step, 2 g of this crude saponin acetate was chromatographed on a column (18 × 3 cm, 50 g Mallinckrodt SilicAR-CC7, 100-200 mesh) using $CHCl_3$-Et_2O gradient elution and following the progress of the fractions with TLC on SilicAR-7GF plates. One fraction, 348 mg. eluted with 5 percent Et_2O in $CHCl_3$, was further purified by repetitive chromatography on thick plates (SilicAR-7GF, $CHCl_3$-Et_2O,1:1) finally giving 41.6 mg of the acetate as a colorless glassy solid, R_f 38–46 (SilicAR-7GF, Et_2O), $[\alpha]_D^{23}$ + 17.37± 4.35° (c = 0.576, $CHCl_3$). (Found: C. 59.4; H. 7.19. Calc. for $C_{88}H_{90}O_{28}$: C. 59.89; H. 7.24 percent). MS and NMR analysis show this material to contain 86 percent of the acetate of oleanoglycotoxin-A and 11 percent of the acetate of 3-[2,4-di-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl]- 23-hydroxyolean-12-ene-28-oic acid. Deacetylation of acetate. The acetate (2.3 mg) was treated with an excess of MeOH-conc. NH_4OH (1:1) at 50° for 12 hours, then evaporated. Extraction with n-BuOH, followed by evaporation, gave the biologically active saponin as a white powder. The preparation and structure determination of the faction described in Example 2 is set forth in detail in the article by R. M. Parkhurst, David W. Thomas, W. A. Skinner, and Lewis W. Cary, entitled "Molluscicidall Saponins of Phytolacca Dodecanra: Oleanoglycotoxin-A," Phytochemistry, Vol. 12, (6) pp. 1437 to 1442 (1973) (Pergamon Press. Printed in England).

EXAMPLE 3

Chromatographic Separation Endod Saponon Powder

In the same chromatographic procedure described above in Example 2, another fraction was discovered. In this operation, 2 g of the crude saponin acetate were chromatographed on a column (18 × 3 cm, 50 g, Mallinckrodt SilicAR-CC7, 100–200 mesh) using $CHCl_3$-ether gradient elution and following the progress of the fractionation with TLC on SilicAR-7GF plates. A fraction, 1015 mg, eluted with 1 percent ether in $CHCl_3$, was further purified by repetitive chromatography on thick plates (SilicAR-7GF, $CHCl_3$:ether, 1:1), finally giving 20.9 mg of a colorless glassy solid, R_f 50-54 (SilicAR-7GF, ether), $[\alpha]_D^{23}$ = +7.38° (1.26 percent

$CHCl_3$) which, as determined by MS analysis, was found to be made up of about 98 percent or more of the acetate of 3-0-[4'-0-(β-D-glucopyranosyl)-3'-0-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-olean-12-ene-28-oic acid. 6.6 mg of the acetate was dissolved in 3 ml of methanol and 1 ml of conc. ammonium hydroxide and maintained at 50° for 24 hours. The solution was evaporated in a slow stream of nitrogen and extracted with n-butanol. Evaporation of the butanol gave the desired saponin fraction as a colorless solid.

EXAMPLE 4

Chromatographic Separation of Endod Saponin Powder

In the same chromatographic procedure described in Examples 2 and 3 above, still another fraction was discovered. In this operation, 2 g of the crude saponin acetates were chromatographed on a column (18×3 cm, 50 g, Mallinckrodt SilicAR-CC7, 100–200 mesh), using $CHCl_3$-ether gradient elution and following the progress of the fractionation via TLC on SilicAR-7GF plates. A fraction, 1015 mg, eluted with 1 percent ether in $CHCl_3$, was further purified by repetitive chromatography on thick plates (SilicAR-7GF $CHCl_3$, $CHCl_3$:ether (1:1), ether), finally giving 35.2 mg of the acetate as a colorless glassy solid, R_f = 62–68 (SilicAR-7GF, ether), $[\alpha]_D^{23}$ = +11.87° (1.87 percent $CHCl_3$). This material was identified by NMR analysis as being made up of about 70 percent of the acetate of 3-0-{2'-0-[2''-0-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-β -D-glucopyranosyl} olean-12-ene-28-oic acid and about 30 percent of the acetate of 3-0-{2'-0-[2''-0-(α-L-rhamnopyranosyl)-β-D-galactopyranosyl)-β-D-glucopyranosyl} olean-12-ene-28-oic acid. This acetate fraction was then deacetylated using the general method employed for this same purpose in Example 3 above.

The saponin fractions described in the foregoing examples were employed in connection with various sperm motility tests, as well as in other tests conducted on blastocysts. The methodology employed in these procedures was as follows:

Sperm Motility Tests

Rat epididymal sperm - Sperm were collected from a freshly decapitated 70–90 day old rat. Each epididymus was excised, cut and the sperm permitted to escape into 1 to 1.5 ml of tissue culture medium 199. Dilutions were adjusted to contain 1 to 1.5 million sperm per ml test. Test extracts and compounds were dissolved in and appropriately diluted with TC-199.

Human sperm tests - Sperm tests were performed within 1 to 4 hours after collection. Original sperm counts ranged from 42 to 176 million per cc. Dilutions of 1 cc of semen ranged from 9 to 15 ml in a modified Ringers solution. Test extracts and compounds were appropriately diluted with the same solution.

Incubation procedures - In tests with either rat or human sperm, 0.1 ml of each solution of the saponin under test was placed in a test tube measuring 10×75 mm. To each test tube, while undergoing shaking in Dubnoff shaker, 0.9 ml of diluted sperm solution was added. At appropriate intervals small amounts of sperm samples in each test tube were removed. Placed between a slide and cover slip for microscopic examination. Percent motility and in most cases the speed and vigor of motility (ranging from 1+ to 4+) were recorded.

The following examples present data obtained in carrying out one or another of the foregoing sperm motility tests, using given saponin portions prepared in accordance with Examples 1-4, as indicated:

EXAMPLE 5

The following data were obtained in conducting rat epididymal sperm tests using both a saponin-free control as well as others in which the n-butanol extract of endod berries, as described in Example 1, was employed in varying concentrations:

Incubation Time	Control		5 μg/ml		10 μg/ml		20 μg/ml		50 μg/ml	
Min.	%	Grade	%	Grade	%	Grade	%	Grade	%	Grade
<3	60	4+	60	4+	60	4+	10	4+	0	—
15	50	3+	40	3+	30	2+	0	—	0	—
30	60	4+	25	2+	5	1+	0	—	0	—
60	50	3+	30	2+	10	1+	0	—	0	—
100	40	1+	40	2+	20	1+	0	—	0	—

EXAMPLE 6

The procedure of Example 5 is repeated except that the saponin fractions employed were those prepared as recited in Example 4.

EXAMPLE 7

The following data are those obtained on conducting human sperm tests using a control as well as various concentrations of the saponin fraction obtained by the n-butanol extraction of endod berries as recited in Example 1:

Incubation Time	Control		10 μg/ml		20 μg/ml		40 μg/ml	
Min.	%	Grade	%	Grade	%	Grade	%	Grade
<3	70	4+	70	4+	10	4+	0	—
15	80	4+	40	4+	10	4+	0	—
30	70	4+	30	3+	10	3+	0	—
60	70	3+	30	3+	20	1+	0	—
90	50	3+	30	2+	20	1+	0	—
120	50	2+	5	2+	10	1+	0	—

EXAMPLE 8

The operation of Example 7 was repeated, but using the saponin fraction prepared in Example 3.

Incubation Time	Control		5 μg/ml		20 μg/ml		80 μg/ml	
Min.	%	Grade	%	Grade	%	Grade	%	Grade
<3	70	4+	70	4+	40	4+	3	1+
15	70	4+	60	4+	40	4+	0	—
30	70	4+	40	4+	30	4+	0	—
60	60	4+	40	4+	30	4+	0	—
90	50	4+	40	4+	30	4+	0	—
120	50	4+	30	4+	30	4+	0	—

EXAMPLE 9

The operation of Example 7 was repeated, but using the freeze-dried, water extract of endod berries as described in Example 1.

Incubation Time	Control		50 μg/ml		200 μg/ml		800 μg/ml	
Min.	%	Grade	%	Grade	%	Grade	%	Grade
<3	70	4+	80	4+	2	3+	0	—
15	70	4+	70	4+	0	—	0	—
30	70	4+	60	4+	0	—	0	—
60	70	4+	60	4+	0	—	0	—
90	60	4+	60	4+	0	—	0	—
120	60	4+	60	4+	0	—	0	—

EXAMPLE 10

The operation of Example 7 was repeated, but using n-butanol extract of whole marigold flowers as described in Example 1.

Incubation Time	Control		20 μg/ml		100 μg/ml		500 μg/ml	
Min.	%	Grade	%	Grade	%	Grade	%	Grade
<3	60	4+	60	4+	30	2+	0	—
15	60	4+	60	4+	5	3+	0	—
30	60	4+	50	4+	0	—	0	—
60	50	4+	50	4+	0	—	0	—
90	60	4+	50	4+	0	—	0	—
120	50	4+	50	4+	0	—	0	—

EXAMPLE 11

The operation of Example 7 was repeated, but using n-butanol extract of whole marigold plants as described in Example 1.

Incubation Time	Control		20 μg/ml		100 μg/ml		500 μg/ml	
Min.	%	Grade	%	Grade	%	Grade	%	Grade
<3	60	4+	70	4+	70	4+	0	—
15	60	4+	70	4+	60	4+	0	—
30	60	4+	70	4+	50	4+	0	—
60	50	4+	60	4+	60	4+	0	—
90	60	4+	70	4+	60	4+	0	—
120	50	4+	70	4+	60	4+	0	—

EXAMPLE 12

The operation of Example 7 was repeated except using the saponin fraction prepared in Example 2.

Incubation Time	Control		40 μg/ml		80 μg/ml		100 μg/ml		400 μg/ml	
Min.	%	Grade	%	Grade	%	Grade	%	Grade	%	Grade
<3	50	4+	50	4+	60	4+	50	4+	0	—
15	60	4+	50	4+	40	4+	50	4+	0	—
30	60	4+	50	4+	40	4+	30	4+	0	—
60	60	4+	50	4+	50	4+	40	4+	0	—
90	60	4+	60	4+	50		30	4+	0	—
120	50	4+	50	4+	20	4+	25	4+	0	—

EXAMPLE 13

Termination of Pregnancy Before Blastocyst Implantation

Nine- to ten-week-old nonparous female rats were coupled with males whose fertility had been proven by a commercial breeder. The following day was designated Day 1 of pregnancy if vaginal sperm or plugs were observed. Bilateral ovariectomies were performed on most rats on Day 3 and on a few on Day 4 of pregnancy.

Pregnancy was maintained, but implantation was prevented by subcutaneous injections of 2 mg of progesterone per day for 2 or 3 days. On the day following the last injection, the uterus was exteriorized by a midventral incision, and either the *Phytolacca dodecandra* butanol extract of Example 1 or the oleanoglycotoxin-B was injected into each uterine horn by means of a 1-ml tuberculin syringe and a 27-gauge needle. Following intrauterine injection, the animals received 1 μg of estrone and 2 or 4 mg of progesterone in 0.1 or 0.2 ml of sesame oil for 3 or 4 days to induct implantation and maintain pregnancy. On the day following the last injection, the rats were sacrificed, and viable fetuses or implantation sites were counted and recorded.

EFFECT OF (A) THE MIXTURE OF SAPONINS IN THE BUTANOL EXTRACT OF *P. DODECANDRA* AND (B) OF OLEANOGLYCOTOXIN-B, ON PREGNANCY BEFORE BLASTOCYST IMPLANTATION

Group	Intrauterine Injection mg/Horn	Substance*	No. Pregnant/ No. Treated	Average No. Fetuses in Pregnant Animal
1	5	Butanol extract	0/2	—
2	2	Butanol extract	0/6	—
3	1	Butanol extract	2/3**	6.5
4	1	Oleanoglycotoxin-B	1/3**	4.0
5	—	Control (saline)	8/8	9.9

* Preparations used in Groups 1 to 4 were dissolved in saline, and 0.05 to 0.1 ml solutions were injected in utero.

**In each of these groups, there was one animal with fetuses in only one uterine horn. It has been reported that with intrauterine contraceptive devices (IUD), it is possible to prevent pregnancy in rats in only the horn that contains the IUD (Parr, Fertil. Steril. 17, 797, 1966)

The data of the foregoing table show that injections of the crude endod saponin extract or that of oleanoglycotoxin-B are highly effective in terminating pregnancy prior to the occurrence of implantation of the blastocysts in the rat.

EXAMPLE 14

Termination of Pregnancy After Blastocyst Implantation

The animals were prepared in the same manner as described above, with one difference. The 2-mg subcutaneous injections of progesterone administered for 3 days were followed by injections of 1 μg of estrone and 2 mg of progesterone for 2 days to induce implantation before the intrauterine injections of test material. The estrone/progesterone injections were continued for an additional 3 days, followed by autopsy on Day 12. The following results are obtained:

EFFECT OF A BUTANOL EXTRACT OF *P. DODECANDRA* ON PREGNANCY AFTER BLASTOCYST IMPLANTATION

Group	Intrauterine Injection mg/Horn	Substance	No. Pregnant/ No. Treated	Average No. Fetuses in Pregnant Animal
1	5	Butanol extract	1/5	13
2	2	Butanol extract	1/3	9
3	—	Control (saline)	6/6	9.8

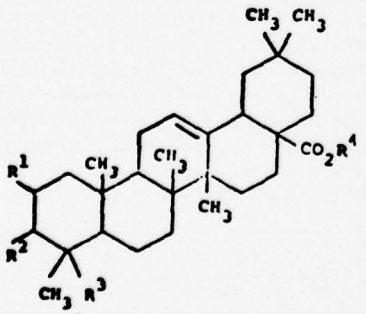
Group	No. with Implantation Sites*/No. Treated	Average No. Implantation Sites in Aborted Animals
1	4/5	8.0
2	2/3	7.7
3	0/6	—

*Implantation sites represent the points of blastocyst attachment to the uterine endometrium of animals in which pregnancy was terminated.

The data of the foregoing table show that the crude saponin extract of endod berries are capable of terminating pregnancy in the rat after implantation of the blastocysts has occured.

We claim:

1. As a spermatocide adapted to be introduced into the vagina prior to intercourse and thus prevent the occurrence of pregnancy, a composition comprising at least one spermatocidally active saponin material having the structure



wherein R^1 is hydrogen or hydroxyl, R^2 is a saccharide unit made up of from two to five sugar moieties selected from the group consisting of glucose, xylose, arabinose, galactose and rhamnose, R^3 is methyl or hydroxymethyl and R^4 is hydrogen or a glucose sugar moiety.

2. The composition of claim 1 wherein the saponin material represents an extract obtained from endod berries.

3. The composition of claim 1 wherein the saponin material represents an extract obtained from marigold plants.

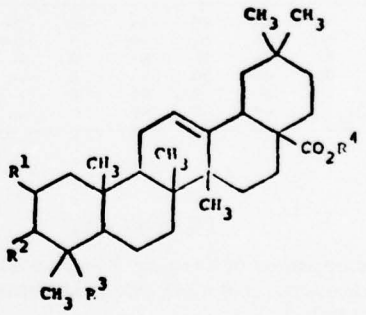
4. The composition of claim 1 wherein the saponin material represents oleanoglycotoxin-A.

5. The composition of claim 1 wherein the saponin material represents 3-0-{4′-0-(β-D-glucopyranosyl)-3′-0-(β-D-galactopyranosyl)-β-D-glucopyranosyl}-olean-12-ene-28-oic acid.

6. The composition of claim 1 wherein the saponin material represents 3-0-{2′-0-{2″-0-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl}-β-D-glucopyranosyl} olean-12-ene-28-oic acid.

7. The composition of claim 1 wherein the saponin material represents 3-0-{2′-0-{2″-0-(α-L-rhamnopyranosyl)-β-D-galactopyranosyl}-β-D-glucopyranosyl} olean-12-ene-28-oic acid.

8. The method of preventing the occurrence of pregnancy in a mammal which comprises introducing into the vagina, prior to intercourse, a spermatocidally effective dosage of a composition comprising at least one spermatocidally active saponin material having the structure



wherein R^1 is hydrogen or hydroxyl, R^2 is a saccharide unit made up of from two to five sugar moieties selected from the group consisting of glucose, xylose, arabinose, galactose and rhamnose, R^3 is methyl or hydroxymethyl and R^4 is hydrogen or a glucose sugar moiety.

9. The method of claim 8 wherein the saponin material has the structure of that extractable from endod berries.

10. The method of claim 8 wherein the saponin material has the structure of that extractable from marigold plants.

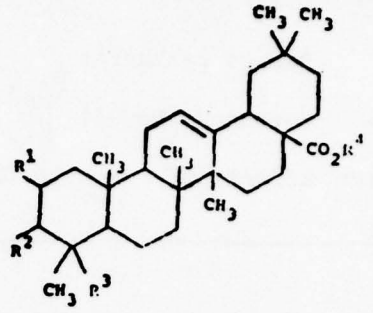
11. The method of claim 8 wherein the saponin material is oleanoglycotoxin-A.

12. The method of claim 8 wherein the saponin material is 3-0-[4'-0-(β-D-glucopyranosyl)-3'-0-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-olean-12-ene-28-oic acid.

13. The method of claim 8 wherein the saponin material is 3-0-{2'-0-[2''-0-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranosyl} olean-12-ene-28-oic acid.

14. The method of claim 8 wherein the saponin material is 3-0-{2'-0-[2''0-0-(α-L-rhamnopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranosyl} olean-12-ene-28-oic acid.

15. The method of terminating pregnancy in a mammal following conception either before or after blastocyst implantation, which comprises introducing into the uterus of said mammal an amount effective to terminate pregnancy of a composition comprising at least one saponin material having the structure



wherein R^1 is hydrogen or hydroxyl, R^2 is a saccharide unit made up of from two to five sugar moieties selected from the group consisting of glucose, xylose, arabinose, galactose and rhamnose, R^3 is methyl or hydroxymethyl and R^4 is hydrogen or a glucose sugar moiety.

* * * * *

MEMO

TO: Robert Parkhurst

DATE: 7 March 1978

FROM: Gordon W. Newell

LOCATION: 253

SUBJECT: Effects of Endod on Cholesterol Blood Levels

CC: W. Skinner
M. Tanabe

During an AAALAC site visit at the National Oregon Primate Research Center last year I discussed with Dr. Malinow studies he was doing with various products that might affect blood cholesterol levels. At that time the product of primary interest was a saponin obtained from alfalfa.

I told him about your work with Endod and subsequently you sent samples of various Endod preparations to him.

Today he phoned and stated that Endod sample No. 11140-37 was very effective in inhibiting cholesterol absorption from the gut. In fact, it was as effective as any material he has studied. I understand the treatment regimen used was a single administration of the product, at a level of 20 mg/kg to the rat.

He would like to extend these studies to the primate (possibly the squirrel monkey) and if the results can be confirmed in the nonhuman primate, an extended study with repeated administrations would be considered.

We should call him (503/645-1141) either Monday or Tuesday morning, or on Friday. He will be away from the Center at other times this week.

It looks like there may be an exciting and new application for Endod(s).

Cable Address: RECON
ST. LUCIA

Castries Tele. 2501

RESEARCH & CONTROL DEPARTMENT
P O BOX 93
CASTRIES, St. Lucia
WEST INDIES.

Staff/4/125

4th August 19 72.

Dr. A. Lemma,
Institute of Pathobiology,
Haile Selassie University,
Addes Abeba, Ethiopia,
Africa.

Dear Dr. Lemma,

At long last we have the data on the effect of endod on our local snail Biomphalaria glabrata, which is enclosed.

Are you planning to summarise these various tests in a single paper? If not, we might try to get our results published as a research note somewhere.

I am sorry to have taken so long to get this work done.

Best wishes.

Yours sincerely,

Robert F. Sturrock, Ph.D.

RFS:mm

The Efficacy of Endod Against St. Lucian B. glabrata

E.S. Upatham, Research and Control Department
Castries, St. Lucia, West Indies

Experiments were conducted according to the standard WHO procedures. Solutions of endod were made up 16-18 hours prior to use. Groups of 40 laboratory-bred B. glabrata were exposed to the solutions for 6 and 24 hours, and were allowed to recover for 48 hours. For each exposure period, 2 sizes of snails were used: 5-6 mm and 9-10 mm.

Results are shown in the table, and are based on probit analysis.

LC_{50} and LC_{90} concentrations of endod at different exposures

	Snail size			
	5-6 mm		9-10 mm	
Exposure time	LC_{50}	LC_{90}	LC_{50}	LC_{90}
6 hours	9.6	11.9	9.7	11.4
24 hours	7.0	9.3	6.3	8.7

TELEPHONE:
SITTINGBOURNE 4444.
TELEX: 96181

WOODSTOCK AGRICULTURAL RESEARCH CENTRE,
SITTINGBOURNE,
KENT.

2 February 1972

Dr Aklilu Lemma,
Life Sciences Division,
Stanford Research Institute,
Menlo Park,
California,
94025 - U.S.A.

Dear Dr Aklilu,

Thank you very much for your letter. We are pleased to hear that the samples of FRESCON for your studies arrived safely.

Mr Crossland is currently away from Woodstock and has asked me to reply to you since I am working with him on many aspects of our molluscicide project. He sends his kindest regards to you and looks forward to seeing you in Sittingbourne whilst on your way to Ethiopia in March.

In answer to your findings concerning the effect of U.V. light on FRESCON, we would agree that being a highly aromatic compound it will absorb short wavelength U.V. light strongly. It is very probable, therefore, that the accompanying excitation of the molecule will lead to rapid decomposition. However, as the appended data shows, FRESCON does not exhibit any appreciable absorption of long wavelength U.V. light (> 290nm) such as found in normal sunlight at ground level. Indeed, there is no evidence to suggest that FRESCON is unstable to sunlight under field conditions.

Before we can comment fully on your results, we would like to have details of the type of lamps used and the wavelengths emitted by them. Standard commercial U.V. lamps emit radiation at wavelengths below the limit found in natural sunlight at ground level and are therefore not very useful for measuring the stability of compounds under natural conditions. Specialised U.V. lamps are available from which the shortwave lengths are cut out and some of these can give more reliable results.

I am pleased to inform you that we have been able to carry out a limited number of bioassays with the butanol extract of Endod you kindly sent to us. We carried out the tests using B. glabrata in accordance with your suggested procedure, and I hope

.../....

that the data will be of use to you. We did experience some difficulty in the first 24 hour test exposure due to an 'overkill' at the higher dose levels; additional dose levels were therefore tested.

This form of Endod is indeed far more active than the previous formulation tested by us, and we should be most interested to learn of your success with the preparation of an EC formulation.

I hope that the above information will be of use to you, and we look foward to seeing you again in the near future.

Yours sincerely,

A P Warley

A.P. Warley
Biological Evaluation Division

SWD/13/APW/24.7/78/R

1. Light absorption by FRESCON in Methanol solution

Wavelength (nm)	Molar Extinction Coefficient (1000 cm^2 / mole)
300	6
280	79
270	547
265	1150

2. Bioassay with Endod against Biomphalaria glabrata

Number of snails dead out of 10 per concentration level.

Concentration ppm.	6 hour exposure		24 hour exposure	
	Test 1	Test 2	Test 1	Test 2
25	10	9	10	-
12.5	4	-	10	-
10.0	-	3	-	-
6.25	2	-	10	-
5.0	-	0	-	9
4.0	-	-	-	4
3.125	0	-	1	-
3.0	-	-	-	0
	LD_{50} 11.5 ppm LD_{90} 23 ppm (plotted on log probit paper)		LD_{50} 3.9 ppm LD_{90} 4.8 ppm (plotted on log probit paper)	

LABORATORY EVALUATION OF ENDOD-EXTRACT AS MOLLUSCICIDE

E. Paulini (Ph.D.), Universidade Federal de Minas Gerais, Bela Horizonte, Brazil

Butyl-alcohol extract of endod was sent to us by Dr. A. Lemma, of the Stanford Research Institute, California, in the form of a white powder, which is completely soluble in water.

The water solution was tested with mature and with newly hatched snails. No tests were made with eggs, because of the resistance of the latter form to endod.

Exposure times of 24 hours and 6 hours were employed, at room temperature (25±1°C). Mortality count was made after 24 and 48 hours.

Results of the biological tests are shown in tables 1-4.

When the LC50 and LC90 values are compared, it is striking that newly hatched snails are about four to five times more resistant to endod than mature snails.

Another interesting fact emerging from the results is that the lethal dose (concentration x exposure time) increases with increasing exposure time and is not constant, in contrast with some molluscicides. This is well demonstrated in the experiment with newly hatched snails, where the LC values were identical both with 6 hours and 24 hours of exposure. The interpretation of this results is that within 6 hours the lethal quantity has been absorbed by the snail, and apparently no further absorption of molluscicide accurred.

It was also observed that at the end of the exposure period (24 hours) (first day) the mortality count was practically identical to the count made after 24 hours of recovery period (day two). From these results it is inferred that the toxic action is rather rapid.

Table 1. Biological tests with mature B. glabrata-24 hours of exposure

Product	Conc.	Diameter	No. of snails	No. of tests	% mortality after days			
					1		2	
					%	No. dead	%	No. dead
Endod	0.0	15-20	10	1	0	0	10.0	1
	1.0	15-20	20	2	10.0	2	20.0	4
	1.5	15-20	20	2	10.0	2	45.0	0
	2.0	15-20	20	2	85.0	17	100.0	20
	2.5	13-19	10	1	70.0	7	90.0	9
	3.0	16-19	10	1	100.0	10	100.0	10
	4.0	15-20	20	2	100.0	20	100.0	20
PCF	0.2	13-20	30	4	66.5	20	97.0	29
	0.3	16-19	10	1	80.0	8	100.0	10
	0.4	16-19	15	2	73.5	11	100.0	15
Control	-	13-20	30	-	3.3	1	3.3	1

Water temperature: 26±1°C

	LC_{50}	LC_{90}
Endod	1.40 ppm	2.30 ppm
PCF	not computable	

Table 2. Effect of Endod on B. glabrata, 6 hours of exposure

Product	Conc. ppm	Diameter (mm)	No. of snails	No. of tests	Percent mortality after days 1		2	
					Percent	No. dead	Percent	No. dead
Endod	1.5	13-19	20	2	5.0	1	5.0	1
	2.0	13-19	20	2	5.0	1	5.0	1
	2.5	14-17	10	2	30.0	3	60.0	6
	3.0	16-19	10	1	50.0	5	80.0	8
	4.0	13-19	20	2	85.0	17	95.0	19
	5.0	14-17	5	1	100.0	5	100.0	5
	6.0	16-19	10	1	40.0	4	40.0	10
PCF	0.6	13-19	20	2	5.0	1	5.0	1
	0.8	13-19	5	1	20.0	1	20.0	1
	1.0	13-19	30	3	23.4	7	26.5	8
	2.0	13-19	20	2	10.0	1	10.0	1
Control	-	13-19	40	-	2.5	1	2.5	1

Water temperature $26 \pm 1°C$

	LC_{50}	LC_{90}
Endod	1.40 ppm	2.30 ppm
PCF	not computable	

Table 3. Biological tests with newly hatched B. glabrata - 24 hours of exposure

Product	Conc. ppm	Age (days)	Number of snails	Number of tests	% of mortality after days 1		2	
					%	No. dead	%	No. dead
	5.0	1-3	90	3	2.2	2	3.3	3
	6.0	1-3	90	3	17.8	16	17.8	16
Endod	8.0	1-3	90	3	69.0	62	71.0	64
	9.0	1-3	30	1	90.0	27	90.0	27
	10.0	1-3	30	1	80.0	24	80.0	24
	0.1	1-3	90	3	4.4	4	5.5	5
PCF	0.2	1-3	90	3	36.6	33	40.0	36
	0.4	1-3	60	2	62.0	56	62.0	56
Control	-	1-3	90	-	-	-	10.0	9

Endod LC_{50} 7.80 ppm LC_{90} 10.0 ppm Temperature: 25 $\pm$ 1°C

PCF 0.27 ppm

Table 4. Biological tests with newly hatched B. glabrata - 6 hours of exposure

Product	Conc. ppm	Age (days)	Number of snails	Number of tests	% of mortality after days 1		2	
					%	No. dead	%	No. dead
	4.0	1-3	30	1	6.6	2	6.6	2
	8.0	1-3	90	3	44.5	40	44.5	40
Endod	10.0	1-3	90	3	92.0	83	92.0	83
	12.0	1-3	90	3	99.0	89	100.0	90
	15.0	1-3	60	2	100.0	60	100.0	60
	0.4	1-3	120	4	37.5	45	39.0	47
PCF	0.6	1-3	90	3	63.0	57	65.4	59
	0.8	1-3	120	4	74.0	89	76.0	91
Control	-	1-3	120	-	-	-	12.4	15

Endod LC_{50} 7.80 ppm LC_{90} 10.0 ppm Temperature: 25 $\pm$ 1°C

PCF 0.45 ppm

A PRELIMINARY REPORT ON THE MOLLUSCICIDAL PROPERTY OF ENDOD (*PHYTOLACCA DODECANDRA*)

AKLILU LEMMA

During a study of the distribution and ecology of bilharzia transmitting snails in the River Assam in the town of Adwa (Aklilu Lemma, 1965), it was noticed that in areas next to where people washed clothes there were many dead snails and small fish floating in the water. Some distance down-stream or immediately up-stream from such washing places, abundant live snails were present. This lead to the idea that the substance which the people used for laundering might have some snail killing property. A further investigation showed that most people in that area use "Endod" instead of soap. In an attempt to confirm whether this substance kills snails or not, some live snails were collected in a beaker and a handful of a solution of Endod was added to it.. After a few minutes all the snails were found dead. Since then, we have been interested in studying Endod and its molluscicidal property in more detail.

Endod is a native herb of Ethiopia and it is found in every part of the country growing wild. Lemordant (1960) explains that this plant is referred to by three different botanical names: *Phytolacca abyssinica* Hochst, *Pircunia abyssinica* Moq.-Tand, and *Phytolacca dodecandra* L'Herit var *latifolia*. However, Mooney (1963) and other plant taxonomists believe that *Phytolacca dodecandra* should be its correct name.

Endod has small brown berry-like fruits which when dried, powdered and put in solution give off foam and can be used for washing clothes. In Ethiopia it is extensively used instead of soap. People in the rural areas prefer it for three reasons: (i) It is cheap in the markets and they can collect any amount of it themselves without much difficulty; (ii) Clothes (particularly linen) washed with it are thought to be much whiter than those washed with soap; and (iii) After clothes are washed with Endod no lice, ticks, or mites remain on them. In addition to its use as soap, Endod is thrown into bodies of water to kill leeches which cause much damage to domestic animals. A description of Endod and its wide use in Ethiopia for laundering was given by Ferret and Galinier as early as 1847. Mérab (1912) noted that the fruit of this plant is used for the termination of pregnancy and is a very strong purgative. Baldrati (1946) reported that it is taken by mouth for the treatment of ascaris and externally used for killing lice.

We are at present engaged in making quantitative controlled laboratory studies on the molluscicidal properties of Endod. The effects of various environmental factors such as temperature, sunlight, soil, organic matter and pH of the water, on the molluscicidal potency of the active ingredients are now being studied. The present paper is a preliminary report on some of our findings.

Tap-water extract of the dried and powdered fruit of Endod has a potency of killing snails (*Biomphalaria*, *Lymnea*, and *Bulinus* spp.) at a concentration of

Reprinted from the Ethiopian Medical Journal, 3, 187-190, by permission.

10 p.p.m. (parts per million) within 24 hours at room temperature (about 23°C). Comparisons of the molluscicidal potency of the green leaves, flower and buds, ripe dried fruits and the black seeds inside the fruit were made. The ripe dried fruit extract killed at 10 p.p.m., the dried flower and buds killed at 10 p.p.m., the dried green leaves killed at 25 p.p.m., and the black seeds killed at 150 p.p.m., all within 24 hours exposure at room temperature.

It is interesting to note that copper sulphate and sodium penthachlorophinate (the two most widely used molluscicides in the world) also act at 10 p.p.m. (Gönnert & Strufe, 1962). Since the crude extract of Endod also kills at 10 p.p.m., the purified active ingredient in it might kill at a considerably higher dilution and thus be more potent than either one of these molluscicides. Further, it has been found that the moluscicidal potency of Endod is not affected by the presence of vegetables (lettuce) and different concentrations of soil ranging from 1000 p.p.m. to 50 p.p.m.. This is particularly interesting in view of the fact that the molluscicidal potency of copper sulphate is greatly reduced in the presence of such material (Gönnert & Strufe loc. cit.). Another interesting property of Endod, compared with the other moluscicide, sodium pentachlorophinate, which repels snails, is that snails feed on the green leaves of the plant until they die. At death the snail's body shrink back into the shell and invariably some blood-like substance can be seen released inside the shell.

The effect of heat on the active ingredient in Endod was tested by boiling the solution for five minutes, and it was found that this did not have any effect. Studies on the stability of the molluscicidal power of Endod in solution have revealed that it remains fully active for a period of at least seven days after preparation but then it decreases with time. Similarly, though in terms of months, it appears that the longer the dried fruits are kept in storage the less effect the extract has in killing snails. We have noticed that different lots of Endod fruits bought from the market have different moluscicidal potencies. Endod did not kill any mosquito larva at a concentration of 2000 p.p.m. but it killed all small fish (about an inch long) within 24 hours. Domestic animals have been seen feeding on the leaves of this plant without any apparent harm.

Studies on the isolation and identification of the active moluscicidal ingredient in Endod are presently under way. According to Mozley (1952) two of the known agents which are active molluscicides of vegetable origin are saponin (present in *Balanites aegyptiaca*) and rotenone (present in *Derris elliptica*). Mozley studied the effect of "saponin" as obtained in pure form from chemical manufacturers and reported as follows: Concentrations of saponin in water of 0.0025 percent (25 p.p.m.) had no apparent effect; 0.015 percent (150 p.p.m.) caused the death of the snails within 24 hours; concentrations of strengths between those two killed some snails and caused others to become torpid. In our studies with Endod only 0.001 percent (10 p.p.m.) of the crude extract killed all snails within 24 hours, and therefore it is most probable that the active ingredient in Endod is not saponin. Also, since up to 2000 p.p.m. of Endod in tap water did not kill any mosquito larva, it appears that the active ingredient is not rotenone because this would have killed the larvae (Mozley, loc. cit.). Comparison of the molluscicidal property of Endod with that of soap, since both are surface tension reducing agents, showed that the *mlc* 100 (minimal lethal concentration to kill 100 percent of the snails) of soap was 1000 p.p.m. whereas that of Endod was only 10 p.p.m.

The toxicity of Endod to mammalian tissue, its potency in killing leeches, and the quantitation of the doses needed to kill different sizes of fish are presently under study. Experiments in the field will also be done very shortly.

Summary.

The author observed dead snails and small fish in the River Assam. These were found near places where people washed their clothes with Endod, the dried fruits of *Phytolacca dodecandra*, instead of using soap.

Preliminary studies have shown that a tap-water extract of Endod is capable of killing bilharzia transmitting snails at a concentration of 10 p.p.m. within 24 hours at room temperature.

Further studies are being undertaken to find out Endod's molluscicidal ingredient, toxicity to mammalian tissue, potency in killing leeches and the doses needed to kill different sizes of fishes.

Resumé.

Dans la rivière Assam l'auteur a trouvé des escargots et des petits poissons morts dans des endoits où les habitants qui venaient faire leur lessive employaient le ENDOD, (fruits sechés de *Phytolacca dodecandra*), au lieu de savon.

Des études préliminaires ont montrés qu'un extrait composé d'eau du robinet et d'ENDOD est capable de tuer en 24 heures les escargots qui transmettent la bilharziose á une concentration de 10 pour 1000 à temperature normale.

Des études ont été entreprises pour mettre en évidence le composant capable de tuer les mollusques, pour déterminer la toxicité vis-à-vis des tissus des mammiféres et les doses nécessaires pour tuer les poissons de différentes grandeurs.

REFERENCES

1. Aklilu Lemma, (1965), Schistosomiasis in Adwa: A report on an ecological study. Eth. Med. J. vol. III No. 2 pp. 84-92.
2. Lemordant, D. (1960), Les Plantes Ethiopiennes, pp. 23-24, Central Printing Press, Addis Ababa.
3. Mooney, H.F. (1963), A Glossary of Ethiopian Plant Names, Dublin University Press Ltd.
4. Ferret et Galinier (1847), Voyage en Abyssinie, Paris. Paulin éd. (Quoted by Lemordant).
5. Mérab, (1912), Médicine et Médicins en Ethiopie, Paris 1912 Vigot Frères éd. (Quoted by Lemordant).
6. Baltradi, I.(1949). Piane officinale dell'Africa orientale. Firence.
7. Gönnert, R. and Strufe, R. (1962). Comparitive investigations of some molluscicides. Ciba Fdt. Symposium,pp. 326-338.
8. Mozley, A. (1952). Molluscicides. pp.29-31. London. Lewis + Co.

Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1970, 42, 597-612

Laboratory and Field Evaluation of the Molluscicidal Properties of *Phytolacca dodecandra*

AKLILU LEMMA, Sc.D.[1]

Dried berries of endod (Phytolacca dodecandra) *(known also as soapberry) are widely used in Ethiopia instead of soap for laundering clothes. It was observed that in natural bodies of water where* endod *had been used there was a high mortality of snails. Subsequently, the molluscicidal potencies of various parts of male and female* endod *plants were determined and the berries were found to be the most potent. The potency of* endod *remained stable over a wide range of temperatures and pH values, in the presence of various concentrations of river-bed mud and after ultraviolet irradiation of solutions. The toxicity of* endod *to mammals and plants has been shown to be very low. Its toxicity to snail eggs also is low but it has been shown that this difficulty can be overcome in the field by repeated treatments.* Endod *kills leeches and schistosome cercariae and miracidia at very low concentrations. Comparative tests with* endod *and several standard molluscicides have given encouraging results.*

Being a natural product, endod *could become a cheap and effective means of controlling schistosomiasis in certain areas since, under suitable climatic conditions, the plant grows rapidly and bears fruit twice a year.*

The molluscicidal properties of *Phytolacca dodecandra* were first observed during a study of the distribution and ecology of schistosomiasis-transmitting snails in the town of Adwa in the northern part of Ethiopia about 800 km from Addis Ababa (Lemma, 1965a). It was noticed that in places along rivers or streams where people washed clothes, there were comparatively more dead snails than there were in adjacent areas, whether upstream or downstream from these washing places. Subsequently, it was shown that a preparation of *P. dodecandra*, widely used in Ethiopia instead of soap for laundering clothes, possesses molluscicidal properties.

P. dodecandra (L'Herit) (synonyms: *P. abyssinica* Hoffm., *Pircunia abyssinica* Moq.), a member of the Phytolaccaceae, is known in Ethiopia as *endod* and elsewhere may be referred to as soapberry. The distribution of this plant is East, West, Central and South Africa and parts of South America and Asia (Dalziel, 1963). *Endod* has small berries which when dried, powdered and placed in water, yield a foaming detergent solution. In Ethiopia, *endod* exists as two main varieties, *arabe* with pinkish, and *ahiyo* with greyish, berries. *Arabe* (possibly meaning " from Arabia ") has more powerful detergent properties than *ahiyo* (meaning " donkey " and implying that it is less active than the other type). The plant is a climber with hanging branches; it grows very rapidly, reaching a height of up to 10 m but the average height is 2 m–3 m (Fig. 1). Under favourable climatic conditions in Ethiopia the plant bears fruit twice a year, in January and July.

Although some substances of vegetable origin are known to be lethal to snails, little investigation has been carried out in this field. Mozley (1939, 1952) tested a number of different vegetable substances against schistosome-transmitting snails, and listed the fruits of *Balanites aegyptiaca* (Del.) (Zygophyllaceae), *Sapindus saponaria* (L.) (Sapindaceae), and *Swartzia madagascariensis* (Desv.) (Leguminosae) among the most promising vegetable molluscicides. The active ingredients in the fruits of these plants are known to be saponins. Msangi & Zeller (personal communication, 1965), working in Tanzania, further studied the fruits of *Sapindus saponaria*, confirming Mozley's original observations and recommending the use of this plant for snail control in

[1] Dean, Faculty of Science, and Director, Institute of Pathobiology, Haile Sellassie I University, Addis Ababa, Ethiopia.

FIG. 1
AN *ENDOD* PLANT (*PHYTOLACCA DODECANDRA*) WITH (INSET) FRUITS AND LEAVES

small bodies of water. In the UAR, Sherif & El-Sawy (1969) reported the possible use of the herb known locally as *damsissa* for snail control. In all these studies, no quantitative approach was made but, in general, large quantities of the plants had to be used to show any molluscicidal effect.[1]

A preliminary report on the molluscicidal properties of *endod* has already been published (Lemma, 1965b). The present report is a summary of studies conducted primarily in the laboratory, but also on a limited scale in the field, on the quantitative evaluation of the molluscicidal properties of *endod*.

[1] In the case of *damsissa*, more than 1000 ppm is needed to produce a molluscicidal effect.

MATERIALS AND METHODS

Except where otherwise stated, standard test procedures (WHO Expert Committee on Bilharziasis, 1960; *Bull. Wld Hlth Org.*, 1965) were followed throughout the study covered in this report.

Source and size of snails used in toxicity tests

The following species and populations of snails were used in toxicity tests:

(1) Freshly collected ***Bulinus (B.) truncatus sericinus*** (Jickeli) (hereinafter referred to as ***B. t. sericinus***) from Lake Hora Abijata, a relatively large crater lake near the town of Debre Zeit about 45 km south of Addis Ababa.

(2) *Biomphalaria pfeifferi ruppellii* (Dunker) from Lake Aba Samuel (about 25 km south of Addis Ababa) and from the irrigation canals at the HVA Sugar Estate in Wonji-Shoa (about 110 km south of Addis Ababa).

(3) *Lymnaea natalensis* (Krauss) obtained from Debre Zeit.

(4) *Physa acuta* (Drapavnaud) from the irrigation canals in Wonji-Shoa.

As far as possible, snails of uniform size were used. The average shell diameter of the ***B. p. ruppellii*** was 7.0 mm, the average height of shell for ***B. t. sericinus*** was 6.7 mm, for *L. natalensis* 11.5 mm and for *P. acuta*, 6.5 mm.

Sources of endod

Most of the *endod* used in the present study was bought from the market at an average cost of US $0.12 (Eth. $0.30) per kg. This price was, however, highly variable, depending upon the place and season, and ranged from US $0.06 to US $0.20 per kg. Most of the material used in the present study was collected in, or bought from areas, at or above 2000 m above sea level.

Preparation of endod *solutions for molluscicidal testing*

The ripe berries of *endod* were harvested and allowed to dry in the sun for 3 or 4 days. The dried fruits were ground to a fine powder manually or with an electric grinder. The same procedure was used also to prepare powders of different parts of the *endod* plant. Weighed amounts of powdered *endod* were then added to different volumes of standard reference water (prepared as described by Freeman, 1953) to make up the desired weight/volume concentrations, expressed in terms of parts per million (ppm).

Other molluscicides

Niclosamide ethanolamine salt (Bayluscide, 70% wettable powder), copper sulfate crystals, sodium pentachlorophenate (NaPCP) pellets, and *N*-trityl-morpholine (18.2% w/v emulsifiable concentrate) were used in the comparative study.

Number of replicates, determination of lethal concentrations and exposure and recovery periods in each test.

The number of replicates of each test was 4 and the average number of snails per test was 10. The average of the 4 replicates was taken as the final result.

The lethal concentrations of molluscicides and their 95% confidence limits, where quoted below, were determined according to the method of Litchfield & Wilcoxon (1949).

Except when otherwise stated, 24-hour exposure and recovery periods were used in all the tests.

RESULTS

Comparison of the molluscicidal properties of the different parts of male and female endod *plants*

Since only the *endod* berries were originally seen to have molluscicidal properties, it was thought desirable to make a comparative evaluation of the potencies of various parts of the plant so that the most active part could be identified. Therefore, comparisons of the molluscicidal properties of different parts of the female and male plants, after they had been dried and reduced to powder, were made. For this, the following parts of the plants were used: ripe pinkish berries, unripe greenish berries, flowers, leaves, stem, bark and root.

The results (Table 1) show that the ripe berries are the most active part of the plant. Comparison of different parts of male and female plants reveal that while the potency of the flowers is about the same, the leaves, stem and bark of the male plant seem to have higher molluscicidal activity than those of the female plant.

In order to determine if any seasonal variation occurs in the concentration of the active principles in the *endod* plant, samples of the bark, leaves, stem, roots, flowers and berries were taken from the same plants every month for a 12-month period and the molluscicidal potency of each sample was determined. The results showed no differences due to seasonal variation.

Since the berries were found to be the most active part of the *endod* plant, the possibility that berries from plants growing at various altitudes and under different climatic conditions might show differences in their molluscicidal potencies was studied. *Endod* berries collected from 10 different parts of Ethiopia were used for this purpose. The results (Table 2)

TABLE 1

COMPARISON OF THE MOLLUSCICIDAL POTENCIES[a] OF DIFFERENT PARTS OF FEMALE AND MALE *ENDOD* PLANTS

Parts of *endod* plant tested	Percentage mortality of *B. t. sericinus* at following concentrations (ppm) of *endod* from female (F) and male (M) plants														0 (control)
	1 000		500		250		100		50		25		15		
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	
Ripe berries	100	—	100	—	100	—	100	—	100	—	100	—	25	—	0
Green berries	100	—	100	—	100	—	100	—	80	—	40	—	—	—	0
Flowers	100	100	100	100	100	100	100	95	45	55	0	0	—	—	0
Leaves	30	100	0	100	0	100	0	80	0	40	0	0	—	—	0
Stem	0	80	0	60	0	5	0	0	0	0	0	0	—	—	0
Bark	0	80	0	60	0	0	0	0	0	0	0	0	—	—	0
Root	0	0	0	0	0	0	0	0	0	0	0	0	—	—	0

TABLE 2

COMPARATIVE MOLLUSCICIDAL POTENCIES OF *ENDOD* FROM DIFFERENT PARTS OF ETHIOPIA

Origin of *endod*		Percentage mortality of *B. t. sericinus* at following concentrations (ppm) of *endod*							
Town	Distance and direction from Addis Ababa, and altitude (m)	40	30	25	20	15	10	5	0 (control)
Adwa	800 km N; 1800 m	100	100	100	80	60	10	0	0
Dessie	400 km NE; 2703 m	100	100	90	60	20	0	0	0
Holeta I	40 km W; 2500 m	100	100	100	90	60	10	0	0
Holeta II	45 km W; 2450 m	100	100	100	70	10	0	0	0
Addis Ababa (Merkato v)	2355 m	100	95	80	70	30	10	0	0
Harar[b]	500 km E; 1800 m	100	100	*100*	*80*	*40*	*10*	0	0
Menagesh	30 km W; 2520 m	100	100	80	50	30	10	0	0
Debre Libanos	80 km N; 2300 m	100	100	100	100	85	35	20	0
Ginchi	90 km W; 2500 m	100	100	100	70	50	10	0	0
Entoto	6 km N; 2600 m	100	100	100	90	60	40	10	0

[b] LC_{50} with 95 % confidence limits = 14.3 (17.6–11.6); LC_{90} with 95 % confidence limits = 21.6 (29.2–16.0).

show no significant differences in their potencies. A comparison of the molluscicidal potencies of the *arabe* and *ahiyo* varieties of *endod* also showed no differences.

Comparative susceptibility of different species of snails to the molluscicidal action of endod *and some other molluscicides*

Since it is known that different species of snails vary in their susceptibility to different molluscicides, it was necessary to determine their comparative susceptibility to the action of the berries of *endod*. From the results presented in Table 3, it appears that there are some significant differences in the susceptibility of the snails tested to several molluscicides during various exposure times. For example, *P. acuta* was killed by *endod* at a concentration of 26 ppm whereas 100 ppm were required to kill *B. p. ruppellii* during 6-hour exposures. However, when the exposure period was increased to 24 hours,

TABLE 3

COMPARATIVE SUSCEPTIBILITY OF DIFFERENT SPECIES OF SNAILS TO VARIOUS MOLLUSCICIDES

Molluscicide	Exposure period (hours)	LC_{90} (ppm) with 95 % confidence limits for following species of snails			
		B. p. ruppellii	*B. t. sericinus*	*L. natalensis*	*P. acuta*
Endod	6	102.7 (140–75)	56 (94–34)	33.4 (40–28)	26.0 (44–17)
	24	25.0 (32–20)	17.6 (22–14)	28.9 (75–11)	13.8 (18–10)
N-tritylmorpholine	6	—	0.091 (0.167–0.051)	—	25.8 (31–23)
	24	—	0.078 (0.13–0.046)	—	4.73 (8–3)
Copper sulfate	6	29.9 (40–22)	11.2 (20–6)	—	—
	24	4.9 (8–3)	1.5 (2.8–0.8)	—	—
Niclosamide ethanolamine salt	6	0.23 (0.5–0.1)	0.59 (1.3–0.3)	—	0.55 (0.9–0.3)
	24	0.053 (0.1–0.03)	0.072 (0.1–0.05)	—	0.199 (0.3–0.1)

each species died with an LC_{90} below 30 ppm and no significant difference in the susceptibility of the four different species of snails to the action of *endod* was observed. *P. acuta*, on the other hand, was about 260 times more resistant than *B. t. sericinus* to *N*-tritylmorpholine during a 6-hour exposure, and about 60 times more so during a 24-hour exposure. *B. p. ruppellii* was about 3 times more resistant than *B. t. sericinus* to copper sulfate during both 6- and 24-hour exposures. *B. t. sericinus* and *P. acuta* were both about twice as resistant as *B. p. ruppellii* to niclosamide ethanolamine salt during a 6-hour exposure, but during a 24-hour exposure, *B. t. sericinus* and *B. p. ruppellii* died in about the same concentration whereas about twice that amount was required to obtain the same kill of *P. acuta*.

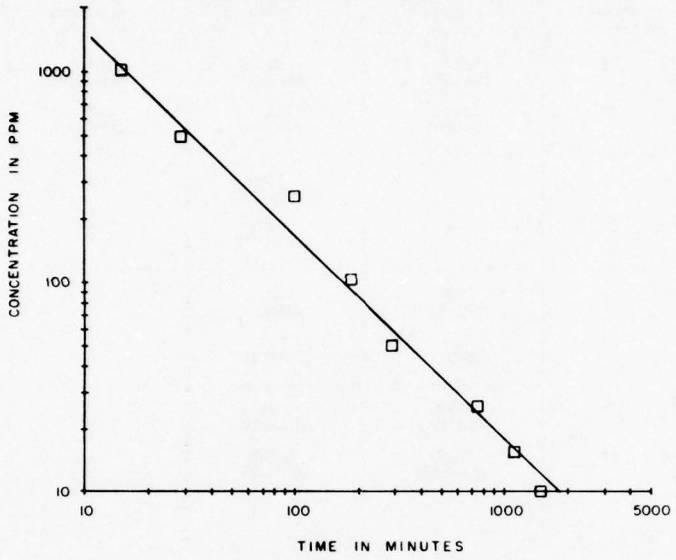
Time-concentration relationships of the molluscicidal activity of endod

In order to investigate further the molluscicidal action of *endod* and also to gather more information on the length of time that applications of *endod* would require to be continued in the field, time–concentration relationships of *endod* on *B. t. ruppellii* were studied (for discussions of the methods, see World Health Organization, 1965). In these experiments, batches of snails were exposed to various concentrations of *endod* and the mean time of death was plotted against the concentration. From the results presented in Fig. 2, it appears that snails take up, and react to, the active principle of *endod* at a constant rate. The steep slope of the curve may be indicative of the fact that the longer is the exposure of snails to *endod*, the more sensitive the snails become to its molluscicidal action.

Comparative toxicities of endod, N-*tritylmorpholine and niclosamide ethanolamine salt for the eggs of* B. t. sericinus

Batches of 10–20 eggs of *B. t. sericinus* at different stages of development were exposed for 24 hours to different concentrations of *endod*, *N*-tritylmorpholine and niclosamide ethanolamine salt. The results showed that whereas the LC_{90} of *endod* against adult snails is about 18 ppm, for eggs it is about 500 ppm. *N*-tritylmorpholine had an LC_{90} of 0.078 ppm for adults and over 15 ppm for eggs. Niclosamide ethanolamine salt, a well-known molluscicide with ovicidal properties, killed both adults and eggs at a concentration of 0.75 ppm. It is

FIG. 2
TIME-CONCENTRATION RELATIONSHIPS[a] OF THE MOLLUSCICIDAL EFFECT OF *ENDOD* ON *B. T. SERICINUS*



[a] Average time to death of batches of *B. t. sericinus* in various concentrations of *endod*.

apparent that *endod*, like *N*-tritylmorpholine, has a very low ovicidal potency by comparison with the toxicity for adults.

Susceptibility of B. p. ruppellii *of various ages to the molluscicidal action of* endod

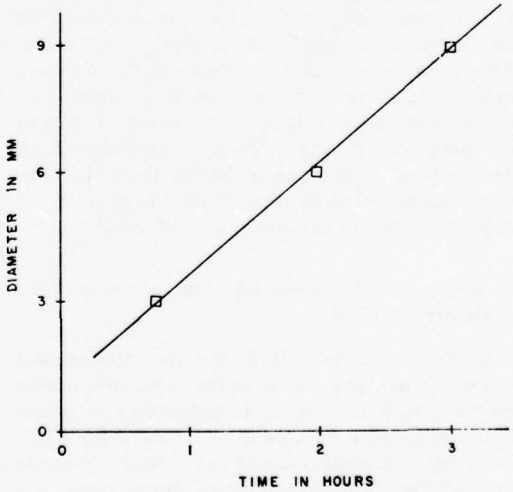
Newly hatched (3 mm diameter), juvenile (6 mm) and adult (9 mm or larger) *B. p. ruppellii* were exposed to 100 ppm of *endod* solution. Newly hatched snails died within 45 minutes, juveniles within 2 hours and adults within 3 hours of exposure (Fig. 3).

Effect of temperature on the molluscicidal potency of endod

Three main points were studied. The first was the effect of water temperature on the molluscicidal action of *endod*. The second and third aspects were the effect of heat on *endod* as powder and in solution.

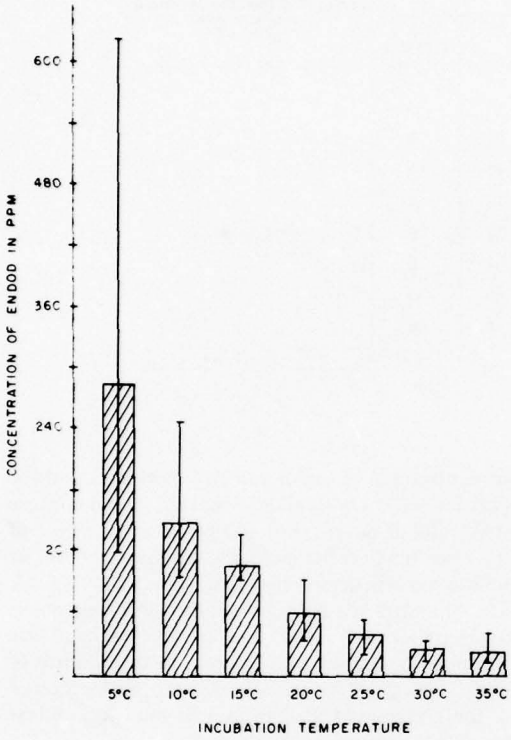
In using *endod* for snail control, the effect of variations in water temperature during daytime and at night must be considered. Therefore, the mortality rate of snails exposed to different concentrations of *endod* and incubated at temperatures ranging from 5°C to 35°C in 5-degree stages was determined. Exposure periods of 6 hours and recovery periods

FIG. 3
SUSCEPTIBILITY OF *B. P. RUPPELLII* OF VARIOUS SHELL DIAMETERS (AGES) TO THE MOLLUSCICIDAL EFFECT OF *ENDOD*



[a] Each small square represents the average 100 % mortality time of 30 snails exposed to a 100-ppm solution of *endod*.

FIG. 4
EFFECT OF VARIATION IN INCUBATION TEMPERATURE ON THE MOLLUSCICIDAL POTENCY OF *ENDOD* ON *B. T. SERICINUS* [a]



[a] LC_{90} with 95 % confidence limits.

of 24 hours were used. From the results presented in Fig. 4, it is evident that the molluscicidal action of *endod* is temperature-dependent. At higher water temperatures (30°C–35°C) less than 20 ppm is needed to kill all snails within a 6-hour exposure period, whereas about 3 times that concentration (60 ppm) is needed to produce the same effect at room temperature (20°C±2°C) and about 6 times as much (ca. 250 ppm) at 5°C.

In an attempt to discover more about the nature of the active principle in *endod*, powdered berries were incubated for 24 hours in drying ovens which were regulated to different temperatures ranging from 50°C to 105°C. The molluscicidal potencies of the incubated berries were then determined at room temperature (22°C) using the regular procedure. The results (Table 4) show that the exposure

TABLE 4
MOLLUSCICIDAL POTENCIES OF POWDERED *ENDOD* NCUBATED FOR 24 HOURS AT VARIOUS TEMPERATURES

Incubation temperatures (°C)	Percentage mortality in 40 specimens of *B. t. sericinus* exposed for 6 hours [a] to following concentrations of *endod* (ppm)				
	100	75	50	40	0 (control)
105	0	0	0	0	0
100	70	15	0	0	0
90	90	35	21	0	0
80	100	90	40	10	0
70	100	100	63	34	0
60	100	100	83	83	0
50	100	100	100	46	0
22 (room temperature; control)	100	100	100	52	0

[a] Recovery period of 24 hours.

of berries to temperatures of up to, and including, 50°C for a 24-hour period has no effect on the molluscicidal potency of *endod*. However, when incubated at 60°C and above, the powder progressively loses its molluscicidal potency until at 105°C it is completely inactivated.

In another approach, solutions of *endod* were boiled for different lengths of time and their molluscicidal potencies were then determined. An *endod* solution was also evaporated to dryness by boiling, then reconstituted, and the molluscicidal potency determined. It was found that a reconstituted *endod* solution has the same molluscicidal potency as one prepared from fresh berries.

Effect of pH on the molluscicidal potency of endod

According to Strufe & Gönnert (1962), the pH of the water used for preparing test solutions of various molluscicides influences their activity. It was therefore necessary to determine the pH values at which such effects on the molluscicidal activity of *endod* could be detected. For this purpose, different concentrations of *endod* were prepared in standard reference water previously adjusted (with $NaHCO_3$, NaOH or HCl) to pH values of 3, 4, 5, 6, 7, 8, 9 and 10. Tests using 6-hour exposure and 24-hour recovery periods were made, and the mortality of

TABLE 5

EFFECT OF pH ON THE MOLLUSCICIDAL POTENCY OF *ENDOD*

pH values of standard and adjusted reference water	Percentage mortality of *B. t. sericinus* exposed for 6 hours [a] to following concentrations of *endod* (ppm)					LC_{90} of *endod* (ppm) for *B. t. sericinus* with 95 % confidence limits in standard reference water (pH 7.2)
	100	75	50	40	0 (control)	
3.1	20	15	10	—	0	
4.0	100	100	70	20	0	
5.2	100	100	85	65	0	
6.1	100	100	97	62	0	
7.2 [b]	*100*	*100*	*95*	*57*	*0*	*56.0 (94–34)*
8.1	100	100	72	50	0	
9.1	100	100	75	40	0	
10.1	65	15	0	0	0	

[a] Recovery period of 24 hours.
[b] Standard reference water.

snails was determined in each test. The pH measurements were made only once in this experiment, namely, before the *endod* solution was added; no follow-up measurements were made during the exposure period since they would not be made in field trials of *endod*. The results of this experiment are given in Table 5. Molluscicidal activity was not significantly affected by pH in the range 4–9; at pH 3 and pH 10, however, much of the activity was lost.

Effect of river-bed mud containing organic and inorganic matter on the molluscicidal potency of endod

It is well known that some molluscicides become absorbed into or adsorbed on to organic and inorganic matter in the water to which they are applied (Dobrovolny & Barbosa, 1953; Paulini, 1956). In such situations, it would be necessary to use larger quantities of the molluscicide to make up for the amounts lost by absorption or adsorption. Experiments were therefore designed to determine whether or not the molluscicidal potency of *endod* is affected by the presence of different concentrations of river-bed mud.

Under natural conditions, flowing water has sufficient turbulence to cause rapid mixing of particles in the water. In an attempt to simulate this condition in the laboratory, solutions of *endod* were prepared with water containing different concentrations of river-bed mud. A number of *B. t. sericinus* were then put into beakers containing different concentrations of *endod* and river-bed mud and the beakers were continuously shaken on an electric shaker for 6 hours. For comparison, the effect of river-bed mud on the molluscicidal action of copper sulfate was also determined in the same way (Table 6). The concentration of *endod* required to kill all snails in the presence of 10 000 ppm of river-bed mud was 100 ppm compared with 60 ppm in the absence of mud. Using copper sulfate, 80 ppm were required in the presence of 10 000 ppm of mud in contrast to only 20 ppm without the mud.

Effect of ultraviolet irradiation on the molluscicidal potency of endod

Some molluscicides such as NaPCP are known to be partially inactivated by the ultraviolet portion of sunlight (Hiatt, Haskins & Olivier, 1960). As the stability of molluscicides is very important in field trials, the effect of ultraviolet radiation on the molluscicidal potency of *endod* was determined. Solutions containing different concentrations (40 ppm–100 ppm) of *endod* were exposed to ultraviolet irradiation[1] for periods of 4 and 8 hours at a distance of about 30 cm from the light source. For comparative purposes, the effects of ultraviolet irradiation on the molluscicidal potency of NaPCP were also determined in the same manner (Fig. 5). Ultraviolet irradiation for 4 and 8 hours does not have any

[1] From a Hanovia 30-W ultraviolet lamp, model CH.1

TABLE 6

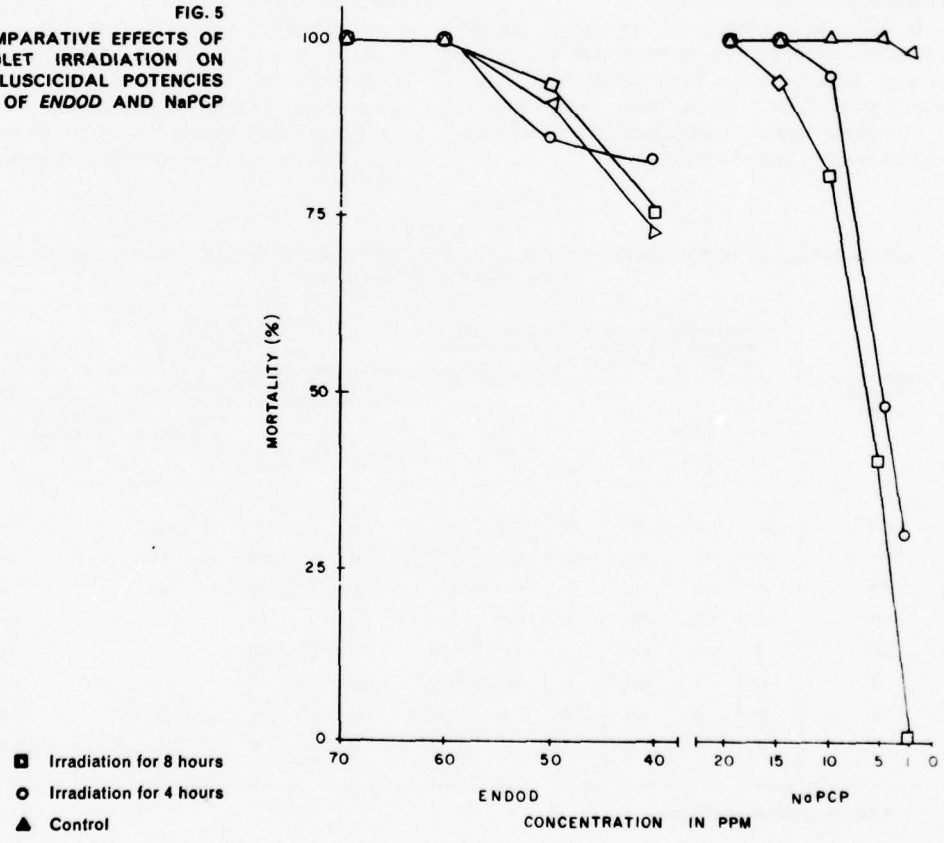
COMPARATIVE EFFECTS OF DIFFERENT CONCENTRATIONS OF RIVER-BED MUD ON THE MOLLUSCICIDAL POTENCIES OF *ENDOD* AND COPPER SULFATE ON *B. T. SERICINUS* FOR 6-HOUR EXPOSURE AND 48-HOUR RECOVERY PERIODS

Concentration of molluscicide (ppm)	Percentage mortality of snails in *endod* with following concentrations of river-bed mud (ppm)[a]				Percentage mortality of snails in copper sulfate with following concentrations of river-bed mud (ppm)[a]			
	10 000	5 000	1 000	0 (control)	10 000	5 000	1 000	0 (control)
100	*100*	100	100	100	100	100	100	100
80	90	*100*	100	100	*100*	100	100	100
60	75	90	*100*	*100*	80	*100*	100	100
50	40	82	65	90	80	92	*100*	100
40	30	57	60	45	87	85	92	100
20	—	—	—	—	52	77	79	*100*
10	—	—	—	—	35	57	52	90
7	—	—	—	—	—	—	—	80

[a] Values in italics indicate lowest concentration of molluscicide to produce 100 % mortality.

FIG. 5

COMPARATIVE EFFECTS OF ULTRAVIOLET IRRADIATION ON THE MOLLUSCICIDAL POTENCIES OF *ENDOD* AND NaPCP



apparent effect on the molluscicidal potency of *endod*. However, there was a progressive and significant reduction on the molluscicidal potency of NaPCP with increased exposure to ultraviolet irradiation.

Storage stability of the molluscicidal property of endod

An important property of a good molluscicide is its stability in storage under different environmental conditions. Therefore, the stability of *endod* in storage at working dilutions (10 ppm–100 ppm), and as whole and powdered berries, was studied.

When *endod* at working dilutions is left at room temperature (22°C), its molluscicidal potency deteriorates over a period of 3 days. However, if such a solution is kept in a refrigerator at 4°C, or if it is sterilized by boiling and then kept at room temperature, its molluscicidal potency remains constant for more than a month.

In order to determine the stability of the molluscicidal potency of *endod* berries after prolonged storage, whole and powdered berries, kept at room temperature (22°C) for 4 years, were tested at 6-monthly intervals. It was found that their molluscicidal potency had not changed.

Solubility of endod *in water*

An experiment was conducted to find the rate at which the active principle from whole berries, crushed berries and finely powdered berries of *endod* is released in water. This was determined under different conditions simulating those in rivers or lakes, with and without filtration (Table 7).

In tests with whole berries, 1-g quantities of dried berries were put into each of 2 flasks containing 1 litre of standard reference water. One of the flasks was left undisturbed and the other was put on an electric shaker revolving at about 130 rev/min. Samples of the solutions were taken at regular intervals and filtered to remove the berries. Serial dilutions of the filtrates were then made and the molluscicidal potency in 6-hour exposures determined. For shaken whole berries it took 48 hours for enough of the active ingredients to dissolve to cause 100% mortality at a concentration of 50 ppm but for unshaken berries 60 hours soaking was necessary. Thus, as would be expected, shaking leads to quicker solution of the active principle.

To determine the rate of diffusion of the active ingredient from crushed berries, 1-g quantities of mortar-crushed berries were put in each of 2 flasks containing 1 litre of standard reference water. One of the flasks was left undisturbed while the contents

TABLE 7

COMPARATIVE DIFFUSION RATES OF THE ACTIVE PRINCIPLE FROM WHOLE BERRIES, CRUSHED BERRIES AND FINELY POWDERED *ENDOD*

Concentration of *endod* (ppm)	Percentage mortality of *B. t. sericinus* exposed for 6 hours [a] to filtered *endod* solutions in which whole berries were soaked or shaken for the following periods (hours)						Percentage mortality of *B. t. sericinus* exposed for 6 hours [a] to filtered and unfiltered solutions of crushed *endod* berries after following extraction periods (min)			Percentage mortality of *B. t. sericinus* exposed for 6 hours [a] to unfiltered solutions of finely powdered *endod* extracted for 30 seconds
	Soaked			Shaken			Filtered		Unfiltered	
	24	36	48	36	48	60	1	5	5	
1 000	60	100	100	70	100	100	100	100	100	100
500	30	100	100	40	100	100	100	100	100	100
250	0	100	100	0	100	100	100	100	100	100
125	0	100	100	0	100	100	100	100	100	100
100	0	100	100	0	100	100	55	100	100	100
75	0	70	100	0	30	100	50	100	100	100
50	0	0	100	0	0	90	42	95	90	100
0	0	0	0	0	0	0	0	0	0	0

[a] Recovery period of 24 hours.

of the other were immediately filtered through No. 1 filter-paper. Serial dilutions of the filtrate and of the unfiltered solution were made at different intervals after adding the crushed berries to the water. Determination of percentage mortality of snails placed in the two solutions made after the first minute of mixing showed that the unfiltered solution was more potent than the filtered one. This seems to be have been due to the removal of the crushed berries from the filtered solution before the active principle had dissolved completely. When samples of the same solution were taken 5 minutes later and filtered, the filtrates had the same potency as the unfiltered solution. The active ingredient appears to dissolve completely in water within 5 minutes of mixing and the suspended fragments of crushed berries have no molluscicidal properties after the first 5 minutes or so.

To determine the rate at which the active principle in finely powdered *endod* berries would dissolve, 1 g of powder was placed in 1 litre of water and within 30 seconds serial dilutions were made. The snail mortality record after a 6-hour exposure showed that even 50 ppm of powdered *endod* berries were enough to kill 100% of the snails.

From these results it appears that the active principle of *endod* is highly water-soluble; powdered or crushed *endod* berries yield an active solution almost immediately after the powder is put in water. Intact whole berries, however, release the active ingredient slowly and this may be an advantage when this molluscicide is used in the field.

Preliminary studies on toxicity of endod

The toxicity of *endod* to mammals, birds, fish, leeches, insects, plants, and to cercariae and miracidia of schistosomes, was determined.

Toxicity of endod *to birds and mammals.* The acute oral toxicity of *endod* to standard laboratory mice and rats, domestic chickens, domestic sheep and rhesus monkeys was quantitatively determined (Table 8). The results show that all these animals have very high tolerance to *endod* and that the concentrations of *endod* used in the field (about 100 ppm) should have no harmful effects on them. A mouse, for example, would have to drink more than 26000 litres of water treated with 100 ppm of *endod* to obtain an LC_{50} dose. The possible chronic effects of *endod* have, however, yet to be studied.

Skin-irritation tests were performed with a paste made from *endod* placed in large quantities on human volunteers and on the shaved skin of guinea-pigs. No harmful effects were detected. This finding is, of course, supported by the circumstantial evidence that the many people who use high concentrations of *endod* for washing clothes appear to suffer no skin irritation, not even on the hands.

Preliminary phytotoxicity studies. A molluscicide should have no serious effect on crops; the toxicity of *endod* to different kinds of economically important plants was therefore determined. Barley, corn, wheat, millet, beans and peas were grown in pots and treated with different concentrations (100 ppm, 500 ppm and 1000 ppm) of *endod* at different stages of their development. The results showed that *endod* had no effect on the germination and growth rates, or on the apparent physiomorphological condition of these plants. Further tests on the possible chronic effects of *endod* are presently being carried out both in the laboratory and under natural conditions in the field.

Fish toxicity studies. Fish constitutes a major part of the diet in many developing countries and in

TABLE 8

ACUTE ORAL TOXICITY OF ENDOD TO ANIMALS

Test animals	Total no. of animals used	Highest concentration of *endod* tolerated by test animals (g/kg of body-weight)	Lowest concentration of *endod* needed to kill test animals (g/kg of body-weight)	LC_{50} of *endod* (g/kg of body-weight)
Mice	48	2.0	3.25	2.6
Rats	18	1.8	2.5	2.2
Chickens	10	2.2	2.5	—
Sheep	6	6.5	7.8	—
Monkeys	2	2	3	—

TABLE 9

COMPARATIVE TOXICITIES TO FRESHWATER FISHES[a] OF *ENDOD* AND NICLOSAMIDE ETHANOLAMINE SALT

Exposure period (hours)	Percentage mortality of *Tilapia* sp. after exposure to following concentrations of *endod* (ppm)						Percentage mortality of *Tilapia* sp. after exposure to following concentrations of niclosamide (ppm)				
	150	100	75	50	25	0 (control)	1	0.75	0.5	0.25	0 (control)
6	100	100	100	0	0	0	100	100	0	0	0
24	100	100	100	100	70	0	100	100	70	30	0

[a] Using 20 specimens of *Tilapia* sp. in 4 replicates of each test.

some it is also of major importance in the national economy. Thus, from both the public health and the economic point of view, it is important that fishes should not be adversely affected by molluscicidal treatments. Since fishes and snails have, to some extent, a common habitat, the choice of a molluscicide which spares fishes is obvious. Almost all the currently used molluscicides are known to be toxic to fishes.

With this limitation in view, the susceptibility of certain important freshwater fishes (*Tilapia* sp.) to the presence of *endod* was investigated. For comparative purposes, the susceptibility of the same species to niclosamide ethanolamine salt was also determined (Table 9). It was found that the concentrations of both *endod* and niclosamide which kill *Tilapia* sp. are about the same as those needed to kill snails.

Effects on insects. Ethiopians who have traditionally used *endod* for washing clothes believe that clothes so washed will have no lice or ticks remaining on them (Baldrati, 1946). Since this belief has not been proved experimentally, appropriate experiments were performed on lice and mosquito larvae. Clothes containing containing large numbers of lice were thoroughly washed with an emulsion of *endod*, rinsed and dried. Also, mosquito larvae belonging to the species *Culex pipiens fatigans* were suspended in solutions containing different amounts of *endod*, and the number of larvae affected after different periods of exposure was determined. The results showed that clothes washed with *endod* still contained live and active lice and that even concentrations of *endod* as high as 2000 ppm had no effect on mosquito larvae.

Effect of endod *on cercariae and miracidia of schistosomes.* The possibility that *endod* applied to natural bodies of water in concentrations sufficient to kill snails would also kill cercariae and miracidia, thereby contributing to the control of schistosomiasis, was considered. Results of an experiment revealed that all cercariae and miracidia of *Schistosoma haematobium* die within 10 minutes in concentrations of 1000 ppm of *endod* in standard reference water, within 1 hour at 100 ppm and within 2 hours at 50 ppm.

Toxicity of endod *to aquatic leeches.* Aquatic leeches are of tremendous economic importance in Ethiopia and many other parts of the world. In Ethiopia, the species *Lymnotis nilotica* Savigny is a serious nuisance to both cattle and man. Since the habitat of these leeches is commonly the same as that of snails, their susceptibility to *endod*, *N*-tritylmorpholine and niclosamide ethanolamine salt was determined.

The results of the tests (Table 10) show that a concentration of *endod* as low as 4 ppm (compared with about 60 ppm for snails) is sufficient to kill all leeches within 6 hours. Using *N*-tritylmorpholine, 15 ppm were required (compared with less than 0.05 ppm for snails) and the concentration of niclosamide needed to kill both leeches and snails was less than 1 ppm.

Preliminary field trials with endod

After promising results were obtained in laboratory experiments, some limited field trials were conducted. These were carried out in 3 different situations: in the Assam, the small river flowing through the town of Adwa, in Lake Hora Abijata, and in the irrigation canals at the Wonji-Shoa sugar plantation. In the Wonji-Shoa irrigation canals, 2 experiments were performed. The first was to determine the total length of canal which could be made free of snails by

TABLE 10

COMPARATIVE TOXICITIES TO LEECHES [a] OF *ENDOD*, *N*-TRITYLMORPHOLINE AND NICLOSAMIDE ETHANOLAMINE SALT

Concentration of molluscicide (ppm)	Percentage mortality of *L. nilotica* exposed for 6 hours to following molluscicides [b]		
	Endod	*N*-trityl-morpholine	Niclosamide
15	100	*100*	100
12	100	55	100
10	100	0	100
5	100	0	100
4	*100*	0	100
3	55	—	100
2	30	—	100
1	14	—	*100*
0.5	—	—	62
0.25	—	—	30
0 (control)	0	0	0

[a] Using 300 specimens of *Lymnotis nilotica* in 10 replicate tests.

[b] Values in italics indicate lowest concentrations of molluscicide to produce 100 % mortality.

the application of a single treatment of *endod* from a single site. The second was to study the effect of 2 treatments, the first to kill adult snails and the second to kill the young ones hatching from eggs unaffected by the first treatment, on the long-range repopulation rate (due to snails being introduced from other areas).

In all instances, powdered *endod* berries were used. Measured amounts in the form of paste or as highly concentrated (10 000 ppm–100 000 ppm) solutions were dropped into the body of water at regular intervals of time to maintain a concentration of 100 ppm in the entire body of water. In the River Assam, and in a 5-km-long irrigation canal in Wonji-Shoa, this concentration was maintained for 6 hours; in another irrigation canal at Wonji-Shoa a concentration of 50 ppm was maintained for 6 hours. In Lake Hora Abijata, a concentration of 50 ppm was tested over a period of 3 hours. To establish the required concentration in the lake, the approximate volume of water in the first 5 m from the shore along a 200-m test strip was determined. The rate of mixing and diffusion caused by wind action and convection currents in the water was estimated by adding a fluorescent dye to the water and taking samples over a 3-hour period. In this way the initial amount of *endod* required to maintain a concentration of 100 ppm for 3 hours could then be calculated.

Appropriate pretreatment and post-treatment counts of snails were made and the reduction in the snail population due to the molluscicidal action of *endod* was determined. In addition to the natural population of snails, in all except one of the areas where field trials were made (the small irrigation canal in Wonji-Shoa), caged snails were used; appropriate numbers of snails (usually 5 of each species) being placed in pieces of cheesecloth (ca 20 cm × 20 cm) which were firmly tied with string, dropped into the water at various places, and anchored to the shore with string.

The results showed that the performance of *endod* in the River Assam, in Lake Hora Abijata and in the irrigation canals in Wonji-Shoa was very satisfactory. The 5-km-long River Assam, the 200-m strip along the shore of Lake Hora Abijata and the 5-km- and 500-m-long irrigation canals in Wonji-Shoa were all freed of snails by the application of 50 ppm–100 ppm of *endod* during an exposure period of 3–6 hours.

DISCUSSION

For a crude natural product such as *endod*, an LC_{90} of about 20 ppm is noteworthy. It has been shown that this potency remains stable over a wide range of pH values and temperatures, under ultra-violet irradiation and in various concentrations of river-bed mud. The stability of dried *endod* berries in prolonged storage and the slow solution in water of the active principle from the whole berries are also important properties. The main disadvantage of *endod* is that it is not ovicidal at the concentrations which kill adult snails. However, this difficulty can be overcome by repeated treatments, as has been demonstrated in the field.

It has been shown that the acute oral toxicity of *endod* to mammals, birds and plants is very low, and there should, therefore, be no risk to them from the use of *endod* in molluscicidal concentrations in the field. On the other hand, *endod* is lethal to fishes as are all the other presently available molluscicides. From the small number of experiments conducted so far, *endod* does not seem to possess any insecticidal property but is exceedingly effective against leeches. Since leeches are pests of some economic and public health importance, it may be possible to use this natural product in their control. *Endod* is also

TABLE 11

SUMMARY OF FIELD TRIALS WITH *ENDOD*

	River Assam, Adwa	Lake Hora Abijata, Debre Zeit	Irrigation canals at Wonji-Shoa sugar plantation	
			KWR3-KWR8 canal	Drainage canal near O camp on Plot 215
Length of test area	4.5 km	200 m along the shore	5 km	500 m
Approximate flow rate of water or volume treated	25 l/sec	Total volume of water treated was 500 m^3	150 l/sec	Total volume of water treated was 260 m^3
Pre-treatment estimates of natural populations of snails	Very abundant throughout the test area. In some places more than 200 snails were counted in 5 minutes	Very abundant throughout the test area, In some places over 100 snails were counted in 5 minutes	Very abundant throughout the test area. Average of 20 snails per standard WHO scoop	Very abundant throughout the test area. Average of 20 snails per standard WHO scoop
Species of snails present	*B. p. ruppellii*, *B. t. sericinus*, *Lymnaea* sp.	*B. t. sericinus*	*B. t. ruppellii*, *B. t. sericinus*, *Lymnaea* sp., *Physa* sp.	*B. p. ruppellii*, *B. t. sericinus*, *Lymnaea* sp., *Physa* sp.
Caged snails used	5 of each of the above per cage placed at 100-m intervals	5 *B. t. sericinus* placed at 10-m intervals	5 of each of the above per cage at 100-m intervals	None used
Concentration of *endod* used	100 ppm for 6 hours	100 ppm for 3 hours	70 ppm for 6 hours	50 ppm for 6 hours
Total amount of *endod* used (kg)	54	50	227	13
Climatic conditions during tests	October–November 1966: no rain, average temperature about 23°C, clear water	February–March 1966: no rain, average temperature 20°C, clear water	January–February 1966: no rain, average temperature 22°C, muddy water	October–February 1967–68: no rain, average temperature 22°C, muddy water
Post-treatment counts of snail populations	18 hours after treatment, only the snails in the first 2½ *km were dead*; 7 days later there were no snails in the entire 4.5 km of test area	24 hours after treatment no live snails or leeches were found	24 hours after treatment no live snails were found in the entire area	24 hours after treatment all except a few *B. p. ruppellii* were dead; the few live snails found were killed on the spot by an application of highly concentrated *endod* (100 000 ppm in paste form)
Comments on other aquatic animals and plants affected by *endod*	Many small fishes and tadpoles were found dead; no water insects were affected; there was no apparent effect on vegetation	No fishes were found dead; all leeches in the treated area were found dead; there was no apparent damage to the vegetation	Some small fishes and tadpoles were dead; neither the weeds in the canal nor the sugar-cane irrigated with the treated water were affected	No fishes present: no apparent effect on the vegetation
Summary of post-treatment examination	About *1 month* later many small snails were observed; they were presumed to have hatched from eggs not affected by the treatment	*6 weeks* later abundant numbers of very small *B. t. sericinus* were observed	*4 weeks* later many small and also a few adult snails were present (presumably the adults were carried down from other parts of the estate)	*4 weeks* later many small snails of different species were present
Second treatment	Not done	Not done	Not done	Done 4 weeks after the first treatment
Duration of absence of snails after second treatment				5 months; after this time, adult snails presumably carried down from upstream were seen establishing colonies

lethal to schistosome cercariae and miracidia in much lower concentrations than those used for the control of snails.

The comparative molluscicidal activity of *endod* against different species of snails in 24-hour exposure periods shows that it has approximately equal potency against all species tested; in a 6-hour exposure, however, *B. p. ruppellii* were more resistant than the other species, *P. acuta* being the most susceptible. In this study, a special attempt was made to use snails of the same size since younger snails are known to be more susceptible to the action of *endod* than the adults are. The differences in susceptibility between different species of snails to various molluscicides, and differences in sensitivity associated with the duration of exposure must be considered when molluscicides are used in field trials.

The information obtained from a study of the time–concentration relationship is valuable since it provides essential information which allows progress from laboratory experiments to field trials. The volume and rate of flow of the body of water are two important factors which determine the quantity of molluscicide to be used. Depending on various local conditions such as length of the working-day, availability of automatic dispensers or other mechanical means of applying molluscicide, an appropriate exposure time can be chosen. Once such a time is selected, the amount of *endod* to be used can be extrapolated from Fig. 2. The preliminary field trials reported in this article were consistent with the laboratory trials.

Investigations into the nature of the active principle in *endod* have been carried out at the suggestion of the World Health Organization and the present author in several laboratories in different parts of the world. Professor W. J. Horton, University of Utah, USA, was the first to attempt such a study; he concluded that the active part of *endod* was a saponigen and traced it to oleanolic acid (W. J. Horton, personal communication, 1966). Studies made by Woldeab Isaac (personal communication, 1967) under the supervision of Professor J. H. Robertson, University College of Dar es Salaam, Tanzania, are in agreement with Professor Horton's conclusions and chemists in the Stanford Research Institute, Calif., USA, have also confirmed them. A more detailed study carried out by Dr K. Jewers, Tropical Products Institute, London, has shown that a freeze-dried aqueous extract was actively molluscicidal at concentrations between 1.25 ppm and 2.5 ppm (K. Jewers, personal communication, 1968) Extractions of *endod* with other common organic solvents have not yet yielded such an active product but the work is still in progress.

The active principle in *endod* may be a relatively simple molecule that can be easily extracted but if, on the contrary, the extraction procedure proves to be complicated and costly, it may not be economically worth while to extract *endod* berries. In any case, the ultimate goal is to use *endod* in the most convenient and cheapest way so that it is available not only to governmental and private organizations but also to rural communities. *Endod*, being a natural product and easily grown in suitable climatic conditions, could play a very important role in the rural economy of some developing countries. At the same time, farmers and other members of rural communities may be taught to use *endod* for the control of schistosomiasis in their areas.

Both intestinal and urinary schistosomiasis are widely distributed in small foci all over Ethiopia. *S. mansoni* is particularly frequent in the northern highlands of Ethiopia whereas *S. haematobium* is at present limited to low, warm areas such as the Awash and Wabi Shebelle valleys. In some parts of these valleys large-scale agricultural development projects are being undertaken and the prevalence of schistosomiasis is increasing very rapidly. Therefore, the introduction of control measures is essential and the possibility of using *endod* for this purpose is now being explored.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Haile Hagos, Legesse Wolde Yohannes, Fekadu Gemetchu, Woldeab Isaac, Shibru Tedla, Haile Tecle, John Stauffer, Tekeste Fekadu and Tsega Bereket for the valuable technical assistance they have given at different times; to Dr H. S. Hopf and Dr K. Jewers, Tropical Products Institute, London, Dr H. Hoogstraal and Dr J. Kent, US Naval Medical Research Unit No. 3, Cairo, UAR, and Professor J. H. Fischthal for editorial comments; and to Dr J. Duncan, Professor R. M. Baxter, Professor S. P. Hutton and Mrs T. Haile for making valuable suggestions on the presentation of the manuscript.

The author also wishes gratefully to acknowledge the donation of a vehicle by the Shell Chemical Company of East Africa, Nairobi, Kenya, and of gasoline by Shell (Ethiopia) Ltd., which greatly facilitated the field work.

RÉSUMÉ

ÉVALUATION, AU LABORATOIRE ET SUR LE TERRAIN, DES PROPRIÉTÉS MOLLUSCICIDES DE *PHYTOLACCA DODECANDRA*

En Ethiopie, on constate une mortalité plus élevée parmi les mollusques dont les habitats sont proches des endroits où les riverains lavent le linge en utilisant en guise de savon une poudre de baies de *Phytolacca dodecandra*, plante de la famille des Phytolaccacées, connue dans le pays sous le nom d'*endod*. Le fait a incité l'auteur à étudier quantitativement, au laboratoire et par des essais limités sur le terrain, les propriétés molluscicides de ce végétal.

De toutes les parties de l'*endod*, les baies font preuve de l'activité molluscicide la plus forte: la CL_{90}, exprimée en parties par million, atteint en moyenne 13,8 pour *Physa acuta*, 17,6 pour *Bulinus (B.) truncatus sericinus*, 25,0 pour *Biomphalaria pfeifferi ruppellii* et 28,9 pour *Lymnaea natalensis*, après 24 heures d'exposition et 48 heures de récupération. On ne note aucune variation de la toxicité en fonction de l'origine géographique des fruits et du moment de la récolte.

Les propriétés molluscicides de l'*endod*, de la *N*-trityl-morpholine, du sulfate de cuivre et du sel d'éthanolamine de la niclosamide ont été comparées par exposition de diverses espèces de mollusques pendant 6 et 24 heures. Exposés à l'*endod* pendant 24 heures, tous les vecteurs étudiés sont affectés dans une mesure sensiblement égale et tués par des concentrations inférieures à 30 parties par million. L'activité molluscicide de l'*endod* s'est maintenue à un niveau stable au cours d'une série d'essais pratiqués dans des conditions très diverses de température de l'eau, de pH, de turbidité, ainsi qu'après traitement des solutions du produit par le rayonnement ultraviolet. La conservation pendant 4 ans de l'*endod* sous forme de baies ou de poudre n'altère pas ses propriétés molluscicides et sa solubilité est très satisfaisante.

D'études préliminaires de la toxicité de l'*endod* à l'égard des mammifères et des oiseaux, il ressort que la souris, le rat, le poulet, le mouton et le singe rhésus y sont très peu sensibles, la CL_{50} étant dans tous les cas supérieure à 2 g/kg de poids corporel. Les premiers résultats des tests de phytotoxicité indiquent que l'*endod* n'exerce aucune influence nocive sur la germination, le rythme de croissance et les caractéristiques morphologiques des végétaux. L'*endod* est par ailleurs dépourvu d'activité insecticide et larvicide et sa toxicité pour les poissons est du même ordre que celle du sel d'éthanolamine de la niclosamide. A la concentration de 4 parties par million, il tue les sangsues aquatiques en 6 heures. Au cours d'une expérience, les cercaires et les miracidiums de *Schistosoma haematobium* ont été tués en 10 minutes par 1000 parties par million, en 1 heure par 100 parties par million et en 2 heures par 50 parties par million du produit. Par contre, l'*endod* ne possède qu'un très faible pouvoir ovicide, la CL_{90} en ce qui concerne les œufs de *B. t. sericinus* étant de l'ordre de 500 parties par million.

On a procédé à des essais limités sur le terrain au moyen de pâte ou de solutions très concentrées d'*endod*. Ces préparations ont été déversées dans un cours d'eau, un lac et des canaux d'irrigation en quantités assurant une concentration de 50-100 parties par million pendant 3 à 6 heures. Les premiers résultats ont été très satisfaisants, l'élimination des mollusques ayant été obtenue dans tous les cas.

L'*endod*, produit naturel, facile à cultiver et de croissance rapide, pourrait représenter un moyen efficace et peu coûteux de lutte contre la schistosomiase dans certaines régions.

REFERENCES

Baldrati, I. (1946) *Piante officinale dell'Africa orientale*, Firenze, Centro di Studi Coloniali

Bull. Wld Hlth Org., 1965, **33**, 567-581

Dalziel, J. M. (1936) *The useful plants of west tropical Africa*, London, Crown Agents

Dobrovolny, C. G. & Barbosa, F. S. (1953) *Publ. avuls Inst. Aggeu Magalhães*, **11**, 121

Freeman, L. (1953) *Sewage ind. Wastes*, **25**, 845

Hiatt, C. W., Haskins, W. T. & Olivier, L. (1960) *Amer. J. trop. Med. Hyg.*, **9**, 527

Lemma, A. (1965a) *Ethiop. med. J.*, **3**, 84

Lemma, A. (1965b) *Ethiop. med. J.*, **3**, 187

Litchfield, A. & Wilcoxon, B. (1949) *J. Pharmacol. exp. Ther.*, **96**, 99

Mozley, A. (1939) *Trans. roy. Soc. Edinb.*, **59**, 721

Mozley, A. (1952) *Molluscicides*, London, Lewis

Paulini, E. (1956) *Bull. Wld Hlth Org.*, **14**, 617

Sherif, A. F. & El-Sawy, M. F. (1969) *Using* damsissa *in a field trial as a control method for schistosome snail vectors.* In: *First African Symposium on Bilharziasis*, Cairo, Government Printing Offices, p. 113

Strufe, R. & Gönnert, R. (1962) *Höfchenbr. Bayer Pfl-Schutz.-Nachr.*, **1**, 50

WHO Expert Committee on Bilharziasis (1961) *Wld Hlth Org. techn. Rep. Ser.*, No. 214

World Health Organization (1965) *Snail control in the prevention of bilharziasis*, Geneva (*World Health Organization: Monograph Series*, No. 50)

PRINTED IN SWITZERLAND

Lemma, A. (1971) *Eth. med. J.* 9, 113.

PRESENT STATUS OF ENDOD AS A MOLLUSCICIDE FOR THE CONTROL OF SCHISTOSOMIASIS

Aklilu Lemma, Sc.D.*

Institute of Pathobiology, Haile Selassie I University, P.O. Box 1176, Addis Ababa, Ethiopia

INTRODUCTION

A preliminary study of the molluscicidal properties of Endod (*Phytolacca dodecandra*) was first reported by Lemma (1965). This was followed by a comprehensive laboratory and field evaluation (Lemma, 1970). The molluscicidal activity of Endod has also been studied or confirmed by King *et al.* (1968), Powell and Whalley (1969), and Montagne and Crossland (1968) in England; by Horton (1968), Skinner and Parkhurst (1967) in the United States; Dawood (1965) in the United Arab Republic; Fenwick (1968) in Tanzania; and Yasuraoka (1970) in Japan.

Snail toxicity

The crude ground berries** of Endod have a molluscicidal potency (LC_{100}) of 10-25 ppm in 24 hours exposure against *Biomphalaria glabrata, B. pfeifferi, Bulinus truncatus, Lymnæa natalensis, Physa acuta, Bulinus globosis, Biomphalaria alexandrina* and other species of aquatic snails in the laboratory. Yasuraoka (1970) has found an LC_{50} of 1.62 ppm in a partially refined extract for the amphibious snail *Oncomelania nosophora* in 48 hours exposure in an immersion test.

The molluscicidal potency of Endod remains unaffected at a pH range of 4 to 9; the activity is directly increased by increase in water temperature from 5°C to 35°C; it is unaffected by ultraviolet light from the sun; and its effect is not reduced by adhesion to different concentrations of organic and inorganic matter in water. The molluscicidal potency of the dry berries is unaffected by storage for over five years, but in water solution the material decomposes rapidly (primarily due to microbial activity) and loses its molluscicidal potency in 2 to 3 days. This biodegradability of Endod could be a useful advantage because of the serious residual effects that other pesticides impose on the ecology of the environment.

---

* *Present address:* Stanford Research Institute, Menlo Park, California 94025, U.S.A.

** Hand-collected berries dried in the sun for several days, mortar ground to fine powder, and dissolved in deionized water on a weight/volume basis. This was used in all of the tests reported herein, but a freeze-dried material of the filtrate from the above solution is more than twice as potent as the ground berries.

Effect on snail eggs

Endod is not ovicidal at molluscicidal concentrations. The LC_{50} for eggs is 300 ppm in 24 hours exposure; but this shortcoming can be successfully overcome by two consecutive treatments, the second being applied two weeks after the first, in time to kill all young snails which hatch from unaffected egg masses before they mature to lay new batches of eggs.

Toxicity to aquatic leeches

Endod kills aquatic leeches (*Lymnatis nilotica*) at 2-3 ppm in 6 hours exposure. In many developing countries, aquatic leeches cause serious animal health and some public health problems, mainly due to loss of blood as a result of their attack. The development of methods for the control of such leeches with the use of Endod, a natural product within easy reach to the farmer, could contribute to the animal welfare (both domestic and wild) and also increase the economic return from them. Further studies on this are being planned.

Cercaricidal and miracidicidal properties

Miracidia and cercariae of schistosomes, as well as other species of trematodes, are readily killed by Endod at considerably lower than molluscicidal concentrations. This property of Endod can be used to an advantage for temporary cleaning of infected bodies of water for emergency crossing by military personnel, engineers, public health people, and other field workers.

The major public health measures being used in the control of schistosomiasis so far are limited to three: (1) treatment; (2) killing the intermediate host snail; and (3) health education. The application of large quantities of chemicals in bodies of water to kill snails entails the risk that such chemicals may unfavorably affect the micro- or even macro-ecology of the environment. An alternative to this method or at least an additional measure could be to aim at breaking the life cycle of the parasite by killing the cercaria with "low" (sublethal to snails) doses of Endod or other chemicals which are lethal to them. It could even be possible to "treat" or "free" the snail from the developmental stages of the parasite without necessarily using the high dose needed to kill the snail. The possible use of Endod for such a new approach in the control of schistosomiasis is now under investigation.

Fish toxicity

All the available molluscicides kill fish, and Endod is not an exception. However, generally speaking, fish (*Tilapia* spp.) appear to tolerate slightly higher concentrations of Endod than snails. In field trials with Endod, particularly small fish have been noted to be affected, but they normally repopulate in a few weeks, presumably from surviving adults or egg masses unaffected by the molluscicide. Further quantitative toxicity studies using different species of fish are now being carried out at the Stanford Research Institute.

Mammalian toxicity

The acute oral mamalian toxicity of Endod berries is generally low. The LC_{50} for mice is 2.6 g/kg. body weight; for sheep 6.5 g./kg. body weight; for monkeys 2.5 g./kg. body weight, and birds normally feed on the berries in the forest.

The fact that in general saponins have hemolytic properties and that the active property in Endod is a saponin (although the structure is not yet fully known), may suggest a possible high mammalian toxicity. However, as it has been shown by mammalian toxicity studies, this is not the case with Endod *berries*. The *root* of the Endod plant, however, has high hemolytic saponin and it is known to be very toxic to humans (Watt and Breyer-Brandwijk, 1962). The chopped root boiled in water is drunk as medicine for venereal diseases, anthelmintic treatments and abortion. People are known to suffer considerably or even die from such treatments; they normally take too large quantities of the material. But the same root with its active saponin does not have any effect on snails even at very high (1000 ppm) concentration. This suggests that the saponin which is present in the berries is different from that present in the roots of the Endod plant, as it is definitely less toxic to mammals and highly toxic to snails.

Phytotoxicity

Endod has no phytotoxicity. It does not affect the germination of seeds, the growth rate of various economic plants tested, or the production of fruit. In some cases, it has been observed to act as a fertilizer, as evidenced by increased growth rate of the test plants.

The Endod plant

The Endod plant is a climbing shrub which can easily be cultivated from either seeds or cuttings. Under favourable climatic conditions, it yields fruit in about 6 months from planting and in some areas in Ethiopia, it gives fruit twice a year: December-January and May-June. It is primarily a highland plant growing wild and sometimes cultivated in back yards for its use as soap. The agronomical characteristics of the plant have not yet been fully studied, but as in the case of some insecticides of plant origin, a strain of Endod with high molluscicidal potency could possibly be selected out of the "wild" varieties presently being used. The ecology and botanical characteristics of this plant should be studied in full.

Chemical studies on Endod

Horton (1967) first in Addis Ababa and later in Utah (U.S.A.), was the first to work on the isolation of the active principle in Endod. He determined that the base molecule is an oleanolic acid and speculated that there may be some sugar groups attached to it to make it water soluble. Since then, organic chemists at the Tropical Products Institute in London (King *et al.*, 1968), and at Stanford Research Institute in California (Skinner and Parkhurst, 1967) have worked on it.

Stanford Research Institute has undertaken a systematic approach to develop a procedure for the purification of the ground Endod berries to concentrate the active principle, to determine the structure of the active molecule and the role of the sugar moieties in its activity, and to develop a chemical assay to determine concentrations of the active principle in water. The study will also include a quantitative determination of the biological activity of the purified material against different species of snails, fish, insects, mammals, plants and some microbial and parasitic organisms.

Various analogs of oleanolic acid and other related triterpenes with water-solubilizing groups will be synthesized and evaluated, in the hope of further improving the biological properties of the natural toxin.

Application

Stress is being made on developing simple methods and procedures for the application of Endod in schistosomiasis control schemes so that the whole project can be managed by rural communities on a self-help basis. Crude ground berries or partially purified powder, if the cost of purification is not too high, can be mixed with water to form highly concentrated solutions which can be applied quantitatively on pre-determined volume to the infected body of water to make desired concentrations of the toxin for specified periods.

Studies on the agronomical characteristics of the plant, including the possibilities of growing the plant on shores of streams and swamps for natural control of snails, are yet to be done.

The Adwa Project

After having done the necessary laboratory and some field tests with Endod, the molluscicide was subjected to a large-scale field trial in the control of schistosomiasis on a pilot project basis in Adwa, northern Ethiopia. Adwa represents a relatively isolated focus of schistosomiasis where the human infection rate is about 65%, and two relatively small streams are responsible for its transmission.

Through the use of aerial photographs a detailed map of the town and its vicinity was made and all houses were numbered. With the use of randomly picked numbers, houses (habitats) were traced and stool samples were collected from everyone living there (all sexes and ages of people were asked to give samples). In this way, baseline infection rate data were detained on 10% of the 17,000 inhabitants of the area. The snail populations and infection rates, as well as possible transmission sites along the two rivers in the area were also made and recorded. Adequate procedures which are being repeated in post-treatment surveys were established. In some cases up to 60% of the *Biomphalaria pfeifferi* were found infected with *S. mansoni*, the only species of schistosome affecting man in the area. After adequate pre-treatment baseline data were compiled, the full participation of the community through the Governor General of the Province and the District Governor and Municipality Council was secured,

and the campaign to eradicate the snails with the use of Endod was launched in March 1969. The first annual report on the project has been written in a monograph form (Lemma and Duncan, 1970) and a summary of the project has been published.

At first Endod collected by volunteers from wild plants in the vicinity of Adwa, or material bought from the market, were used. Since the market value for the berries considerably increased as a result of our demand, the plant is now being grown on a large-scale to supply berries for the pilot control project.

For the initial application of Endod, the berries were ground with a mill, made into a highly concentrated emulsion, and applied (in pre-determined quantities) to make 100 ppm for 6 hours in the stream. The concentrated solution was applied from regulated barrels at the head water, or applied directly on the shores in watering cans in much the same way as Bayluscide is used in other countries.

A few days after the first application, 100% kill of snails was obtained and some small fish and tadpoles were noted to be affected. The highly polluted water was considerably cleared up by the application of Endod. A second treatment was made two weeks after the first but this time only 50 ppm was used to kill young snails which hatched out from un-affected eggs. Within a few weeks after these applications, the frog and fish populations in the treated streams had returned to the high level noted prior to treatment. Survey for snails in the treated streams for several months after second treatment showed no snails. A weekly surveillance is at present being carried out and any snails found are eliminated by the application of Endod either in sacs deposited on the spot or by repeated sprinkling of concentrated material over the area. Such surveillance will continue for at least 5 years and the efficacy of such molluscicide on the incidence and prevalence of the disease will be assessed by repeated surveys of the human population. If the control is successful, in five years time the children between 1-5 years should be free from the infection and a significant decrease in the incidence of the disease in the adult population should also be seen. The results from Adwa will be compared with that of a control village about 40 miles away.

If positive results are obtained from this pilot project, it is hoped to demonstrate the value of Endod in the control of schistosomiasis on a local self-help basis. The cost of application and maintenance of such a control measure is being kept to minimum, and the methodology involved in its application is continuously being simplified and standardized.

SUMMARY

Current knowledge on the potential value and use of Endod (*Phytolacca dodecandra*) as a molluscicide for the control of schistosomiasis is presented. Specific subjects discussed are: toxicity of Endod for snails and snail eggs; toxicity to leeches, cercaricidal and miracidicidal properties; toxicity to fish, mammals and plants; a description of the Endod plant; chemical characterization of the active principle; application methods; description of a pilot field trial in northern Ethiopia.

RESUME

Des conaissance courrantes sont presentéer sur la valeur potentielle et utilisation de l'Endod (*Phytolacca dodecandra*) comme un molluquicide pour le control de la schistosomiase.

Les sujets specifiques sont discutés: la toxicité de l'Endod envers les mollusques et oeufs des mollusques; la toxicité envers les sangsues; les proprietés cercaricide et miracidicide; la toxicité envers les poissons, mammiféres et plantes; un description de la plante d'Endod; caractère chimique du principe actif; methodes d'application; description d'un champ d'essai dans le nord de l'Ethiopie.

ACKNOWLEDGEMENT

We are much indebted to His Highness Prince Ras Mengesha Seyoum for his sincere and continued support of the Adwa Project. Without his assistance we would not have been able to do the work.

REFERENCES

Dawood, I. K. (1965) Unpublished data. Egypt-49, Alexandria, United Arab Republic.

Fenwick, A. (1968) Unpublished data. Tropical Pesticides Research Institute, Arusha, Tanzania.

Horton, J. (1968) Preliminary report of investigation of molluscicidal components of *Phytolacca dodecandra*. Wld. Hlth. Org. Mol./Inf./68.24.

King, T. A., Jewers, K., Richardson, H. and Falshaw, C. P. (1968) Endod saponin, the molluscicide of the fruit of Endod (*Phytolacca dodecandra*). Paper presented at the 5th International Symposium on the Chemistry of Natural Products, London, 8-13 July, 1968.

Lemma, A. (1965) Preliminary Report on the molluscicidal properties of Endod, *Phytolacca dodecandra*. *Eth. med. J. 3*, 187.

Lemma, A. (1970) Laboratory and field evaluations of the molluscicidal properties of *Phytolacca dodecandra*. *Bull. Wld. Hlth. Org. 42*, 597.

Lemma, A. and Duncan, J. (1970) First Annual Report of Schistosomiasis Pilot Control Project in Adwa, Ethiopia. Institute of Pathobiology Monograph, Haile Sellassie I University, Addis Ababa.

Montagne and Crossland, N. O. (1968) Unpublished data. Woodstock Agricultural Research Centre, Kent, England.

Powell, J. W. and Whalley, W. B. (1969) Triterpenoid saponins from *Phytolacca dodecandra*. *Phytochemistry 8*, 2105.

Skinner, W. A. and Parkhurst, R. M. (1967) Unpublished data. Stanford Research Institute, Menlo Park, California.

Watt, J. M. and Breyer-Brandwijk, M. G. (1962) Medical and Poisonous Plants of Southern and Eastern Africa. 2nd ed., London: E. and S. Livingstone Ltd.

Yasuraoka, K. (1970) Unpublished data. National Institute of Health, Kamiosaki, Shinagawa-Ku NIH, Tokyo, Japan.

WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTÉ

WHO/SCHISTO/71.8

ENGLISH ONLY

LABORATORY EVALUATION OF THE MOLLUSCICIDAL PROPERTIES OF TRITERPENOID SAPONIN, A MOLLUSCICIDAL FRACTION FROM ENDOD (PHYTOLACCA DODECANDRA), AGAINST ONCOMELANIA NOSOPHORA

by

K. Yasuraoka
Department of Parasitology, National Institute of Health,
Tokyo, Japan

1. INTRODUCTION

Aklilu Lemma (1965) discovered that a molluscicidal fraction occurs naturally in the berries of the Ethiopian plant Endod, Phytolacca dodecandra, which grows in East, West, Central and South Africa and in some parts of South America and Asia. Analytical work on the molluscicidal fraction by hydrolytic degradation, by thin layer chromatography, and by infra-red spectroscopy indicated that triterpenoid saponin was the molluscicidal agent (National Research Development Corporation, London, 1968). Laboratory tests were carried out to evaluate its molluscicidal properties against Oncomelania nosophora.

2. MATERIALS AND METHODS

The triterpenoid saponin used in this test was received in March 1970 through the courtesy of Takeda Chemical Industries Ltd., Tokyo, in whose laboratories it had been extracted from the dried berries of Endod, Phytolacca dodecandra, following the patent procedure originated at the National Research Development Corporation, London. The chemical was dissolved in deionized tap water and a series of appropriate twofold dilutions was prepared under constant stirring, unless otherwise specified. The testing procedure was essentially as described by Komiya et al. (1962). For each concentration, 30 freshly collected adult snails were used, with 10 snails per dish. In all tests a 48-hour exposure period at a temperature of 25°C was applied. Mortality count was made after a 48-hour recovery period in deionized tap water, and the LC_{50} and LC_{90} values were determined by Litchfield & Wilcoxon's (1949) method. The reduction of molluscicidal activity under the influence of various physicochemical factors, such as sunlight, pH and hardness of water, and presence of $CaCO_3$ or yeast, was calculated according to the formula described by Paulini & de Souza (1970).

3. RESULTS

3.1 Efficacy of triterpenoid saponin against O. nosophora in the immersion method

From the snail mortality obtained in a series of dilutions ranging from 0.3125 ppm to 40 ppm of the chemical, the LC_{50} and LC_{90} were calculated as 1.85 (1.5-2.4) ppm and 4.6 (3.4-6.2) ppm respectively.

3.2 Influence of sunlight

Petri dishes each containing 100 ml of the dilutions, in a layer 0.5 cm thick, were exposed to direct sunlight for four hours. Parallel tests were run with NaPCP as reference compound. The irradiated samples and corresponding unirradiated controls were tested for their molluscicidal activity. Table 1 shows the results with the irradiated and control dilutions.

The LC_{50} values in the irradiated and unirradiated dilutions were in the proportion of 3:1, that is to say, there was an apparent deterioration in toxicity and after a four-hour exposure to sunlight a decline in efficacy down to one-third was found in the biological tests. The results with NaPCP showed a more pronounced influence of sunlight on the deterioration in toxicity.

3.3 Influence of pH of water

The pH levels were obtained using sodium hydroxide and hydrochloric reagents. The water was first adjusted to the desired pH, then triterpenoid saponin was added. Oncomelania snails were introduced and the pH was again recorded. The test results (Table 2) indicate that there is no definite dependence on pH.

3.4 Influence of hardness of water

The hard water used in this test was composed of 600 ppm calcium chloride and 139 ppm magnesium chloride. Parallel tests were run with deionized water. The LC_{50} and LC_{90} values obtained and the reduction of molluscicidal activity observed are given in Table 3. Only a moderate loss could be observed with hard water.

3.5 Influence of yeast cells

Commercial yeast has been selected as a test organism for investigating the absorption of molluscicides by living cells in water (Paulini & de Souza, 1970). Experiments were performed to determine whether the efficacy of triterpenoid saponin is reduced by the presence of yeast cells. Experimental techniques were in accordance with those of Paulini & de Souza (1970). Tests consisted of three sets of containers: (1) molluscicide in deionized tap water, without yeast, (2) molluscicide and yeast in deionized tap water, and (3) only yeast in deionized tap water. Table 4 shows the LC_{50} and LC_{90} values obtained in both the presence and absence of yeast extract. This table refers to the experiments in which the proportion of yeast to molluscicide was 1000:1, i.e. 1000 parts yeast for one part molluscicide. As seen in the table, triterpenoid saponin showed no appreciable loss in the presence of yeast cells. In this series of tests lower LC_{50} and LC_{90} values were obtained because after mixing the molluscicide with yeast cells only the supernatant was used for the tests.

3.6 Sorption by calcium carbonate

Paulini & de Souza (1970) studied the influence of calcium carbonate on the toxicity of some molluscicides because natural waters may pass through limestone formations, and also because in certain water treatment processes calcium carbonate may be precipitated. It seemed of interest to determine whether this naturally occurring substance had any influence on triterpenoid saponin. The molluscicide was made up in stock solution of 0.1 per cent. (w/v) in ethanol (99.5 per cent.). Techniques similar to those described by Paulini & de Souza (1970) were used in this test series. Tests were carried out with three parallel runs: (1) deionized tap water with molluscicide; (2) deionized tap water with 0.1 per cent. calcium carbonate; and (3) deionized tap water with molluscicide and calcium carbonate. The LC_{50} and LC_{90} values obtained and the reduction of molluscicidal activity observed are given in Table 5. There was no apparent loss in toxicity in the presence of calcium carbonate. The much lower LC_{50} and LC_{90} values obtained in this series of tests may be ascribed to the fact that the molluscicide was first made up in stock solution of 0.1 per cent. (w/v) in ethanol (99.5 per cent.) instead of water.

TABLE 1. INFLUENCE OF SUNLIGHT ON THE MOLLUSCICIDAL ACTIVITY OF TRITERPENOID SAPONIN AND NaPCP

Product	LC	Irradiated (ppm)	Untreated (ppm)	% reduction
Triterpenoid saponin	50	4.2 (3.1-5.8)	1.5 (1.0-2.4)	64
	90	9.7 (6.7-14.2)	5.5 (2.7-11.3)	43
NaPCP	50	More than 4	0.3 (0.2-0.4)	More than 94
	90	Not computable	0.6 (0.3-1.1)	Not computable

TABLE 2. INFLUENCE OF pH OF WATER ON THE MOLLUSCICIDAL ACTIVITY OF TRITERPENOID SAPONIN

LC	pH of water	
	5.8-6.0	7.6-8.0
50	1.5 (1.3-1.8) ppm	1.5 (1.2-1.9) ppm
90	2.3 (1.9-2.8) ppm	2.5 (2.0-3.2) ppm

TABLE 3. INFLUENCE OF HARDNESS OF WATER ON THE MOLLUSCICIDAL ACTIVITY OF TRITERPENOID SAPONIN

LC	Hard water* (ppm)	Deionized water (ppm)	% reduction
50	2.0 (1.4-2.9)	1.1 (0.8-1.5)	45
90	6.8 (3.9-11.8)	2.2 (1.6-3.1)	68

* 600 ppm calcium chloride plus 139 ppm magnesium chloride.

TABLE 4. INFLUENCE OF YEAST CELL SUSPENSION (1000 ppm) ON THE MOLLUSCICIDAL ACTIVITY OF TRITERPENOID SAPONIN (1 ppm)

LC	With yeast (ppm)	Without yeast (ppm)	% reduction
50	6.8 (5.2-8.8)	5.7 (4.8-6.8)	16
90	22.0 (13.5-35.9)	17.0 (12.3-23.5)	23

TABLE 5. INFLUENCE OF SUSPENDED CALCIUM CARBONATE ON THE MOLLUSCICIDAL ACTIVITY OF TRITERPENOID SAPONIN

LC	With $CaCO_3$ (ppm)	Without $CaCO_3$ (ppm)	% reduction
50	0.78 (0.68-0.89)	0.75 (0.66-0.81)	4
90	1.08 (0.94-1.24)	1.01 (0.85-1.20)	6

4. SUMMARY AND CONCLUSION

Triterpenoid saponin, a molluscicidal fraction from Endod (Phytolacca dodecandra), has been submitted to laboratory tests. The LC_{50} against O. nosophora snails was 1.85 (1.5-2.4) ppm and the LC_{90} 4.6 (3.4-6.2) ppm, for a 48-hour exposure period at 25 °C. The working stability in the presence of various physicochemical factors appears to be satisfactory. Toxicity to plants, fishes and mammals should be taken into consideration in future evaluation.

REFERENCES

Lemma, A. (1965) Ethiop. med. J., 3, 84-92

Lemma, A. (1965) Ethiop. med. J., 3, 187-190

Lemma, A. (1970) Bull. Wld Hlth Org., 42, 597-612

Komiya, Y., Hosaka, Y. & Yasuraoka, K. (1962) Jap. J. med. Sci. Biol., 15, 41-51

Litchfield, J. T. & Wilcoxon, F. (1949) J. Pharmacol. exp. Ther., 96, 99-113

National Research Development Corporation (1968) Patents Act, 1949, Provisional Specification 29760/68

Paulini, E. & de Souza, C. P. (1970) WHO mimeographed document, PD/MOL/70.12

Laboratory evaluation of the molluscicidal potency of a butanol extract of *Phytolacca dodecandra* (endod) berries

S. S. BAALAWY[1]

Abstract

The effect of butanol extracts of endod against Biomphalaria choanomphala, B. pfeifferi, *and* Bulinus (Physopsis) nasutus *was tested at different concentrations and for different exposure periods. Exposure to 19–25 ppm for 6 hours or to 6–7 ppm for 24 hours caused about 100% mortality.*

A number of substances of vegetable origin have been found to have molluscicidal activity. Mozley (1939, 1952) reported that the fruits of *Balanites aegyptiaca*, *Sapindus saponaria*, and *Swartzia madagascarensis* were among the most promising natural molluscicides. An investigation of the molluscicidal properties of the fruits of *Sapindus saponaria* on the snail *Bulinus* (*Physopsis*) *africanus* was made by Msangi & Zeller (1965).

In many developing countries in the tropics, schistosomiasis is endemic and the snails that transmit the disease are widespread. Therefore, large-scale schistosomiasis control programmes based on the control of snails by means of conventional molluscicides may be expensive. Further studies on the possibilities of using vegetable molluscicides are therefore desirable.

Dried berries of *Phytolacca dodecandra* (endod) are widely used in Ethiopia instead of soap for washing clothes. It was observed that in natural bodies of water where endod had been used there

[1] East African Institute for Medical Research, Mwanza, Tanzania.

Reprinted from the
Bulletin of the World Health Organization,
47, 422-425, by permission.

was a high mortality of snails. Subsequently, the molluscicidal effects of various parts of the plant were determined, and the berries were found to have the greatest activity (Lemma, 1970). Lemma (personal communication) has found that butanol extracts of endod are toxic to *Biomphalaria glabrata* snails.

This report describes experiments made to evaluate the molluscicidal effect of butanol extracts of endod against snails collected from Lake Victoria and from streams and ponds in Mwanza, Tanzania. Mature snails of the following species were used: *B. choanomphala* (average shell diameter, 6.5 mm), *B. pfeifferi* (average shell diameter, 11.0 mm), and *Bulinus* (*Physopsis*) *nasutus* (average shell diameter, 10.5 mm). Snails for experimental purposes were kept in the laboratory for 2 days in basins containing lake water at pH 6.4. A total of 20 snails of each species were used for each dosage. Groups of 10 snails were placed for 6 hours in 250-ml plastic glasses containing the following concentrations (ppm) of endod extract: 25, 19, 12, 10, 6, 5, and 3. Other groups of 10 snails per container were exposed to the following concentrations (ppm) for 24 hours: 7, 6, 5, 4, 3, 2, and 1. Concentrations of 3.6 ppm and 3.3 ppm were included in the series for trials with *B. pfeifferi*, and concentrations of 2.6 ppm and 2.3 ppm were included for trials with *B.* (*P.*) *nasutus*. The temperature during exposures was 23° ± 1°C.

After exposure, all snails were washed in clean lake water and left for 24 hours to recover in clean lake water before being reexamined. Death was determined by the absence of movement and failure of the snail to withdraw inside its shell when touched with a needle; characteristic bleeding was frequently also a useful indication of death.

Statistical estimations of the LD_{50} and LD_{90} values were calculated from the probit transformation as described by Finney (1947), and the fiducial limits of the median lethal dose at the 95% probability level were obtained.

Results

The death rates for snails exposed for 6 and 24 hours to different concentrations of endod extract are shown in Table 1. Clear differences in mortality rates at the two exposure periods were observed. While exposure of snails for 6 hours to higher concentrations (19–25 ppm) caused a mortality rate of about 100%, the same effect was obtained with exposure for 24 hours to concentrations of 6–7 ppm.

Table 1. Number of dead snails out of 20 exposed to different concentrations of endod extract [a]

Species of snail	Exposure for 6 hours							Exposure for 24 hours									
	Concentration (ppm) of endod extract							Concentration (ppm) of endod extract									
	25	19	13	9	6	5	3	7.0	6.0	5.0	4.0	3.6	3.3	3	2.6	2.3	2.0	1.0
B. choanomphala	20 (100)	19 (95)	13 (65)	10 (50)	4 (20)	4 (20)	1 (5)	20 (100)	19 (95)	16 (80)	15 (75)	—	—	10 (50)	—	—	5 (25)	1 (5)
B. (P.) nasutus	20 (100)	20 (100)	20 (100)	18 (90)	15 (75)	2 (10)	1 (5)	20 (100)	20 (100)	20 (100)	20 (100)	—	—	15 (75)	13 (65)	13 (65)	6 (30)	0 (0)
B. pfeifferi	20 (100)	18 (90)	14 (70)	8 (40)	7 (35)	2 (10)	1 (5)	20 (100)	20 (100)	20 (100)	17 (85)	16 (80)	14 (70)	4 (20)	—	—	0 (0)	0 (0)

[a] Percentage mortality rates in parentheses.

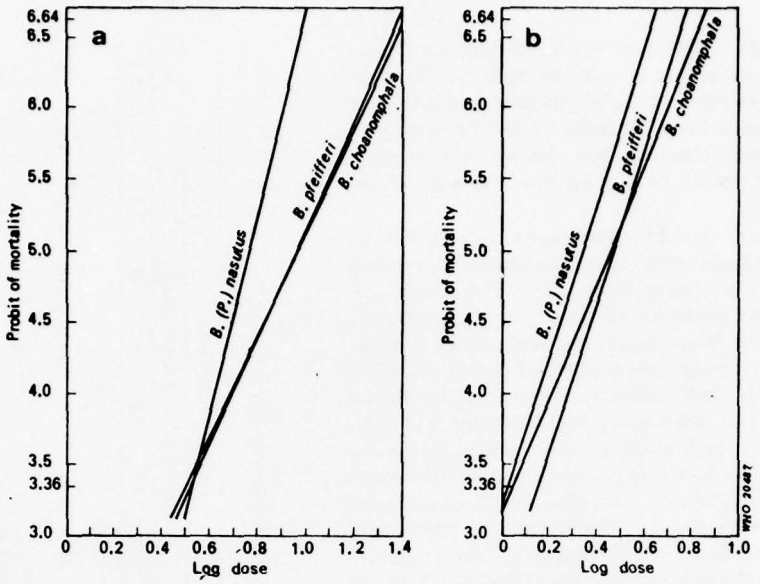


Fig. 1. Relationship of log dose to mortality (probit scale) for snails exposed to endod extract for (a) 6 hours, and (b) 24 hours.

The log-dose–probit lines plotted from the test data are given in Fig. 1. The LD_{50} and LD_{90} values calculated from the data are shown in Table 2.

An analysis of the results showed that the calculated LD_{50} and LD_{90} values for both species of *Biomphalaria* were nearly the same for both exposure periods. For *B.* (*P.*) *nasutus* the LD_{50} and LD_{90} values were about half those for the other two species of snail for the 6-hour exposure period but only slightly less than those for the other species for the 24-hour exposure period. In general, the endod extract was more effective against *B.* (*P.*) *nasutus*.

For an exposure period of 24 hours the slope function values for all species of snail are nearly the same and for an exposure period of 6 hours the values for *B. pfeifferi* and *B. choanomphala* are almost the same, but compared with that for *B.* (*P.*) *nasutus* the slopes are less steep.

Discussion

An LD_{90} value of about 20 ppm for a 6-hour exposure period and a value of about 5 ppm for a 24-hour exposure period in a natural molluscicide are noteworthy results. Since endod is a natural material that can be produced under suitable climatic conditions in the tropics, and since the extraction

Table 2. Statistical analysis of expected effective doses of endod for 3 species of snail

Species of snail	Exposure for 6 hours				Exposure for 24 hours			
	LD_{50} (ppm)	LD_{90} (ppm)	Slope function (S)	Lower and upper values of LD_{50} at 95 % probability level (ppm)	LD_{50} (ppm)	LD_{90} (ppm)	Slope function (S)	Lower and upper values of LD_{50} at 95 % probability level (ppm)
B. choanomphala	9.0	20.4	1.9	7.6–10.7	2.8	5.9	1.8	2.4–3.3
B. (*P.*) *nasutus*	5.8	8.8	1.5	5.2–6.5	2.1	3.9	1.6	1.7–2.4
B. pfeifferi	9.9	19.2	1.7	8.7–11.1	2.9	5.2	1.6	2.3–3.5

procedures are neither complicated nor expensive, it can be used with reasonable economy in field programmes for the control of snail vectors of disease.

The effective dose of endod varies somewhat for the different species of snail tested; in exposures for 6 hours 100% mortality was recorded at 25 ppm in both species of *Biomphalaria* but only half that concentration was needed to kill all *B.* (*P.*) *nasutus*. Responses to the extract by the three species of snail were approximately the same after 24-hour exposures.

These differences in toxicity will result in different field application costs, depending on the species of snail to be controlled. In the laboratory, low concentrations of the extract apparently irritated snails of the species *B.* (*P.*) *nasutus* inducing them to crawl out of the container. Under field conditions, therefore, care will be needed to choose a suitable dosage level since a sublethal dose may induce the snails to avoid contact with the treated water.

ACKNOWLEDGEMENTS

The author thanks Dr Aklilu Lemma, Stanford Research Institute, Calif., USA, for supplying the sample of endod extract, and Mr H. G. Moyo, laboratory assistant, for his help during the experiment. Dr V. M. Eyakuze, Director, East African Institute for Medical Research is thanked for his encouragement. The report is published by kind permission of the Secretary-General of the East African Community.

REFERENCES

Finney, D. J. (1947) *Probit analysis*, Cambridge, University Press

Lemma, A. (1970) *Bull. Wld Hlth Org.*, **42**, 597

Mozley, A. (1939) *Trans. roy Soc. Edinb.*, **59**, 721

Mozley, A. (1952) *Molluscicides*, London, Lewis

Msangi, A. S. & Zeller, C. (1965) *Proc. E. Afr. Acad.*, **3**, 52

Unpublished Research Note No. 12

ANTIMICROBIAL AND ANTHELMINTIC ACTIVITIES OF ENDOD (PHYTOLACCA DODECANDRA)

Aklilu Lemma, Andrew Maxwell and Gerald Brody
Institute of Pathobiology and Stanford Research Institute, 1972

Endod is the Ethiopian name for the pokeberry plant, Phytolacca dodecandra, whose berries are ground and used in various parts of the world as a soap for washing clothes. This use of the plant in Ethiopia led to the discovery of its molluscicidal properties (1). Endod is now being developed for use in the control of snail-borne diseases such as schistosomiasis and fascioliasis. A simple method for butanol extraction of the active principle from the berries has been developed (2); the active principle has been found to be the glycoside of oleanolic acid (3,4,5).

In addition to its use as a soap, endod is widely used as "folk medicine" for a variety of ailments. It is believed to be a hemostatic agent, a remedy for urinogenital conditions (including gonorrhea), and for tapeworm infections (6).

In the present study, endod was evaluated for antimicrobial and anthelmintic activities.

MATERIALS AND METHODS

Endod. Endod berries collected in Ethiopia and extracted with butanol according to the procedures described by Lemma et al. (2) were used in all experiments reported herein. In this paper, the term endod is used for the butanol extract of the berries of Phytolacca dodecandra.

Antiviral tests. Possible antiviral activity of endod was tested against six viruses in roller-tube culture systems, using rhesus monkey kidney cells.

Antibacterial and antifungal tests. Five bacteria and 12 fungi (see Table 1) were tested for susceptibility to endod.

Gram-positive bacteria were grown in brain-heart influsion broth and gram-negative ones in trypticase soy broth. After 24 hours of growth at 37°C, the organisms were diluted to a concentration of approximately 1 x 10^8 cells per milliliter with the appropriate growth medium, and 0.05 ml of this suspension was added to the various dilutions of the test compound.

The two yeasts, Cryptococcus neoformans and Candida albicans, were cultured for 48 hours at 37°C in Sabouraud dextrose agar slants. The cells were then suspended in Sabouraud dextrose broth and treated as described for the bacteria. The filamentous fungi were cultured at room temperature (22±2°C) for 14 days. The "mat" of fungi was removed from the agar slant and homogenized to a fine suspension in Sabouraud dextrose broth. This suspension was diluted to a uniform concentration (OD of 0.1 at 600 nm), and 0.05 ml was used for inoculating various dilutions of the test compound.

The compound was tested at concentrations of 100, 50, 10, 5, 1, 0.1 and 0 (control) µg/ml; in some cases, a concentration of 1,000 µg/ml was also tested. Once the test compound was inoculated with an organism, it was incubated for the following periods of time before the minimal inhibitory concentration (MIC) was determined: bacteria, 24 hours;

yeast, 48 hours; and filamentous fungi, 14 days or more--until the controls showed good growth.

The MIC was designated as the concentration at which no visible growth occurred (bacteriostatic or fungistatic concentration). A sample was taken from the tubes in which no growth occurred and was added to fresh medium containing no test compound. The concentration at which no growth occurred was designated as the minimum lethal concentration (MLC). These samples were incubated for at least 14 days to determine if latent growth could be detected before final results were recorded.

Antiprotozoan tests. Active cultures (4-6 days old) of Entamoeba histolytica, grown in diphasic egg medium, and Leishmania enriettii, grown in modified blood-agar medium, were used in these tests. Various concentrations of endod were prepared in buffered balanced salt solution; 1 ml of this test solution was mixed with 1 ml of culture. After incubation at room temperature for 24 hours, mortality (lysis and/or immobilization of the organisms) at each concentration was recorded.

Animal experimentation. For assessment of curative effects of endod against L. enriettii infection in the guinea pig, 10% endod powder in neutral unibase ointment (commercial grade) was mixed manually and applied topically on the ulcerative sores. The medication was applied every other day.

Weanling (about 4 weeks old) Duncan-Hartley guinea pigs were used in all tests. The animals were inoculated in the ear or freshly shaved areas of the back with about 1 to 2 x 10^6 freshly isolated promastigotes. Under normal conditions, such animals progressively develop and maintain a localized ulcerative sore for about 8 to 10 weeks, after which time the guinea pigs build enough immunity to arrest the infection. In some cases (5 to 10%), the immune response of individual guinea pigs fails to respond to the infection; the parasites then continuously multiply and spread to all the extremities of the animal, finally killing it.

The reason for shaving the skin at the site of inoculation is to provide a cooler place for the organisms to develop. They are unable to develop in the internal organs or under hair-covered skin.

Anthelmintic tests. The anthelmintic activities of endod were evaluated against four helminths in the mouse. The sources of the parasites and the test procedures used were those described by Brody and Elward (7).

RESULTS

Effect on viruses. The viruses used in this test were pilio virus (Type 1), ECHO-12, influenza A-2, vesicular stomatitis, adeno virus 7, and vaccinia.

In preparation for evaluation of the antiviral activity, the highest concentration of endod that could be tolerated by the cell culture without incurring obvious cellular damage was determined. The highest nontoxic level, or one-half of the cytotoxic concentration of the compound, was then used as the primary test level.

Endod was found to be cytotoxic at 4 µg/ml. At the test level of 2 µg/ml, it did not exhibit any antiviral activity against any of the six viruses.

Effect on bacteria and fungi (Table 1). Endod did not exhibit any

antibacterial activity against the five species of bacteria tested, even at concentrations as high as 1,000 µg/ml.

A striking selective activity against dermatophytes but not against systemic fungi was particularly interesting. Whereas systemic fungi were unaffected by up to 1,000 µg/ml, dermatophytes were killed by about 10 µg/ml.

Effect on protozoa. Both E. histolytica and L. enriettii were killed at 10 ug/ml within 24 hours. The sensitivity of these protozoa to endod was similar to that of dermatophytes.

Evaluation of antileishmanial therapeutic value of endod. Based on the knowledge of the sensitivity of promastigotes to the lytic action of endod, experiments were undertaken to determine its possible therapeutic value in animals. Cutaneous leishmaniasis caused by L. enriettii in guinea pigs was used as a model for the study.

In the first experiment (Table 2), 15 guinea pigs with 4-week-old active ulcerative leishmanial sores were divided into three groups of five. One group was treated with 10% endod in unibase ointment, another group was treated with unibase ointment alone, and the third group was not given any treatment. After 4 weeks of treatment, 2 of the 5 endod-greated animals were healed whereas all the animals in the two control groups still had active sores.

The second experiment was designed to test any prophylactic or inhibitory effect endod may have on the early developmental stages of leishmanial infection. Nine weanling guinea pigs were each inoculated with 2×10^6 infective promastigotes and were placed into three equal groups. Immediately after infection, one group was treated over the site of inoculation with 10% endod in unibase, another group was treated with unibase ointment alone, and the third was left as untreated controls. Weekly observations on the development of lesions showed that 2 of the 3 endod-treated animals and all of the animals in the two control groups developed ulcerative sores (Table 3). Both treated and untreated animals healed at about the same rate.

In the third experiment, the possibility of enhancing the therapeutic value of endod by using dimethyl sulfoxide (DMSO) as a solvent to carry it into deep tissues was explored. Twenty guinea pigs, each with about 4-week-old ulcerative leishmanial sores, were divided into two groups. One group was treated with 10% endod in a 20:80 (v/w) mixture of DMSO: unibase (endod dissolved readily in DMSO). The other group was treated with only the solvent.

The results after 2 and 4 weeks of treatment are summarized in Table 4. DMSO did not have any enhancing effect on the therapeutic value of endod against leishmanial infections.

Effect on helminths. The anthelmintic activity of endod was tested with Nematospiroides dubius, Hymenolepis nana, Syphacia obvelata and Aspiculuris tetraptera. Two types of in vivo experiments were conducted. In the first, mice were started on medicated rations, containing 1,000 µg/g endod, one day prior to inducing infection and were continued on medication for 17 days in order to detect activity against the various developmental stages of the test helminths. In the second, endod was examined for anthelmintic activity against mature infections of the four test helminths in mice, using a 3-day per os regimen at 320 mg/kg/day. Endod did not exhibit any anthelmintic activity against the four test helminths in either medication regimen.

Under *in vitro* conditions, endod dit not inhibit the embryonation of *Ascaris suum* ova, even at a concentration of 1,000 ppm. The action of endod against *Schistosoma* miracidia and cercariae was also determined. Miracidia were immobilized and killed at 1 ppm within 30 minutes and Cercariae were immobilized and killed at 10 ppm within 1 hour of exposure.

DISCUSSION

In spite of traditional beliefs that endod has some medicinal value, our studies showed that it has no antiviral, antibacterial or anthelmintic activity at the levels tested. However, numerous historical reports indicate that this plant has been used by indigenous people for the treatment of *Taenia saginata* infection (6). Our failure to detect its anthelmintic activity against the mouse tapeworm *H. nana* may be due to various factors, including differences in dosage, hosts, and the drug sensitivity of the worms.

Although endod dit not show any antiviral or antibacterial activity, its selective antifungal properties were pronounced. Dermetophytes were highly susceptible to the lethal action of endod. The reason for this selectivity is unknown, but basic physiological differences between the two groups of fungi are widely recognized. The chemotherapeutic value of endod against dermatophytes may be worth exploring further. In this regard, it is interesting to note that most of the molluscicides now in use (copper sulfate, sodium pentachlorophenate, and Bayluscide^{R} are also known to be fungicides.

Although the *in vitro* antileishmanial activity of endod was very high, the results of the chemotherapeutic trials were not very encouraging. The treatment regimens needed were too long (4 weeks or more). Endod also did not have any prophylactic or suppressive effect in early infections. The use of DMSO as a vehicle for carrying endod into the infected tissues did not enhance its chemotherapeutic value. A likely reason for failure of the topical treatment is the intracellular location of the organisms and the inability of the medication to gain access to the organisms.

One of the drugs used for the treatment of cutaneous leishmaniasis is Amphotericin-B, a well-known, but highly toxic, antifungal drug. This association of antifungal and antileishmanial activities suggests a possible pathway in the search for new antileishmanial agents.

An added objective to our undertaking topical application tests was to determine the toxicity of endod on an open wound, as endod is known to be highly hemolytic. It can completely hemolyze rabbit red blood cells within 30 minutes at a concentration of about 0.1 µg/ml. The possibility of such material penetrating through open wounds of human handlers of this molluscicide is an important consideration. The application of up to 10% endod in unibase, with or without DMSO, on large, ulcerative, and occasionally bleeding sores had no visible harmful effect in the 18 guinea pigs thus treated. In fact, there was some detectable improvement in lesion size and general condition of the animals.

In spite of the emetic properties of endod (6), sufficient quantities (5-10 ppm) of the solution may be passed through the intestinal tract and possibly be useful in the treatment of intestinal amoebiasis. The effect of endod against other intestinal protozoa should also be investigated.

The anti-miracidial and anti-cercarial properties of endod could alsbe usefully employed as one aspect of a multiple approach to the control of schistosomiasis. Application of the molluscicide in contaminated water will render the water free of snails, and also will eliminate miracidia and cercariae, thus preventing infection of any surviving snails and making the water safe for immediate human use.

TABLE 1. Antibacterial and antifungal activity of endod

Species	MIC[1] (µg/ml)	MLC[2] (µg/ml)
Bacteria:		
Staphylococcus aureus ATCC 12600	1,000	1,000
Bacillus subtilis ATCC 6633	1,000	1,000
Pseudomonas aeruginosa ATCC 10145	1,000	1,000
Salmonella paratyphi ATCC 9150	1,000	1,000
Escherichia coli	1,000	1,000
Fungi:		
Candida albicans	1,000	1,000
Cryptococcus neoformans	1,000	1,000
Microsporum audouini	10	10
Microsporum canis	10	--
Trichophyton mentagrophytes	10	50
Epidermophyton floccsum	10	50
Rhizoctonia solani	100	100
Alternaria solani	100	100
Helminthosporium gramineum	100	100
Aspergillus fumigatus	1,000	1,000
Rhizoctonia stolonifer	1,000	1,000
Fusarium roseum	1,000	1,000

[1]Minimum inhibitory concentraion [2]Minimum lethal concentration

TABLE 2. Therapeutic effect of endod against cutaneous leishmaniasis in the guinea pig[a]

Treatment	No. of guinea pigs	Result after 2 wks of treatment		Result after 4 wks of treatment	
		Improved	Healed	Improved	Healed
10% endod in unibase	5	2	0	1	2
Unibase only	5	0	0	0	0[b]
No treatment	5	0	0	0	0[c]

[a]Each of 15 animals was inoculated with 2 x 10^6 L. enriettii promastigotes on the ear and/or back 4 weeks prior to test; all developed active ulcerating sores.

[b]One animal had a severe diffuse desion and died during the third week of observation.

[c]One animal had spreading lesions.

TABLE 3. Prophylactic and inhibitory effect of endod on the development of cutaneous leishmaniasis in the guinea pig[a]

Treatment	No. of guinea pigs	Development of lesions after treatment		
		Lesion beginning (2-4 weeks)	Active sores (6-8 weeks)	Healing (10-12 weeks)
10% endod in unibase	3	2 showed some lesions	2 had active sores	All healed
Unibase alone	3	All developed lesions	All had active sores	All healed
No treatment	3	All developed lesions	All had active sores	All healed

[a]Each of 9 animals was inoculated with 2×10^6 freshly isolated L. enrietti promastigotes on a shaved area of the back. Treatment commenced on the day of inoculation of organisms.

TABLE 4. Therapeutic value of endod in DMSO and unibase on cutaneous leishmaniasis in the guinea pig[a]

Treatment	No. of guinea pigs	State of lesions after treatment	
		2 weeks	4 weeks
10% endod in 20:80 (v/w) DMSO;unibase	10	4 healed 3 "improved" 3 no change	6 healed 2 "improved" 2 no change[b]
20:80 (v/w) DMSO:unibase	10	3 healed 3 "improved" 4 no change	3 healed 3 "improved" 4 no change

[a]All animals had 4-week-old active ulcerated sores at the beginning of the experiment.

[b]One had a metastatic lesion.

LITERATURE CITED

1. Lemma, 1965. A preliminary report of the molluscicidal property of endod (Phytolacca dodecandra). Eth. Med. J. 3: 187-190.
2. Lemma, A., G. Brody, G.W. Newell, R.M. Parkhurst and W.A. Skinner. 1972. Studies on the molluscicidal properties of Endod (Phytolacca dodecandra): I. Increased potency with butanol extraction. J. Parasitol. 58: 104-107.
3. Parkhurst, R.M., D.W. Thomas, W.A. Skinner and L.W. Cary. 1973a. Molluscicidal Saponins of Phytolacca dodecandra. I. Lemmatoxin-A. Phytochem. 12: 1437-1442.
4. Parkhurst, R.M., D.W. Thomas, W.A. Skinner and L.W. Cary. 1973b. Molluscicidal saponins of Phytolacca dodecandra. II. Lemmatoxin-B. Canad. J. Chem. 52: 702-705.
5. Parkhurst, R.M., D.W. Thomas, W.A. Skinner and L.W. Cary. 1973c. Molluscicidal saponins of Phytolacca dodecandra. III. Lemmatoxin-C. Ind. J. Chem. 11: 1192-1195.
6. Watt, J.M. and Meyer-Brandwijk, M.G. 1962. The medicinal and poisonous plants of eastern and southern Africa. 2nd. ed. Edinburgh: E.& S. Livingston Ltd.
7. Brody, G. and Elward, T.E. 1971. Comparitive responses of 29 known anthelmintics under standardized drug-diet and gavage medication regimens against four helminth species in mice. J. Parsit. 57:1068-1077.

THE JOURNAL OF PARASITOLOGY
Vol. 58, No. 1, February 1972, p. 104–107

STUDIES ON THE MOLLUSCICIDAL PROPERTIES OF ENDOD (*PHYTOLACCA DODECANDRA*): I. INCREASED POTENCY WITH BUTANOL EXTRACTION

Aklilu Lemma,* Gerald Brody, Gordon W. Newell, R. M. Parkhurst, and W. A. Skinner
Life Sciences Division, Stanford Research Institute, Menlo Park, California 94025

ABSTRACT: Dried, ground-up berries of endod (*Phytolacca dodecandra*), suspended in water at 15 to 30 ppm, will kill *Biomphalaria* snails in 24 hr. Butanol extraction of the aqueous suspension of the berries increases its potency by 7- to 10-fold. The extraction procedure is simple and amenable to inexpensive utilization in tropical areas where the plant can be grown, processed, and used as needed. The extract remains stable under various conditions of storage and has very low mammalian oral and skin toxicities. It is not significantly affected by variations in environmental factors such as pH (5 to 9), ambient temperature, ultraviolet light from the sun, and presence of different concentrations of organic matter (up to 1,000 ppm of rabbit feces). As in the case of other available molluscicides, endod is piscicidal at molluscicidal concentrations. The extract is also nonovicidal at molluscicidal levels, but this can be successfully overcome by multiple treatments timed to kill first the adults and then the young snails that hatch out of unaffected eggs.

Schistosomiasis is a parasitic disease that affects over 200 million people in tropical and subtropical areas of the world, where it poses a threat to the health of the people and to the agricultural development of the affected countries. Well-intended development projects in these countries unfortunately provide new breeding habitats for the vector snail. At present, the most effective way to combat schistosomiasis and other snail-borne diseases is to control the snails with safe and available molluscicides. Most of the chemical and synthetic molluscicides presently available commercially are expensive, and difficulties in application and other disadvantages are widely recognized. The high cost and hard currency involved in the purchase and importation of these molluscicides, and the large quantities that must be continually applied in order to have adequate snail control, are some of the major factors limiting their use in developing countries. An effective and safe molluscicide that can be produced locally, rather than imported, would be an important contribution to the struggle against the highly endemic and rapidly spreading schistosomiasis in these countries.

Discovery of the molluscicidal properties of the berries of the Ethiopian herb endod (*Phytolacca dodecandra*) (Lemma, 1965), was followed by comprehensive laboratory and field evaluation of the potency of this natural product for a number of snail intermediate hosts of schistosomiasis and fascioliasis (Lemma, 1970; Lemma and Duncan, 1970; Yasuraoka, 1971). Sun-dried berries, ground up and suspended in water at concentrations of 15 to 30 parts per million (ppm), will kill *Schistosoma*- and *Fasciola*-transmitting snails in 24 hr. This toxicity is unaffected by variation in pH (5 to 9) and the presence of different concentrations of organic and inorganic matter in the test water (Lemma, 1970). Large-scale field evaluation of endod in a schistosomiasis control pilot project in Ethiopia revealed that dry ground berries suspended in water and applied at a concentration of 100 ppm for 6 hr were effective in controlling the vector snail, *Biomphalaria pfeifferi* (Lemma and Duncan, 1970). Several attempts to isolate and characterize the active molluscicidal component in the berries have been made. Horton (1968) speculated that the active principle may be the glycoside or glycuronide of oleanolic acid in the berry. King and co-workers (1968) and Powell and Whalley (1969) found different sugars present but did not show structural relationships of these sugars to the oleanolic acid.

Procedures for development of an effective and simple butanol extract of endod are developed. Preliminary evaluation of the biological activities of the extract against snails,

Received for publication 5 August 1971.

* On sabbatical leave from Haile Selassie I University, Addis Ababa, Ethiopia.

fish, and mammals are reported here. Detailed studies on the chemical structure of the active principle, results of quantitative toxicological evaluations of the butanol extract, and the economics of its production will be published elsewhere.

MATERIALS AND METHODS

I. Source of berries and extraction procedure

Endod berries, collected from wild plants in Ethiopia, were sun-dried for 2 to 3 days, ground to a fine powder, and used as stock material. The butanol extract of the homogenized berries proved to have the highest molluscicidal activity. To obtain 5 kg of this extract, 25 kg of ground berries were extracted with 80 liters of light petroleum ether by percolation. Evaporation *in vacuo* of the petroleum ether gave about 200 g of a green wax, which was inactive against snails. The defatted ground berries were extracted twice with 200 liters of warm water; each time the mixture was allowed to stand overnight. Solids were removed by centrifugation to yield 400 liters of a clear brown solution. The inactive solid residue, half the weight of the original material, was discarded. The remaining aqueous solution was then extracted twice with 200 liters of n-butanol, which yielded 5 kg upon evaporation. After further washing with diethyl ether, the product was a light tan powder. When *Biomphalaria glabrata* were exposed for 24 hr to the butanol extract at 26 C and pH of 7.4, the LC_{90} (lethal concentration to kill 90% of the snails) of the extract was 3.0 parts per million (ppm); the corresponding value for the crude material was 22.3 ppm.

When a small amount of water is added to endod powder, it forms a gummy paste that is difficult to extract and the residue is difficult to filter or centrifuge. Also, when large quantities of aqueous solution of endod are mixed with butanol, an emulsion is formed that is very difficult to separate. To overcome these difficulties, we develop the following simple procedure, one amenable to inexpensive utilization in tropical areas where the plant is endemic and the need for a cheap and safe molluscicide is greatest.

1. Ground berries are extracted with ether to remove inert fat-soluble materials.
2. Five parts of air-dried defatted powder are soaked in 8 parts of warm water. Without preliminary soaking, butanol does not extract active molluscicidal material, but excess water will extract sugars unrelated to molluscicidal action.
3. Butanol is added to the soaked powder (1:2 by volume), gently mixed, and poured into a percolation column, where the butanol extract is allowed to percolate through, then evaporated to dryness.
4. The product can be prepared as a fine powder (by complete evaporation of the butanol), as pellets or briquettes of different size and hardness (by compression), or as a highly concentrated emulsion (by partial evaporation of the solvent). The variety of formulation of this molluscicide permits application under different conditions.

TABLE I. *Toxicity of powdered crude endod berries compared with that of butanol extract against* Biomphalaria glabrata. *(Results are averages of four replicas, or 40 snails per group.)*

Exposure time (hr)	LC_{90} (with 95% confidence limits)	
	Powdered berries	Butanol extract
1	713.9 (550.8–925.3)	71.5 (67.2–74.3)
6	84.1 (69.3–102.2)	13.3 (11.3–15.8)
24	22.3 (18.6–27.2)	3.0 (2.7– 3.4)

II. Molluscicidal test procedures

Molluscicidal tests were conducted according to procedures recommended by the World Health Organization (1961). Five snails were exposed in 200-ml disposable paper cups, 2 cups per test dilution in dechlorinated water (pH 7.4), prepared in twofold serial dilutions. Mortality was recorded after the test snails were allowed a 24-hr recovery period in dechlorinated tap water. The data were analyzed by probit analysis method (Miller and Tainter, 1944).

III. Snails

Test snails were *Biomphalaria glabrata* (albino NIH strain), *Biomphalaria alexandrina* (from Egypt), and *Bulinis truncatus* (from Egypt), kindly supplied by Donald Heyneman, Hooper Foundation, University of California, San Francisco. The snails were reared in dechlorinated tap water at pH 7.4, 26 C, and fed with lettuce. *B. glabrata* of about 10-mm diameter were used in most tests.

RESULTS

A comparison of the molluscicidal activity of crude endod berries and the butanol extract is given in Table I. The butanol extract is about 7 to 10 times more toxic than the crude berries to all snails tested; the LC_{90} (24 hr) of the butanol extract against *B. glabrata*, *B. alexandrina*, and *B. truncatus* was 3.0, 3.2, and 2.8 ppm, respectively.

The potency of the butanol extract is nearly constant in the pH range 6 to 8 (LC_{90} 3.0 ppm), increases slightly at pH 5 (LC_{90} 2.0 ppm), and decreases at pH 9 (LC_{90} 10 ppm). An increase in ambient temperature raises the toxicity of the butanol extract; the LC_{90} was 3 ppm at 22 C compared to 2 ppm at 32 C.

To determine the degree of adsorption of

endod and copper sulfate by organic matter concentrations of 1, 2, 4, and 8 ppm of each molluscicide were prepared in water containing a 1,000-ppm suspension of dried and finely powdered rabbit feces. The solution was allowed to mix and interact for 24 hr, with occasional shaking. The molluscicidal potency of endod before and after such treatment was unchanged (LC_{90} 3 ppm), whereas that of copper sulfate was reduced by 800% (from 1 to 8 ppm).

The molluscicidal potency of the butanol extract was also unaffected by ultraviolet light when different concentrations of it, in 200-ml plastic drinking cups, were exposed for 10 hr to bright sunlight or an artificial ultraviolet light emitting 1,000 ergs/sec/cm^2. In a comparable test, Bayluscide® lost 50% of its activity.

Neither butanol extract nor crude endod affects unhatched snails at molluscicidal concentrations. Because of this shortcoming multiple successive treatments, over a period of 3 to 4 weeks, are required to kill newly hatched snails. Such treatment would be normal procedure in any snail control program and poses no serious problem in the application of endod.

The molluscicidal activity of endod in crude powder form remains stable at room temperature in specimen jars for more than 5 years, the maximum period tested. The butanol extract, similarly stored for 1 year so far, also appears to remain stable. However, an aqueous solution containing 100 ppm of the powdered berries lost molluscicidal potency in 3 to 4 days, primarily due to microbial degradation of the sugars (Lemma, 1970). A 25-ppm aqueous solution of butanol extract remained active up to 45 days. Prolonged stability of the butanol extract in working dilutions and its eventual biodegradability are the very desirable properties of endod.

Our preliminary fish toxicity studies on the butanol extract showed an LC_{90} (24 hr) of 1.5 ppm for sticklebacks (*Gasterosteus aculeatus*), 3.1 ppm for gambusia (*Gambusia affinis*), and 3.6 ppm for goldfish (*Carassius auratus*). Endod is therefore, like all other molluscicides, toxic to fish at molluscicidal levels.

Our mammalian toxicity studies showed that the extract is a potent emetic for monkeys, dogs, and cats. There were no deaths with oral doses as high as 5,120 mg/kg. Emesis occurred at doses as low as 5 mg/kg; lower doses did not appear to have any effect on these animals. The oral LD_{50} in nonregurgitating animals was 1,450 mg/kg for rats, 1,200 mg/kg for mice, and 287 mg/kg for guinea pigs. An aqueous paste of the extract did not cause irritation when applied to abraded or unabraded skin of rabbits. When the dry powder (54 mg) was applied to the rabbit eye, it induced severe irritation in unwashed eyes and milder reactions when the eyes were washed within 2 min after application. When inhaled by humans, the dusty powder was irritating to the mucous membrane, causing transient coughing and sneezing, but no other apparent effect.

TABLE II. *Comparative laboratory and field application doses of different molluscicides.*

Molluscicide	LC_{90} (ppm, 24 hr)	Laboratory (ppm-hr)	Field (ppm-hr)
Crude endod	22	528	600*
Butanol extract of endod	3	72	80 (present work)
Sodium pentachlorophenate	3	72	80†‡
Copper sulfate	1	24	200†‡
Bayluscide	0.2	5	8†§

* Lemma and Duncan (1970).
† WHO (1961).
‡ WHO (1965).
§ Strufe and Gönnert (1962).

The molluscicidal activities of endod and other chemical synthetic molluscicides tested in the laboratory and field are summarized in Table II.

Concentrations of the molluscicide required for a given mortality, multiplied by the time needed, is a constant for each molluscicide tested under standard conditions against a given snail species. This factor, concentrations × time, expressed in ppm-hr, is widely used to compare potencies of different molluscicides (WHO, 1965a).

DISCUSSION

Molluscicidal values for butanol extract of endod are comparable to those for sodium pentachlorophenate, a molluscicide in use for over 50 years in schistosomiasis control. However, the latter has some major disadvantages: it is a skin irritant, it is inactivated by solar ultraviolet light (Strufe and Gönnert, 1962),

and it is a repellent to snails (WHO, 1965b). These disadvantages are not found in endod (Lemma, 1970). Copper sulfate, another molluscicide in use for many years, is rapidly adsorbed by mud and organic matter. In dechlorinated tap water in the laboratory, copper sulfate is about 3 times more active than endod, but in the field the concentration of copper sulfate needed is more than twice that for endod (200 ppm-hr for copper sulfate, 80 ppm-hr for butanol extract). In spite of being partially inactivated by ultraviolet light, Bayluscide still is generally considered the best available molluscicide, being ovicidal at molluscicidal concentrations. An important advantage of the endod extract over Bayluscide, an expensive molluscicide, is that it is a natural product that can be locally grown and purified at nominal cost. This economic factor is of crucial importance in many regions hyperendemic for schistosomiasis. In Ethiopia the crude berry is widely used as a soap and as "home" remedies for various ailments (Lemma, 1965, 1970). This ready acceptance by the population is an additional important factor.

The potency of endod against snails is high (LC_{90} at 3 ppm), although the ovicidal activity is low, requiring multiple administration for snail control. The emetic property and an LC_{50} of 1.4 g/kg provide an essential safety factor for mammals. Lethality of the product to small fish is a disadvantage shared with all molluscicides now in use (WHO, 1961, 1965a, b). The economic advantages, ready availability, and potential usefulness of endod give promise of a new and important weapon against schistosomiasis and other snail-borne diseases.

ACKNOWLEDGMENTS

We thank P. Yau, R. Rittenhouse, T. Jorgenson, D. Kay, and Mike Rossi for technical assistance. This work was in part supported by the Office of Naval Research Contract N00014-71-C-0123, Stanford Research Institute Internal Research and Development fund, and a travel grant (to A. L.) from Wellcome Trust, London.

LITERATURE CITED

HORTON, W. J. 1968. Preliminary report on investigations of molluscicidal components of *Phytolacca dodecandra*. WHO Molluscicide Information Series No. 24.

KING, T. A., K. JEWERS, H. RICHARDSON, AND C. P. FALSHAW. 1968. Endod saponin, the molluscicide of the fruit of endod (*Phytolacca dodecandra*). Fifth International Symposium on the Chemistry of Natural Products, London. (Abstr. F43.)

LEMMA, A. 1965. A preliminary report on the molluscicidal property of endod (*Phytolacca dodecandra*). Ethiopian Med. J. **3**: 187–190.

———. 1970. Laboratory and field evaluations of the molluscicidal properties of endod (*Phytolacca dodecandra*). Bull. World Hlth Org. **42**: 597–612.

———, AND J. DUNCAN. 1970. First annual report of schistosomiasis pilot control project in Adwa, Ethiopia. Institute of Pathobiology Monograph (35 p.). Presented at 2nd International Congress of Parasitology, 6–12 September 1970, Washington, D. C. J. Parasit. **56**: 438–439 (Abstr.).

MILLER, L. C., AND M. L. TAINTER. 1944. Estimation of the ED_{50} and its error by means of logarithmic-probit graph paper. Proc. Soc. Exp. Biol. Med. **57**: 261–264.

POWELL, J. W., AND W. B. WHALLEY. 1969. Triterpenoid saponins from *Phytolacca dodecandra*. Phytochemistry **8**: 2105–2107.

STRUFE, R., AND R. GÖNNERT. 1962. Comparative studies on the influence of environmental factors upon the efficiency of Bayluscide®. Pflanzenschutz-Nachrichten "Bayer" **15**: 50–70.

WORLD HLTH ORG. 1961. Expert Committee on Bilharziasis, World Hlth Org. Tech. Rept. Ser. No. 214.

WORLD HLTH ORG. 1965a. Snail control in the prevention of bilharziasis. World Hlth Org. Monogr. Ser. No. 50.

WORLD HLTH ORG. 1965b. Molluscicide screening and evaluation. Bull. World Hlth Org. **33**: 567–581.

YASURAOKA, K. 1971. Laboratory evaluation of the molluscicidal fraction from endod (*Phytolacca dodecandra*), against *Oncomelania nosophora*. World Hlth Org. Schisto. Inf. Series 8.

THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE Vol. 22, No. 6
 Printed in U.S.A.

ENDOD EXTRACT, A PLANT-DERIVED MOLLUSCICIDE: TOXICITY FOR MOSQUITOES*

ANDREW SPIELMAN AND AKILU LEMMA†
Department of Tropical Public Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115

Abstract. A butanol extract of the soapberry plant, *Phytolacca dodecandra* (endod), is toxic at about 1 part per million to second and third instar *Aedes*, *Culex*, and *Anopheles* mosquitoes. Ova and pupae are not affected and adults die only after ingestion of concentrated endod. Endod extract is considerably more toxic than rotenone and about 100 times as active as a commercial saponin preparation. It is about twice as toxic as DDT when applied against DDT-resistant larval *A. aegypti*: 20 times less toxic for DDT-susceptible *C. pipiens*, and 40 times less toxic for DDT-susceptible *A. quadrimaculatus*. The concentration of endod required to kill mosquitoes is less than that which kills snails and fish.

An extract of the soapberry plant, endod (*Phytolacca dodecandra*), is strongly molluscicidal,[1] and an extraction procedure simple enough for field use has been developed.[2] After preliminary field trials,[3] an intensive attempt to control the snail vectors of schistosomiasis and fascioliasis in Ethiopia was initiated, and this project is now underway. A unique feature of this project involves the indigenous population in the manufacture of the extract as well as in its application in the field.

Since use of endod extract in the field may affect aquatic insects, we investigated the insecticidal properties of endod. Various species of mosquitoes were exposed to endod and resulting mortality recorded. Each instar was studied and the toxicity of endod was compared to that of several related toxicants.

MATERIALS AND METHODS

Butanol extracts of endod were prepared[2] and dissolved in distilled water. Technical grade DDT (E.S.A. Reference Standard, pp' isomer 77.2%, Nutritional Biochemicals Co., Cleveland, Ohio), crystalline rotenone (95% pure, S. B. Penick Co., New York, N.Y.) and Reagent grade Saponin (Nut. Biochem. Co.) were used as reference standards—DDT and rotenone were dissolved in 95% ethanol and saponin in distilled water. All stock solutions contained 10 mg material per ml of solvent.

Aedes aegypti colonies were derived from Grand Bahama Island, *Culex pipiens molestus* from Boston, Massachusetts, and *Anopheles quadrimaculatus* from Alabama. All experimental exposures were done in ½-pint, plastic cups. Ten larval mosquitoes were placed in 100 ml of distilled water containing various concentrations of toxicant and held for 1 day. Larvae were then decanted and transferred to fresh distilled water and fed tetramin fish food. At one day and at 2 days after treatment, dead larvae were removed and counted.

Adult mosquitoes were treated with endod extract by feeding as follows. At 3 days of age, virgin adult females were denied water and food. One day later, they were provided cotton pledgets saturated with solutions containing endod, 10% sucrose, and a trace of carmine. Survival was observed for 1 week thereafter.

Mortality data were analyzed by means of computerized probit-analysis. LC_{50},* LC_{90}, and 95% confidence limits were derived.

RESULTS

The sensitivity to endod of each of the larval instars of *A. aegypti* was determined. Larvae were exposed to semi-logarithmic dilutions for 1 day and mortality was recorded during this and the next 2 days. Mortality was greatest in larvae

Accepted 25 May 1973.

* Supported in part by training grant A10046 and research grant AI-10274 from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service.

† Present address: Haile Sellassie I University, Addis Ababa, Ethiopia.

* LC, lethal concentration.

TABLE 1

*Mortality of mosquitoes after exposure to semi-logarithmic dilutions of endod**

Species	Instar	Concentration of endod extract (ppm)	
		LC_{50}	LC_{90}
A. aegypti	I	2.2 (1.6–3.1)†	8.8 (4.6–16.9)
	II	0.17 (0.14–0.22)	0.43 (0.31–0.59)
	III	0.33 (0.25–0.44)	1.32 (0.91–1.92)
	IV early	9.8 (6.9–14.0)	61.8 (34.4–111.1)
	IV late	14.7 (11.0–19.7)	80.3 (48.2–133.9)
	Adult ♀	27.0 (7.7–95.2)	1,146.6 (140.2–9,379.1)
C. pipiens	II	0.19 (0.14–0.26)	0.79 (0.51–1.22)
	III	0.44 (0.32–0.59)	1.86 (1.14–3.02)
A. quadrimaculatus	II	0.14 (0.26–0.65)	1.78 (1.06–2.99)
	III	0.41 (0.26–0.66)	5.80 (2.98–11.29)

* Each result is based upon 30 mosquitoes in three replicates
† Figures in parentheses represent 95% confidence limits.

exposed during the second instar (Table 1). For these larvae, 0.4 ppm of endod killed 90% of treated mosquitoes. Third instar larvae were somewhat less sensitive while first instar larvae survived in water containing at least 10 times the concentration of endod required to kill second instar larvae. Larvae of the fourth instar were even more resistant to endod: late fourth instar larvae (adult eye falciform, pupal trumpets non-sclerotized) survived at least 100 times the concentration that killed second instar larvae. Pupae and eggs of *A. aegypti* appeared not to be affected by endod solutions.

Adult female *A. aegypti* died within 2 days after ingesting 10% sucrose containing dissolved endod (Table 1) if the toxicant was quite concentrated. The LC_{90} was well in excess of 100 parts per million. Resulting pathology was specific: degeneration of the epithelial lining of the fore-gut and of the anterior mid-gut. Destruction of the tissue was rapid and only the basement lamina remained at 1 day post-treatment.

Larval *C. pipiens* were about as sensitive to endod as were *A. aegypti* (Table 1). *A. quadrimaculatus*, on the other hand, appeared to be somewhat less susceptible. The LC_{90} for second instar larvae of both culicine populations was less than 1 ppm, and for the anopheline larvae the LC_{90} was between 1 and 3 ppm.

Each of the three mosquito populations was tested with the common toxicant DDT. *A. aegypti* larvae were surprisingly resistant to this insecticide, the LC_{90} being 3 to 4 ppm for second and third instar larvae (Table 2). Younger larvae

TABLE 2

*Mortality of mosquitoes after exposure to semi-logarithmic dilutions of DDT, rotenone, and saponin**

	Species	Instar	Concentration of endod extract (ppm)	
			LC_{50}	LC_{90}
DDT	*A. aegypti*	I	0.14 (0.10–0.19)†	0.42 (0.25–0.71)
		II	0.52 (0.33–0.83)	3.99 (1.73–9.18)
		III	0.54 (0.38–0.77)	3.18 (1.68–6.02)
	C. pipiens	III	0.03 (0.03–0.04)	0.08 (0.05–0.01)
	A. quadrimaculatus	III	0.01 (0.005–0.16)	0.08 (0.03–0.12)
Rotenone	*A. aegypti*	III	6.13 (4.6–8.2)	23.44 (15.1–36.4)
Saponin	*A. aegypti*	III	>100	

* Each result is based upon 30 mosquitoes in three replicates.
† Figures in parentheses represent 95% confidence limits.

were more susceptible. Third instar *C. pipiens* and *A. quadrimaculatus* were affected when this toxicant was more dilute (about 0.1 ppm).

The insecticidal properties of endod were compared to those of the plant-derived insecticide, rotenone. The LC_{50} for third instar larvae of rotenone, of *A. aegypti* was about 20 times higher than that of endod (Table 2).

A commercial saponin preparation was tested against third instar larval *A. aegypti*. This material, which is chemically related to endod, was apparently non-toxic (Table 2).

DISCUSSION

Endod extract is toxic to mosquitoes, and this toxicity is not a general property of saponin-like compounds. In adult *A. aegypti*, the mode of toxicity involves destruction of the epithelial lining of the gut. However, this effect requires administration of enormous quantities of toxicant, while death of larvae follows contact with endod in concentrations comparable to those of practical larvicides.

Even rotenone, a saponin that has long been used as an insecticide, is less toxic for larval mosquitoes than is endod. Indeed, larvae of the Grand Bahama strain of *A. aegypti* are destroyed by about half as much endod extract as of DDT. However, these mosquitoes are surprisingly resistant to DDT. (To our knowledge, this population of mosquitoes has not previously been exposed to insecticides.) The *Culex* and *Anopheles* populations were normally susceptible to DDT.[4] DDT was about 20 times more active than was endod against *C. pipiens* and about 40 times more active against *A. quadrimaculatus*.

Endod appears to be a more effective insecticide than a molluscicide. For several species of snails the LC_{90} values were approximately 3 ppm, more than 10 times that of mosquitoes.[2] The comparable value for leeches and fish is about equal to that in snails. The acute oral LD_{50} in non-regurgitating animals is 1.45 g/kg of body weight for rats, 1.2 g/kg for mice and 0.287 g/kg for guinea pigs. A preliminary report describing the non-toxicity of endod against *C. pipiens*[3] should be modified, since only certain developmental stages survive exposure to concentrated endod.

Our finding that endod is highly toxic only for second and third instar mosquito larvae indicates a possible limitation in its use as an insecticide, because it may be necessary to treat a population repeatedly in order to effect control. However, the active moiety is quite stable,[3] which may compensate for this apparent deficiency.

It is apparent that application of endod for control of molluscs may result in mortality among aquatic insects. This possibility requires careful investigation. It may be that concurrent control of some medically important arthropods may be achieved. In areas in which the local manufacture of endod is commercially feasible, its use as an insecticide should be further evaluated.

REFERENCES

1. Lemma, A., 1965. A preliminary report on the molluscicidal property of endod (*Phytolacca dodecandra*). *Ethiop. Med. J., 3:* 187–190.
2. Lemma, A., Brody, G., Newell, G. W., Parkhurst, R. M., and Skinner, W. A., 1972. Studies on the molluscicidal properties of endod (*Phytolacca dodecandra*): I. Increased potency with butanol extraction. *J. Parasitol., 58:* 104–107.
3. Lemma, A., 1970. Laboratory and field evaluation of the molluscicidal properties of *Phytolacca dodecandra*. *Bull. W.H.O., 42:* 597–612.
4. Brown, A. W. A., and Pal, R., 1971. *Insecticide Resistance in Arthropods*. W.H.O. Monogr. Ser. No. 38. World Health Organization, Geneva.

Lemma, A. and Yau, P. (1974) *Ethiop. med. J. 12*, 109

STUDIES ON THE MOLLUSCICIDAL PROPERTIES OF ENDOD (*PHYTOLACCA DODECANDRA*): II. COMPARATIVE TOXICITY OF VARIOUS MOLLUSCICIDES TO FISH AND SNAILS*

Aklilu Lemma, Sc. D. and Percy Yau**

Stanford Research Institute, Menlo Park, California, U.S.A.

* The first article in this series was published in the *Journal of Parasitology*.

This research was supported in part by Office of Naval Research, Contract No. N00014-71-C-0123, United States Navy.

Request reprints to Institute of Pathobiology, Haile Sellassie I University, P. O. Box 1176, Addis Ababa.

** *Present address:* Institute of Pathobiology, Haile Sellassie I University.

As previously reported in this Journal (Lemma, 1965), the berries of the native Ethiopian plant *(Phytolacca dodecandra)*, which have long been used as a detergent, have been found to act also as a natural molluscicide. Since molluscicides are being used increasingly for the control of schistosomiasis and other snail-borne diseases, serious consideration should be given to the effects that such compounds may have on fish, particularly in areas where fish are an important element in the human diet.

The primary objective of the studies being reported was to compare the toxicity of the butanol extract of the berries of Endod (Lemma *et al.*, 1972) to various species and sizes of fish and to mature snails. However, to provide adequate information on which to base a comparison of the piscicidal activity of the leading molluscicides now in use in different parts of the world, the toxicity of *Bayluscide*, pentachlorophenol, copper sulphate, and *Frescon* was also determined.

Materials and methods

The *Bayluscide* (wettable powder) used in the present study was supplied by the Bayer Company in Germany, the *Frescon* (emulsion concentrate) by the Woodstock Agriculture Research Unit, Sittingborne, England. Technical grade copper sulphate and pentachlorophenol were purchased from local suppliers. The molluscicide solutions were prepared in the same way as for testing snails (Lemma, 1970).

Three species of fish were tested: *Gasterosteus aculeatus* (sticklebacks), *Tilapia mossambica* and *Carassius auratus* (goldfish). Except for the goldfish, all specimens used were immature or fingerlings. Their weights and sizes were: sticklebacks, 0.5-1.2 g. and 3.5 cm.; tilapia, 1-5 g. and 4-6 cm.; small goldfish, 0.75-20 g. and 4-6 cm.; mature goldfish 90-130 g. and 20-25 cm. All the fish were obtained from a local pet shop.

Small fish were tested in groups of 10 in 8 litres and large fish in groups of 3 in 24 litres of molluscicidal solution in aerated aquaria. The snails used were laboratory-bred, mature *Biomphalaria glabrata* (maximum diameter 12-15 mm.). They were tested in the same concentration of molluscicides as the fish, either in parallel tests or in cages in the same aquaria with fish.

The number of fish found dead after 3, 6, and 24 hours of exposure were recorded. The 24-hour LC_{90} (concentration that was lethal to 90% of the fish or snails) was calculated on those that survived a 24-hour recovery period in dechlorinated tap water with food.

Results

Table 1 shows the comparative susceptibility of different species and sizes of fish and *B. glabrata* to the five molluscicides.

TABLE 1: Comparative susceptibility of various species and sizes of fish and Biomphalaria glabrata to five molluscicides

Species	Avg. wt. (g.)	Avg. Length (cm.)	LC_{90} (ppm.) after various hours of exposure and 24 hours recovery				
			Fish and snails alone				*B. glabrata* with fish
			1 hr.	3 hr.	6 hr.	24 hr.	24 hr.
			Endod				
G. aculeatus	0.9	4.1		3.5	1.8	1.8	3.5
T. mossambica	3.6	5.9		3.6	3.0	2.4	3.0
C. auratus - small	1.2	5.2		5.4	3.5	3.2	3.2
- large	108.0	21.0		8.8	4.6	3.7	3.4
B. glabrata						3.0	
			Bayluscide				
G. aculeatus	1.0	4.0	0.19	0.18	0.18	0.16	0.17
T. mossambica	2.0	5.4	0.70	0.70	0.70	0.70	< 0.40
C. auratus - small	1.1	4.9	0.50	0.52	0.52	0.52	0.40
- large	97.6	19.7	0.62	0.65	0.65	0.65	0.55
B. glabrata						0.20	
			Frescon				
G. aculeatus	0.9	4.1		0.65	0.65	0.28	0.25
T. mossambica	3.6	5.7		> 3.2	> 3.2	3.60	0.52
C. auratus - small	1.8	5.0		0.65	0.54	0.54	0.54
- large	118.0	23.5		> 1.6	> 1.6	1.00	1.4
B. glabrata						0.18	
			Pentachlorophenol				
G. aculeatus	0.6	4.7		1.2	0.75	0.37	0.98
T. mossambica	2.0	5.0		2.9	2.9	0.80	0.25
C. auratus - small	1.5	4.8		1.8	1.6	1.60	1.70
- large	108.0	22.0		> 2.0	> 2.0	1.60	1.90
B. glabrata						1.00	
			Copper Sulphate				
G. aculeatus	0.6	4.7		> 8.0	4.6	2.8	0.75
T. mossambica	2.0	5.0		> 16.0	23.3	11.3	2.0
C. auratus - small	1.5	4.8		> 8.0	5.4	3.5	1.0
- large	108.0	22.0		> 8.0	> 8.0	6.9	2.5
B. glabrata						0.5	

Endod.

The LC_{90} (24 hours) of the butanol extract of Endod was 1.8 ppm. for sticklebacks, 2.4 ppm. for tilapia, 3.2 ppm. for small goldfish, and 3.7 ppm. for large goldfish. The LC_{90} for snails (3.0 ppm.) was not appreciably altered by the presence of fish in the aquaria.

Bayluscide.

The 24-hour LC_{90} of this molluscicide was 0.16 ppm. for sticklebacks, 0.52 ppm. for small goldfish, and 0.65 ppm. for large goldfish. Tilapia were the most resistant, with a LC_{90} of 0.70 ppm. The comparative value for snails was 0.2 ppm. Unlike the other molluscicides tested, the action of *Bayluscide* on fish depends on concentration only and not on time. At all concentrations, almost all the fish that died did so within the first hour of exposure; as shown in the table, prolonging the exposure to 3, 6, and 24 hours does not appear to alter the LC_{90} values. This action suggests contact poisoning. The mortality of snails in the aquaria with fish exposed to a lethal dose of *Bayluscide* was relatively low. This may be partly due to detoxification of the molluscicide by fish excretion or by physical adsorption of the compound on the fish scales.

Frescon.

The 24-hour LC_{90} of *Frescon* was 0.28 ppm. for sticklebacks, 3.6ppm. for tilapia, 0.54 ppm. for small goldfish, and 1.0 ppm. for large goldfish. Of the five molluscicides, *Frescon* appears to be the most readily detoxified by fish. At all concentrations tested, snail kill was considerably reduced by the presence of fish. The normal LC_{90} (24 hours) of *Frescon* for *B. glabrata*, 0.18 ppm., was increased to 0.25, 0.52, 0.54, and 1.4 ppm. in the presence of sticklebacks, tilapia, small goldfish and large goldfish, respectively.

Pentachlorophenol.

The LC_{90} (24 hours) was 0.37 ppm. for sticklebacks, 0.80 ppm. for tilapia, 1.6 ppm. for both small and large goldfish, and 1.00 ppm. for snails. Some detoxification of the molluscicide, particularly by goldfish, was observed.

Copper sulphate.

This compound appears to be considerably less toxic to fish than to snails. The LC_{90} (24 hours) was 2.8 ppm. for sticklebacks, 11.3 ppm. for tilapia, 3.5 ppm. for small goldfish, and 6.9 ppm. for large goldfish, but only 0.5 ppm. for snails alone. Considerable detoxification occurred; in the presence of fish, the LC_{90} for snails ranged from 0.75 to 2.5 ppm.

Discussion

The results clearly show that different species of fish vary in their susceptibility to different molluscicides. Small goldfish weighing about 1 g.

and large goldfish weighing about 100 g. were almost equally susceptible to the lethal action of all the molluscicides tested. The high LC_{90} of copper sulphate for large goldfish may have been due to adsorption or detoxification of the molluscicide by the fish, as evidenced by snail bioassay tests.

Detoxification appears to be an important factor affecting piscicidal potency of molluscicides. Some of them, such as *Frescon* are more readily detoxified by fish; thus, larger quantities of the compound are required to kill both fish and snails. Of the various molluscicides tested, Endod and pentachlorophenol are least detoxifiable by fish; the others were affected to varying degrees.

Gönnert (1961, 1962) has reported on the piscicidal activities of *Bayluscide* and sodium pentachlorophenate. Berrios-Duran and co-workers (1964) have also studied the comparative piscicidal activities of various molluscicides. Although they used different species of snails from those we did, their results generally agree with ours.

Results of laboratory tests as presented here cannot be accepted as representative of results obtained under field conditions. For one thing, the concentration of molluscicides used in the field usually exceeds that used in the laboratory by several fold. so that the relative resistance of some of the fish to the molluscicides may not mean much. Secondly, the laboratory conditions of testing were unnatural since the fish were crowded and the detoxification process was therefore exaggerated. In any case, the only conclusions one can reach from such data are that different species of fish vary in their susceptibility to different molluscicides and that there is no significant difference in susceptibility of different sizes of goldfish to molluscicides.

There are significant variations in susceptibility of different species of fish to different molluscicides. However, due to detoxification and adsorption of such compounds by various organic and inorganic matter in the test water, the amount of molluscicides usually applied in the field for disease control purposes will not spare any fish. All the molluscicides tested in the present study are toxic to fish at about the same concentration ranges as those normally applied in the field.

Practically speaking, the ecological distribution of disease vector snails is quite different from that of edible fish. *Biomphalaria*, *Bulinus*, and *Lymnaea* snails normally breed and transmit the disease in slow-moving small streams of stagnant water, but large fish are found in bigger streams and larger bodies of water. The molluscicides used in the small streams will be diluted considerably by the time they pass through the small bodies of water and are drained into the bigger bodies of water, and thus will not affect the big fish. However, the small streams and stagnant bodies of water contain small fish that play an important role in controlling insects. Therefore, their destruction during snail control programs could lead to some ecological imbalance. This would be particularly important in the areas where insects such as the mosquito are important disease vectors. In such cases, a search for compounds with both molluscicidal and insecticidal properties may be warranted, or else the molluscicidal compounds should be used in combination with insecticides.

Summary

The piscicidal effects of Endod, *Bayluscide*, *Frescon*, pentachlorophenol and copper sulphate were compared in the laboratory. The susceptibilities of *Gasterosteus aculeatus* (sticklebacks), *Tilapia mossambica* (Tilapia), and *Carassius auratus* (goldfish) to different concentrations of the molluscicides were determined and compared to the susceptibility of *Biomphalaria glabrata* snails under similar conditions. Although some variations in the susceptibility of different species and sizes of fish to different molluscicides were noted, it was concluded that, practically speaking, all the molluscicides tested in the present study are toxic to fish at about the same concentration ranges as those normally applied in the field. However, since fish, especially edible ones, have entirely different habitats from bilharzia-transmitting snails, the application of the molluscicides in small streams to control snails may not significantly interfere with edible fish.

Résumé

On a comparé dans le laboratoire la toxicité envers les poissons d'Endod, *Bayluscide*, *Frescon*, pentachlorophenol et cuivre sulfaté. On a determiné les susceptibilités de *Gasterosteus aculeatus*, *Tilapia mossambica*, et *Carrassius auratus* aux concentrations différentes des mollusquicides et les a comparées à la susceptibilité des mollusques *Biomphalaria glabrata* aux conditions semblables. Bien qu'on a noté des variations de susceptibilité des poissons d'espèces et de grandeurs différentes, on a fini par décider que, pour parler pratiquement, tous les mollusquicides éprouvés dans cette étude sont toxiques aux poissons aux mêmes concentrations que celles appliquées d'habitude dans un champ d'essai. Cependant, parce que les poissons, surtout ceux qui sont mangeable, habitent des lieux tout à fait différents que les mollusques qui transmettent la bilharziose, l'application des mollusquicides dans les petits ruisseaux afin de contrôler les mollusques peut-être ne gênera pas significativement les poissons mangeables.

References

Berrios-Duran, L.A., Ritchie, L.S., Frick, L.P., and Fox, I. (1964) Comparative piscicidal activity of "Stabilized Chevreul Salt" (SCS), a candidate molluscicide, and *Bayluscide*. *WHO Molluscicide Inf. Series* No. 18.

Gönnert, R. (1961) Results of laboratory and field trials with molluscicide Bayer 73. *Bull. Wld. Hlth. Org. 25*, 483.

Gönnert, R. (1962) Bayluscide, a new compound for controlling medically important freshwater snails. *Pflanzenschutz-Nachrichten "Bayer" 15*, 4.

Lemma, A. (1965) A preliminary report on the molluscicidal property of Endod. *Ethiop. med. J. 3*, 187.

Lemma, A. (1970) Laboratory and field evaluations of the molluscicidal

properties of Endod (*Phytolacca dodecandra*). *Bull. Wld. Hlth. Org. 42*, 597.

Lemma, A., Brody, G., Newell, G.W., Parkhurst, R.M. and Skinner, W.A. (1972) Studies on the molluscicidal properties of Endod (*Phytolacca dodecandra*). I. Increased potency with butanol extraction. *J. Parasit. 58*, 104.

Lemma, A. and Yau, P. (1974) *Ethiop. med. J. 13*, 115

STUDIES ON THE MOLLUSCICIDAL PROPERTIES OF ENDOD (*PHYTOLACCA DODECANDRA*). III. STABILITY AND POTENCY UNDER DIFFERENT ENVIRONMENTAL CONDITIONS*

Aklilu Lemma, Sc.D. and Percy Yau**

Stanford Research Institute, Menlo Park, California, U.S.A.

*This research was supported in part by Office of Naval Research Contract No. N00014-71-C-0123, United States Navy.

Request reprints to Institute of Pathobiology, Haile Sellassie I University, P. O. Box 1176, Addis Ababa.

** *Present address:* Institute of Pathobiology, Haile Sellassie I University.

The objectives of this study were to investigate the effects of organic matter, hydrogen ion concentration (pH), and ultraviolet (UV) light on the molluscicidal potency of the native Ethiopian plant, Endod *(Phytolacca dodecandra)*, and to determine the sensitivity of various species and life stages of snails to the lethal action of this plant-derived molluscicide (Lemma *et al.*, 1972). For comparative purposes, other molluscicides with known advantages and disadvantages were used in the study.

Environmental conditions such as the presence of organic and inorganic matter in water, pH, temperature, UV light from the sun and other factors are known to affect the molluscicidal potencies of various compounds. For example, the potency of copper sulphate is known to be decreased by as much as 200-fold due to its adsorption on organic matter in water. Sodium pentachlorophenate is considerably inactivated by UV radiation from the sun; also, it irritates human skin and is therefore difficult to use. Even some of the newer and improved molluscicides have disadvantages: *Frescon* is known to be highly affected by variation in pH of water, and *Bayluscide* is partially inactivated by UV radiation from the sun (WHO, 1965). Although some molluscicides such as *Bayluscide* and sodium pentachlorophenate have the added advantage of killing snail eggs at molluscicidal concentrations, others, like *Frescon*, do not have this advantage.

Materials and methods

In this paper, the term Endod is used for the butanol extract of the berries of *Phytolacca dodecandra*. The procedures for preparing the extract and the snail assay methods used have been described previously (Lemma *et al.*, 1972).

Bayluscide, 70% wettable powder of the thanolamine salt, was supplied by the Bayer Company in Germany; *Frescon*, 16% emulsifiable concentrate of N-trityl morpholine, by Shell International Chemicals, London. Technical grade copper sulphate and pentachlorophenol were purchased from local chemical suppliers.

To collect snail eggs, about 4 by 3 inch plastic (Cellophane) sheets were submerged in the various aquaria where snails bred. When taken out, after about two weeks, each sheet was covered with large numbers of egg masses containing snail embryos in different stages of development. The sheets containing the egg masses were then immersed in petri dishes containing various concentrations of the test molluscicides. After 24 hours, the egg masses were washed in distilled water several times and left up to a week in clean water with some food. Each egg mass was periodically examined under the microscope to ascertain the viability of the embryos.

For determining adsorption of molluscicides or organic matter, faeces collected from laboratory rabbits were dried in an oven for about 24 hours, ground to fine powder with a mortar, and suspended in dechlorinated tap water at various concentrations ranging from 500 to 4,000 ppm. The test molluscicide was then added. During the next 24 hours, the solutions were gently shaken occasionally to allow the molluscicides and the rabbit faeces to interact. These were then poured, in 200ml. quantities, into paper cups, and snails were exposed to them. After 24 hours of exposure, the snails were removed, washed with clean dechlorinated water, and allowed to recover for from 24 to 48 hours. Snail mortality in each dilution was then recorded.

To determine the effect of UV radiation, both from the sun and from a UV lamp, on Endod and the other molluscicides, various dilutions of the molluscicides were prepared. In one series of tests, 100 ml. quantities of the solutions were placed in 9 inch diameter aluminium pans and exposed to bright sunlight for six hours prior to exposing the snails. Solutions kept in a dark place in the laboratory were used as controls. In the other series, the solutions were poured into 12 by 27 cm. plastic pans to a depth of 1.3 cm. and then exposed to radiation from a shortwave UV lamp for various periods. This lamp, built for bacteriological sterilization, emits 2537 A° wavelength at a rate of 1,000 ergs. /sec./cm.^{2} at the point of the solutions. In both series of tests, sufficient distilled water was added to the solutions to make up for the amounts lost through evaporation. The solutions were then assayed for their toxicity to snails.

The stability of Endod in the laboratory was also determined. Large volumes of serial dilutions of the molluscicides, ranging from 4 to 100 ppm., were left to stand at room temperature in a relatively well lighted laboratory. The stability of the molluscicides in the working dilutions stored in the laboratory was then calculated and expressed in terms of LC_{90}.

Results

Effect of molluscicides on different life stages of Biomphalaria glabrata.

The susceptibilities of snail eggs, young snails, and mature large snails to Endod, *Bayluscide*, *Frescon*, pentachlorophenol and copper sulphate are given in Table 1.

Endod was not ovicidal even at 100 ppm., and young snails were more resistant to it than adults. *Bayluscide* was about equiactive against all

life stages, including eggs. *Frescon* did not affect snail eggs even at 40 ppm., but it killed both young and adult snails at about 0.2 ppm. Pentachlorophenol killed all stages at about 1 ppm.; eggs died at about the adult-killing dose, but young snails were slightly more susceptible. Snail eggs were about six times more resistant to copper sulphate than adult snails, but young and adult ones had about the same susceptibility.

TABLE 1: Effects of various molluscicides on the egg and different sizes of B. glabrata expressed as 24-hour LC_{90} (ppm.)

Molluscicides	Eggs	Small (1-3 mm. diam.)	Large (10-15 mm. diam.)
Endod	100.00	4.70	3.00
Bayluscide	0.20	0.20	0.26
Frescon	40.00*	0.20	0.21
Copper sulphate	4.00	0.70	0.50
Pentachlorophenol	1.00	0.65	1.00

* All figures given for *Frescon* should be reduced to 16% to get the actual concentration of active principle. Figures are based on using the 16% emulsion concentrate as stock.

Comparative ovicidal properties of Endod.

In an attempt to determine whether Endod was nonovicidal to only a certain species of snails, eggs of different species of *Biomphalaria*, *Bulinus* and *Lymnaea* snails were tested. The results showed that whereas 100 ppm. did not affect *Biomphalaria* and *Bulinus* eggs in 24 hours of exposure, 5 ppm. killed all *Lymnaea* eggs. This specific susceptibility of *Lymnaea* eggs to Endod may be due to the nature of the membrane covering the egg mass. According to the studies of Beadle (1969), the membranes around *Biomphalaria* and *Bulinus* egg masses are not permeable to material with molecular weight over 500, whereas material with molecular weight up to 3,000 can permeate the membrane around *Lymnaea* eggs. The active principle in Endod has a molecular weight of about 1,000.

Time-concentration relationship effect of Endod on B. glabrata.

Mature *B. glabrata* snails were exposed to various concentrations of Endod for various periods, and the LC_{90} for each exposure time was plotted as a time-concentration curve (Figure 1). For the first 15 hours of exposure, the time-concentration relationship was linear; *i.e.*, the higher the concentration, the shorter the time needed to kill the snails. The threshold concentration at which any snail kill was observed appears to be 3 ppm.

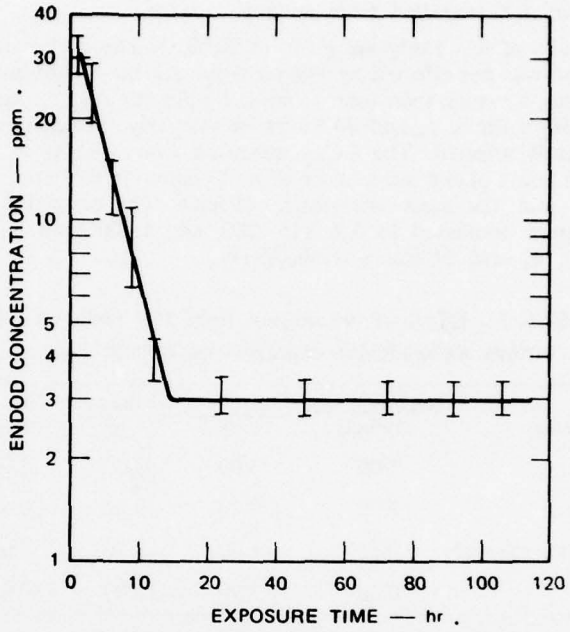


FIGURE 1: Time-concentration relationship of the molluscicidal effect of Endod on B. glabrata.

At this level, all snails are affected within 15 hours of exposure. Prolonged exposures - up to 100 hours - at lower concentrations, did not give lower LC_{90} values.

Effect of sunlight.

The results of this study are presented in Table 2. Endod and copper sulphate did not appear to be affected by exposure to the sun, but pentachlorophenol was considerably inactivated (LC_{90} increased from 1 to 40 ppm.). *Frescon* and *Bayluscide* were also affected somewhat. The LC_{90} of *Bayluscide* increased from about 0.2 ppm. to 0.4 ppm., and the LC_{90} of *Frescon* increased from 0.2 ppm. to 0.5 ppm.

TABLE 2: Effect of sunlight on potency of various molluscicides expressed as 24-hour LC_{90} (ppm.) of molluscicide

Molluscicides	Kept in dark (control)	6-hour exposure to sun
Endod	3.00	3.00
Bayluscide	0.20	0.40
Frescon	0.20	0.50
Pentachlorophenol	1.00	40.00
Copper sulphate	1.00	1.00

Effects of UV radiation from artificial source.

The results of this study are given in Table 3. The molluscicidal potency of Endod was not affected by UV radiation for up to 24 hours. The LC_{90} of *Bayluscide* was increased from 0.2 ppm. to 0.4, 0.5, and 4.2 ppm. by radiation for 3, 6, and 24 hours, respectively. Pentachlorophenol was significantly affected. The LC_{90} increased from 0.65 ppm. to 7.0 ppm. during 6 hours of exposure, and rose to 30 ppm. after 24 hours of exposure. *Frescon* was the most drastically affected. The normal 24-hour LC_{90} of 0.2 ppm. increased to 2.9, 115, 280, and 1,000 ppm. after radiation for 1, 3, 6, and 24 hours, respectively.

TABLE 3: Effect of irradiation from UV lamp on potency of various molluscicides expressed as 24-hour LC_{90} (ppm.)

Molluscicides	Nonirradiated Controls	Irradiation Times			
		1 hr.	3 hr.	6 hr.	24 hr.
Endod	3.00	3.00	3.00	3.00	3.00
Bayluscide	0.20	0.20	0.40	0.50	4.20
Pentachlorophenol	0.65	1.00	1.70	7.00	30.00
Frescon	0.20	2.90	115.00	180.00	1000.00

Effect of pH on molluscicidal potency of Endod.

Dilutions of Endod were prepared in dechlorinated tap water that had been adjusted to pH values of 5 to 9 with sodium hydroxide or hydrochloric acid. The results of the molluscicidal potency of Endod in water of these pH values is shown in Figure 2. It appears that Endod is more active at lower than at high pH values. The LC_{90} was 1.7 ppm. at pH 5 and 12.0 ppm. at pH 9.

Effect of organic matter.

The effects of various concentrations of rabbit faeces on Endod and the other molluscicides are given in Table 4.

TABLE 4: Effect of organic matter on potency of various molluscicides expressed as 24-hour LC_{90} (ppm.)

Molluscicides	Control (no rabbit faeces)	Concentration of rabbit faeces (ppm.)			
		500	1000	2000	4000
Endod	3.00	3.2	3.5	3.6	6.6
Bayluscide	0.20	0.4	0.5	1.7	2.0
Frescon	0.20	1.3	1.4	7.5	12.0
Copper sulphate	0.50	2.0	6.5	20.0	36.0
Pentachlorophenol	1.5	1.5	1.7	1.6	2.1

120

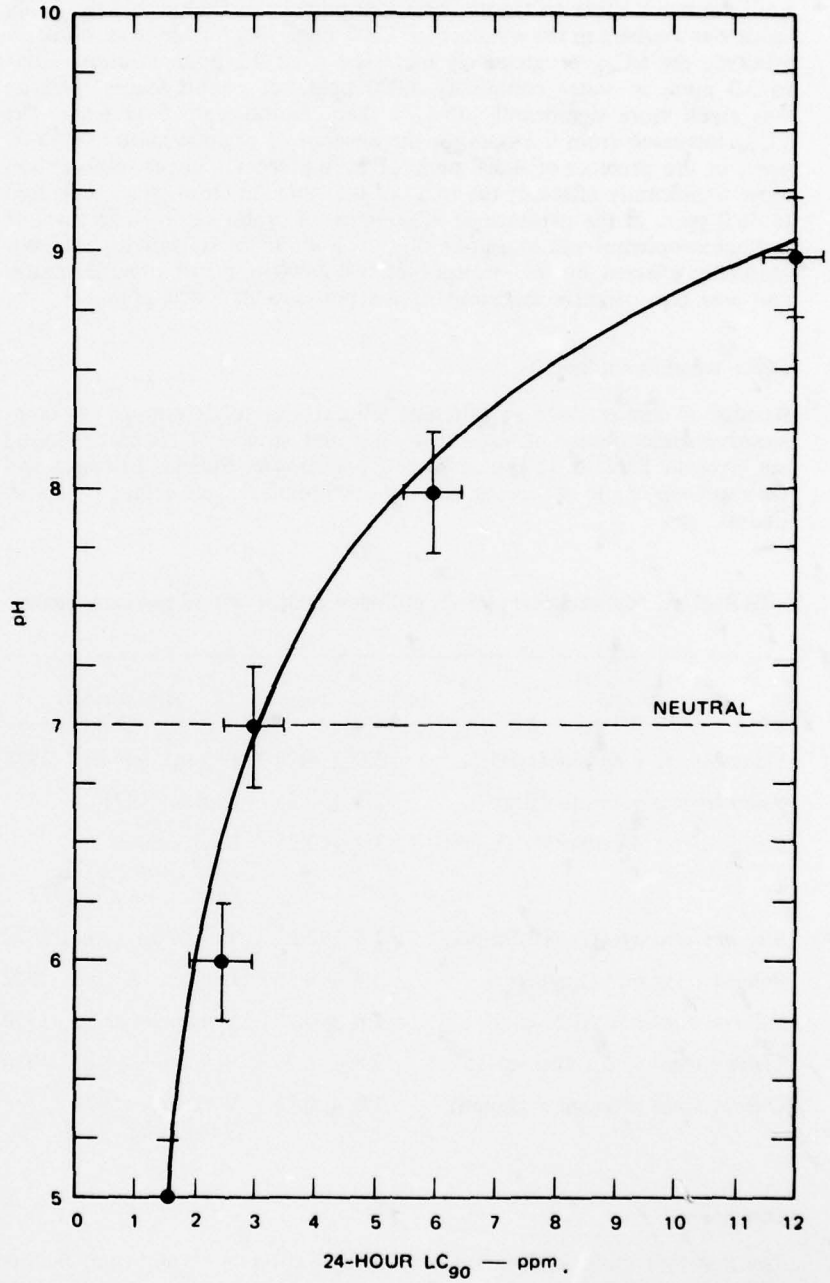


FIGURE 2: Molluscicidal potency of Endod at various pH values.

The presence of up to 2,000 ppm. of rabbit faeces in the diluting water did not have much effect on the molluscicidal potency of Endod, but the LC_{90} value was doubled in the presence of 4,000 ppm. *Bayluscide* was similarly affected; the LC_{90} progressively increased from 0.2 ppm. in clean water to 2.0 ppm. in water containing 4,000 ppm. of rabbit faeces. *Frescon* was much more significantly affected than Endod and *Bayluscide*; the LC_{90} increased from 0.2 ppm. in the absence of organic matter to 12.00 ppm. in the presence of 4,000 ppm. of rabbit faeces. Copper sulphate was most significantly affected; the LC_{90} of 0.5 ppm. in clean water increased to 36.0 ppm. in the presence of 4,000 ppm. of rabbit faeces. The effect of pentachlorophenol was more like that of Endod; *i.e.* its activity was not markedly affected by the presence of 500-2,000 ppm. of organic matter and was only slightly increased in the presence of 4,000 ppm.

Effect on different species.

Results of studies done in different laboratories to determine the comparative susceptibility of various species and strains of snails to Endod are given in Table 5. It appears that *Biomphalaria*, *Bulinus*, *Lymnaea* and *Oncomelania* snails are about equally susceptible to the lethal effects of Endod.

TABLE 5: Molluscicidal potency of Endod against various species of snails

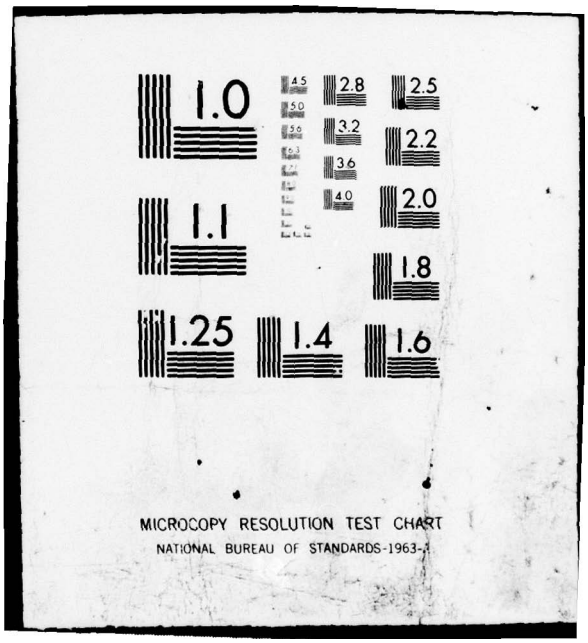
Snail Species (adults)	24-hr. LC_{90} (ppm.)	Investigators
Biomphalaria glabrata (NIH)	3.0 ± 0.25	Lemma *et al.*, 1972
Biomphalaria glabrata (Brazil)	2.3 ± 0.25	Paulini, 1971
Biomphalaria alexandrina (Egypt)	3.0 ± 0.25	Heynemann and Limm, 1971 Lemma *et al.*, 1972
Biomphalaria pfeifferi (Ethiopia)	2.8 ± 0.25	Yohannes *et al.*, 1970
Bulinus truncatus (Egypt)	3.4 ± 0.25	Lemma *et al.*, 1972
Bulinus truncatus (Ethiopia)	1.4 ± 0.25	Yohannes *et al.*, 1970
Lymnaea natalensis (Ethiopia)	2.8 ± 0.25	Yohannes *et al.*, 1970
Oncomelania nosophora (Japan)	1.6 ± 0.25	Wagner, 1971 Yasuraoka, 1971

Discussion

The fact that molluscicides are affected by various environmental factors such as organic matter, pH, and ultraviolet light from the sun has long been established (WHO, 1965). Although the primary purpose of the studies

AD-A080 030 CALIFORNIA UNIV SAN FRANCISCO F/G 6/5
STUDIES ON MOLLUSCICIDAL AND OTHER PROPERTIES OF THE ENDOD PLAN--ETC(U)
JUN 79 A LEMMA, D HEYNEMAN, H KLOOS N00014-76-C-0218
UNCLASSIFIED NL

3 OF 6
AD
A080030


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

reported herein was to determine the effects of such factors on the potency of Endod, we also compared the effects of these factors on all the leading molluscicides in identical tests.

Endod is not ovicidal to *Biomphalaria* and *Bulinus* eggs at molluscicidal concentrations, and the young of these snails appear to be slightly more resistant to it than the adults. However, *Lymnaea* eggs and the young and adult snails are affected at about the same low concentration. This may be due to differences in permeability of the egg-mass membrane of different species of snails.

The time-concentration relationship of Endod was very interesting. For the first 15 hours, the time needed to kill the snail was dose-dependent. However, 3 ppm. appears to be the threshold concentration at which any snail kill can be obtained. Concentrations lower than 3 ppm. did not kill even after exposure for 100 hours, which may be partly due to the rapid degradability of Endod in water.

Endod appears to have very good tolerance to sunlight and various degrees of UV radiation. In comparison, *Frescon* and pentachlorophenol were markedly affected. The effect of short wavelength light on *Frescon* was particularly interesting since it had not been reported before. In a personal communication, Warley of Shell International Chemicals agreed that *Frescon*, being a highly aromatic compound, will readily absorb short wavelength UV light and that this may lead to its rapid decomposition. However, he explained that *Frescon* does not exhibit any appreciable absorption of long wavelength UV light (such as is found in normal sunlight at ground level). This agrees with our finding that *Frescon* was only slightly affected by exposure to sunlight.

Endod is more active in acidic than in alkaline solutions. This may be due to hydrolysis of the sugar components of the active molecule. For practical purposes, the amount of molluscicide to be applied will have to be determined on the basis of the pH values of the water to be treated. However, since most of the snail habitats are more acidic than alkaline, this may not present any problem for Endod.

The effect of organic matter on different molluscicides was very interesting, Endod, *Bayluscide*, and pentachlorophenol were not significantly affected by rabbit faeces up to a concentration of 2,000 ppm.; at 4,000 ppm., they were affected to varying degrees, but copper sulphate and *Frescon* were markedly affected at all levels of concentration of the rabbit faeces.

Studies on the comparative susceptibility of different species of snails have shown that Endod is active against *Biomphalaria*, *Bulinus*, *Lymnaea* and *Oncomelania* snails. The economic advantages, ready availability, and potential usefulness of Endod give promise of a new and important weapon against schistosomiasis and other snail-borne diseases.

Summary

In view of the well established fact that molluscicides vary in their stability

in different environmental conditions, the effects of organic matter (rabbit faeces), pH, and UV light (both from the sun and artificial source) on the molluscicidal potency of the butanol extract of Endod was studied in comparison with other molluscicides. The comparative sensitivity of various species and life-stages of snails to the lethal action of Endod and other reference molluscicides was also determined. The results showed that the activity of Endod is not significantly affected by the presence of up to 2000 ppm. dried and ground rabbit faeces; by pH range of 5 to 9 (though more active in acid rather than alkaline media); and by up to 6 hours exposure to bright sunlight and up to 24 hours exposure to UV lamp. Endod is not ovicidal to *Biomphalaria* and *Bulinus* eggs at molluscicidal concentrations, but *Lymnaea* eggs, juveniles and adult snails all die at the same concentration of about 3 ppm. This appears to be due to permeability of the egg-mass membrane of different species of snails.

Résumé

En perspective du fait bien connu que la stabilité des mollusquicides varie sous des conditions différentes environmentales, on a étudié les effets de la matière organique (féces de lapin), pH, et la lumière ultraviolet (du soleil et aussi d'une source artificelle) sur la puissance mollusquicidale de l'extrait de l'Endod fait part butanol, comparée aux autres mollusquicides. On a aussi déterminé la sensibilité comparative des éspèces varieuses et les phases de la vie des mollusques à l'action meutrière de l'Endod et de quatre autres mollusquicides. Les résultats montrent que l'activité de l'Endod n'est pas significativement affectée par la presence de jusqu'à 2000 ppm. de féces de lapin sechés et poudrés,par pH de 5 à 9 (bien qu'il soit plus actif dans les milieux acides qu'alcalins); et par jusqu'à 6 heures d'exposition au soleil brillant et jusqu'à 24 heures d'exposition à une lampe ultraviolet. L'Endod n'est pas ovicidal aux oeufs de *Biomphalaria* et *Bulinus* aux concentrations mollusquicidales, mais les oeufs de *Lymnaea*, les jeunes et les mollusques adultes, tous meurent à la même concentration de presque 3 ppm. Ceci semble être à cause de la perméabilité des membranes de la masse des oeufs des éspèces différentes des mollusques.

References

Beadle, G.W. (1969) Personal communication.

Heynemann, D. and Limm, H.K. (1971) Personal communication.

Lemma A., Brody, G., Newell, G.W., Parkhurst, R.M. and Skinner, W.A. (1972) Studies on the molluscicidal properties of Endod (*Phytolacca dodecandra*). I. Increased potency with butanol extraction. *J. Parasitol.* *58*, 104.

Paulini, E. (1971) Personal communication.

Wagner, R. (1971) Personal communication.

Reprinted from

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE, Vol. 69. No. 1. pp. 167-168, 1975.

SCREENING FOR MUTAGENIC ACTIVITY OF SOME MOLLUSCICIDES

SIR,—With the increasing dependence on molluscicides to control snail-borne diseases such as schistosomiasis and fascioliasis, and the toxicity of these compounds to fish as well as snails, we felt that it would be interesting to know whether such compounds have any mutagenic activity. A preliminary attempt was made to test this point in a bacterial system specifically designed for the detection of mutagens and carcinogens (AMES, 1971). Tests were performed to detect:

1. Mutagens that cause base-pair substitution, hisG46, using the histidine-requiring mutant of *Salmonella typhimurium* LT-2.
2. Mutagens that add or delete one or two base-pairs (frame-shift mutagen), hisC207, using the histidine-requiring frame-shift mutant of *S. typhimurium* LT-2.

One mg. of the test molluscicide (0·5 ml. in the case of Frescon) was placed in the centre of a petri dish that had been heavily seeded with either the hisG46 or hisC207 histidine-requiring mutant of *S. typhimurium* LT-2 in histidine-deficient medium. Under normal conditions, these organisms will not grow in this medium. If the test compounds have any mutagenic properties, they would be expected to cause some mutation in the bacterial population and reverse the dependency of the bacteria on histidine, thus permitting growth of such mutants. Under such circumstances, a circle of colonies would appear around the spot of the mutagen after about 36 hours of incubation. The diffusion of the test compound in the plate was designed to allow a wide range of concentrations to be tested on a single plate.

The mutagenic activities of the following 5 molluscicides were tested.

1. Endod *(Phytolacca dodecandra)*, an Ethiopian plant-product molluscicide prepared in a wettable powder form according to procedures described in LEMMA et al., (1972). The active molluscicidal component in the berries of this plant is a triterpenoid saponin consisting of oleanolic acid glucoside (PARKHURST et al., 1974a, b).
2. *Bayluscide,*® a wettable powder molluscicide produced by the Bayer Company in Germany and widely used in the fight against schistosomiasis. The active principal is the ethanolamine salt of 5,2'-dischloro-4'-nitrosalicylic anilide.
3. *Frescon,*® a Shell Chemical Company product with very high toxicity to snails. The active principal is N-tritylmorpholine prepared in a 16·5% (w/v) emulsifiable concentration.
4. *Copper sulphate,* a compound that has been used for snail control for the past 60 years.
5. *Sodium pentachlorophenate,* another compound that has been used for snail control for many years.

The results of our bacteriological tests showed that none of these 5 molluscicides have any detectable mutagenic properties *in this system.* In comparable tests run as controls, however, the antischistosomal agent hycanthone and the antimalarial drug quinacrine (atabrine) showed high degrees of frame-shift mutagenic activity. Clearly, caution must be used in interpreting these findings, owing to the limited system employed. What is mutagenic in one bacterial system may not necessarily be so in other systems, and what is not mutagenic in one bacterial system may not necessarily be safe in others. (See AMES, 1971, for additional discussion of this test system).

We are, etc.,

AKLILU LEMMA,
Institute of Pathobiology,
Haile Sellassie I University,
Addis Ababa, Ethiopia.
BRUCE N. AMES,
Biochemistry Department,
University of California,
Berkeley, California.

5 *September,* 1974

REFERENCES

AMES, B. M. (1971). *Chemical Mutagens: Principles and Methods for their Detection.* Vol. 1, Ed. A. Hollaender. New York: Plenum Publishing Corp.
LEMMA, A., BRODY, G., NEWELL, G. W., PARKHURST, R. M. & SKINNER, W. A. (1972). *J. Parasit.*, **58**, 104.
PARKHURST, R. M., THOMAS, D. W., SKINNER, W. A. & CARY, L. W. (1974a). *Can. J. Chem.*, **52**, 702.
———, ———, ——— & ——— (1974b). *Indian J. Chem.*, in press.

Unpublished Research Note No. 11

MOLLUSCICIDAL PROPERTIES OF ENDOD (*PHYTOLACCA DODECANDRA*): PILOT-SCALE PRODUCTION OF THE WATER EXTRACT [1]

Aklilu Lemma (Sc.D.) and Donald Heyneman (Ph.D.)
Institute of Pathobiology and the George Williams Hooper Foundation

This report summarises the work undertaken during the period August 1974 to May 1975 and financed by the Office of Naval Research Contract 00014-69-0200-2006. The work was carried out at the Technology Faculty of Addis Ababa University under the guidance of Dr. Aklilu Lemma by the following personnel:

Prof. Dr. R. Bisanz	Chemical Engineer
Mr. I. McChessney	Chemical Engineer
Ato Asseged Mammo	Mechanical Engineer
Ato Gaim Kebreab	Mechanical Engineer
Ato Osman Abenur	Electrical Engineer
Ato Tesfaye Fefere	Chemist

LIST OF CONTENTS

INTRODUCTION

The berry of the endod plant (*Phytolacca dodecandra*) is known to contain a glycoside compound that possesses strong molluscicidal properties. When used simply in the crude form, ground berries produce a 100% mortality rate in *Biomphalaria* snails at a concentration of 25ppm (in a 24-hour exposure and a subsequent 24-hour recovery period test). Laboratory extractions using water as the solvent have produced an extract that is active at 6-8ppm, and butanol-water extractions are known to be active at 2-3ppm. The endod berry has therefore much potential as a plentiful source of cheap molluscicide for use in the treatment and

---

[1] The authors acknowledge with much appreciation the assistance and support given by the Faculty of Technology in regard to equipment, staff, skilled technicians and facilities supplied by the institution.

control of mollusc-transmitted diseases, and in particular schistosomiasis and fascioliasis.

In order to realize the potential of the endod berry with respect to the control of schistosomiasis a project was commenced three years ago at the Institute of Pathobiology to construct a pilot scale extraction plant that would produce sufficient quantities of extract to enable quantitative field evaluations of its molluscicidal properties to be carried out. The plant that was evolved was basically a scale-up of an existing laboratory technique for producing extract and employed water and butanol as the solvents. The original plant did not, however, reach completion because the difficulties involved in recovering the heat-sensitive molluscicide from solution were not overcome. By August 1974 the project had been resited at the Faculty of Technology and a completely new plant was under construction that was to use the original vacuum extraction technique combined with a new vacuum drying process.

During August and September further laboratory experiments were carried out and this resulted in a total re-design of the plant which was now to feature a centrifugal extractor and spray-dryer combination. The main idea behind the change was to use only water as the solvent and thus make the extraction procedure as simple as possible. This meant accepting a lower figure for the toxicity of the final extract, but it was felt that this figure would be adequate for the purposes of snail control and thus fell in with the objectives that had been set for the project. It was also felt that this process could then at a later date become the first step of a more involved procedure to produce pure molluscicide by providing a more concentrated raw material.

The fundamental principles of the centrifugal extractor-spray dryer pilot plant were very simple, but the small size of the equipment precluded the use of existing data in the design stage. This effectively meant tackling the problem with little or no idea of what results would be obtained and consequently the performance of the original spray dryer was very poor, and after some experiments had been performed it was abandoned and completely reconstructed. At the same time much difficulty was encountered in obtaining a suitable drive mechanism for the centrifugal extractor and it was not until the middle of May 1975 that the assembly of the pilot plant was finally completed.

The testing of the pilot plant has now been commenced, but before the optimum conditions for the production of extract can be known, a comprehensive program of tests and checks has to be completed. To date the pilot plant has shown itself capable of producing a finely-powdered, light brown extract active at 15ppm, and it is expected that the design figure of 6-8ppm will easily be achieved once the characteristics that determine the performance of the pilot plant are properly understood and controlled.

The report traces the evolution of the present pilot plant from its original conception to the current test program, and includes a summary of future plans.

SECTION A: Approach to Pilot Plant Design

The design problem is most conveniently considered in two stages:

1) By using a suitable solvent, the molluscicidal principle has to be removed from the ground endod berries as a solution in that solvent.
2) The solvent has to be evaporated off to leave the molluscicidal principle as a dry product.

In terms of engineering processes:

1) An extraction process
2) A drying process

(1) EXTRACTION

It was decided to restrict the choice of solvents to the two that had previously been extensively used in the laboratory:

1) Water: Using water as the extraction medium it has been found that a dry product toxic at 6-8ppm could be obtained. Water has the advantage that it removes many other non-active water solubles from the berry, e.g. various sugars, that are not required. In addition to this non-specific activity it is also difficult to separate water from the ground endod berry.

2) Butanol: Butanol is a more specific solvent for the molluscicidal principle, but it does not swell the dry ground endod and extraction of the dry ground berries with butanol alone is not feasible.

3) Butanol and Water: By combining the two solvents it has been found possible to produce a dry product toxic at 3-4ppm. This can be done either by soaking the berries with water to swell, then using butanol to extract the wet endod, or by extracting first with water and then extracting the water extract with butanol. To prevent the formation of an emulsion when the butanol is added to the water extract, the extract has to be concentrated quite considerably with the advantage that the quantity of expensive butanol that then has to be used is much less.

So the choice of extraction process seemed to lie between a simple water extraction or two, more complicated, butanol/water processes. However, the major problem that had emerged from the previous work was not the extraction of the molluscicide which is a relatively simple process, but the drying of the liquid extract.

(2) DRYING

The basic problem that had emerged from previous work and laboratory experiments was that the dryed molluscicide is sensitive to temperature, to the extent that heating above 60°C for any length of time leads to a large reduction in toxicity of the final dry product. Boiling the molluscicide solution, however, does not have any effective on its toxicity; it is only the dry molluscicide that is temperature sensitive.

This meant that evaporating the dryness at the normal boiling point of the solvent, the usual method of obtaining a dry product from a solution, could not be used, because the solid material gathering on the sides of the drying vessel would experience a temperature high enough to de-activate it. Freeze drying on this scale could be ruled out on financial considerations alone, so it appeared that the only solution was to reduce the boiling temperature to an acceptable level, by reducing the pressure. Consequently a vacuum dryer was designed in which trays of the liquid extract would be placed in an evacuated chamber and heated by radiation from hot pipes until all the liquid had evaporated off at a temperature of around 60°C. The trays were then to be removed and the dry product scraped off. Even using this arrangement, however, no guarantee could be given that the temperature of the drying product would not rise above 60°C in the last stages of drying. Drying the molluscicide extract in thick layers, necessary because of the quantities involved, produces a thick viscous material that eventually

solidifies to a dark brown semi-brittle layer that can be powderdd. To ensure that such a layer is dry enough to be powdered, this for the moment being the desired form of the product, would not be possible without a big risk of over heating, and de-activation.

The only drying process that seemed to offer clear advantages in all aspects was spray-drying. In this process tiny droplets of solution are sprayed into a hot air stream. The droplets boil immediately, at a temperature corresponding to the normal boiling point of the solvent, and become dry particles at that temperature. By careful design of the dryer involving the liquid flow rate, drop size, air distribution and temperature, it is possible to arrange for the particles to be quickly removed from the hot dryer before they can increase their temperature, and be deposited in a cold collection device where they rapidly cool down. The spray dryer has the advantage of producing a finely powdered product in a continuous process, but the disadvantage is that the solvent, which passes out in the air stream, is generally not recoverable.

It is possible to recover the solvent, but this requires a large amount of extra equipment that is expensive and complicated, and so the use of butanol in the extraction process was ruled out. This meant that only water soluble extracts could at present be dryed by this method. Despite this drawback and the problems anticipated in designing a complete spray dryer, it was decided that for the present investigations a simple and reliable method of producing large quantities of endod extract was the top priority.

SECTION B: Pilot Plant Design

(1) EXTRACTION

Various laboratory experiments were carried out to determine the most efficient way of extracting the endod berries with water. Basically the process involves thoroughly mixing certain quantities of ground endod and water, allowing them to stand, and then filtering off the liquid extract. It has been noted before that there is a problem in separating the water from the endod after extraction. In the laboratory a centrifuge was used that generated a force of 2,500gm, and it was found that to a close approximation endod always retained its own, dry weight, of water. That is, if 3kg of water and 1kg of endod were thoroughly mixed and then centrifuged, it was possible to recover a maximum of 2kg of water extract, with the endod retaining 1kg of water.

Mixing endod and water in the ratio of 2 parts of water to 1 part of endod produces a very thick soup-like mixture which will not filter unless considerable pressure is applied, whereupon it is possible to obtain up to 1 part water extract. The water soluble constituents of endod were noted to pass into solution almost immediately, judged by the color of various extracts, and it was found that using hot water did not make an appreciable difference. The process of filtering, however, was considerably improved by the use of hot water and so subsequently boiling water was generally used for extraction. Increasing the ratio of water to that of endod produced, after mixing, more dilute 'soups' that were still very reluctant to filter even when pressure was applied, and gave a more dilute extract.

For the purpose of spray drying it is necessary to have a continuous supply of feed solution of some 20-25% concentration. This effectively means using as little water as possible to extract the endod, but in such a way that the efficiency of extraction is high as possible, and is

best achieved by washing the endod many times with small quantities of water. Taking these considerations into account it was possible to rule out all mechanical type filter presses, because with a 10-15cm filter cake a very large pressure would be required to give good recovery of the extract and such a filter cake could not be easily washed. By using a centrifuge with a thin filter cake, 1-2cm (giving the same capacity as a comparable mechanical press), considerably less overall pressure is needed to give good recovery and the cake is thin enough to enable wash water to filter through. It was therefore decided to use some form of centrifugal extractor.

After due consideration a second-hand washing machine was purchased. The machine had a horizontally mounted barrel, 55cm in diameter and 30cm wide and was designed for speeds of up to 500rpm. The circumference of the barrel was lined with 5mm wire mesh and removable bags of nylon taffeta (mesh size 40 micron) were fabricated to fit into the barrel, on top of the mesh and provide the filtering medium. Experiments in the laboratory had shown that before efficient extraction could be carried out it was necessary to thoroughly wet the endod berries and allow them to soak and swell for a few minutes. With the laboratory centrifuge this was done before the endod was loaded into the machine. The centrifuge was then run up to maximum speed, 5,000rpm-2,500gm, and several extractions of the endod were performed with small quantities of water. It was obviously impractical to load large amounts of soaked endod into the washing machine while it was stationary and loading the machine while it was running would still have given severe balancing problems. It was therefore decided to drive the washing machine at two speeds. A low speed corresponding to just over 1gm at which powdered endod would be evenly loaded into the machine and by adding sufficient water the soaking could then be carried out while the machine was running. After a period of soaking at the low speed the machine would then be run up to the high speed for extraction.

The original intention was to use a DC motor with continuously variable speeds between 50 and 550rpm, but a suitable motor could not be found, so it was decided that an AC electric motor and some form of speed change should be used. A 0.w5KW, 1385rpm electric motor was available and was used to replace the original 110V drive motor and a clutchless gear change was built. A spring loaded pulley mounted on the motor gave various ratios depending on the tension applied to the drive belt by an idler pulley. Full tension gave the lowest drive ratio and a speed of 180rpm - 10gm and the highest speed available was 540rpm - 90gm. Ideally the low speed should have been around 60rpm - 1.1gm, but in practice it was found to be impossible to get a ratio greater than 1:3 between the lowest and highest speeds because of the inflexibility of the drive belt and the small dimensions of the pulley attached to the washing machine barrel.

A thermostatically controlled 7.2Kw, 60 liter water tank was mounted above the washing machine and a length of 1/2-inch pipe, flexibly mounted so that it could be withdrawn from the machine for bag changing, etc., was drilled with 1mm holes and attached on top of the machine so that it sprayed directly and evenly onto the barrel circumference. Various combinations of endod and water were loaded into the washing machine and a typical procedure was as follows: The washing machine with nylon bag in place was run at slow speed while 1kg of dry powdered endod was carefully loaded (to achieve a good balance) and 1 liter of

boiling water was sprayed on. Similarly two more kg of endod and two more liters of water were also loaded. After five minutes of soaking 3 liters of boiling water were then sprayed on and the machine run up to full speed. A further 3 liters rinse was then executed at the higher speed. This procedure yielded a total of 5.4 liters of dark brown extract of approximately 8% concentration. The original design had called for a directly obtained concentration of between 20-20%, but because the low speed of the machine was giving around 10gm, the water intended to soak and swell the endod was passing quickly through and compacting the filter cake, which caused later additions of water to channel their way through the endod without extracting much of the water soluble material.

This arrangement was suitable for preliminary tests on the spray dryer, but was inflexible for more involved extraction procedures and eventually an ex-aircraft dynamotor was obtained. The dynamotor operated on 27V DC with additional 400V and 750V armature windings and had a rated operating speed of 6,000rpm. By maintaining 27V it was possible to continuously vary the speed of the motor from 0 to 6,000rpm. A 27V supply was constructed to provide current for the field circuit and various attempts are being made to construct a variable high-current supply for the armature, but for the time being it is powered by a 12V car battery. The pulley on the rear of the centrifuge was rebuilt to give a stepdown ratio of 1:6 and at present it is possible to vary the centrifuge speed continuously between 0 and 400rpm. A second-hand car speedometer was connected to the centrifuge barrel to give a direct indication of its speed and various extraction procedures are now being worked on.

(2) DRYING

To overcome the problems of heat sensitivity and de-activation that occurs when endod extract is dried, it was decided to use a spray drying method for this process. The first dryer design was for a co-current dryer with the nozzle spraying fine droplets into a laminar hot air flow that was being sucked through the dryer and then through the cyclone separator by a centrifugal blower.

Preliminary calculations revealed that with an inlet temperature of 250°C and an outlet temperature of 50°C it should be possible to dry 6cc/sec of a 25% solution of extract. This would require 200m^3/hr of air and provided that there were no heat losses 11Kw of heating would be necessary. From the literature it was estimated that droplets of 60 microns diameter would take approximately 0.1-0.2 seconds to dry in such conditions, and the dryer body was dimensioned accordingly.

The whole assembly was constructed from 1mm mild steel sheet. The air heater was double-skinned and insulated with powdered asbestos, and a total heat output of 9Kw was obtained by using twelve 750W firebars. The hot air sucked into the plenum chamber, designed to create a smooth downward air flow in the dryer, travelled through the cylindrical section at approximately 50cm/sec. In the conical section the air flow was accelerated to 10m/sec and passed through the cyclone where particles greater than 5 microns are recovered from the air stream and collected in a plastic container. The cooled air and water vapour pass through the dryer at a rate of approximately 200m^3/hr.

The design of the nozzle had to be tackled empirally and after much experimenting an air injection nozzle was developed that gave a consistently fine spray in the form of wide angled, solid cone. The

fineness of the spray could be controlled by the amount of air pressure applied to the nozzle. Although many calculations were made during the design the comparatively small size of the dryer made it very difficult to predict the performance characteristcis.

The First Spray Dryer in Operation

After allowing the spray dryer sufficient time to warm up to operating temperature spraying of an 8% solution of extract was begun. At the design figure of 6cc/sec no drying was observed to take place and the chamber became coated with a thick gluey covering of semi-dried endod. In subsequent tests the spray rate was reduced until droplets were seen to be drying (1cc/sec), but there was still very heavy depositing of semi-dry material on the walls of the dryer. Eventually some powder was collected from the cyclone, but despite concentrating the endod extract to the design figure of 25% and modifying the air distribution system no significant decrease in the amount of material that built up on the walls was noted. It becamse obvious that there were some fundamental errors in the design of the dryer.

1) There was not sufficient mixing between the spray and the hot air which results in only a small heat exchange between the two. Consequently the drops did not dry sufficiently before they arrived at the walls and stuck to the walls, instead of bouncing off. The hot air, which effectively by-passed the spray, therefore left the dryer at around 140-150°C, a temperature sufficiently high to de-activate the final product.
2) The spray rate could not be effectively monitored and the large fluctuations made accurate control of the drying conditions impossible.
3) The heater casing was buckling badly and dislodging large amounts of scale that were potential sources of short-circuiting. The original wiring system was also inadequate and kept breaking down after only a few minutes of operation.

Despite these problems some of the powder collected during the experiments was found to be toxic at around 6ppm. It was not possible to overcome the faults of the first spray dryer with simple modifications so a second spray dryer was designed and built.

The Second Spray Dryer

To overcome the problem of hot air/spray mixing a countercurrent system was adopted where the extract is sprayed onto a jet of hot air distributed directly beneath it. From experience with the first dryer it was also decided to make the drying chamber very much larger, and the heater elements were built into a 3mm sheet metal case with much improved wiring and no insulation. A small dosing pump was constructed to accurately meter the flow of extract to the nozzle.

After several trials a suitable air distributor was fabricated, which could be raised or lowered by about 15cm, the dosing pump was calibrated, and the conditions necessary to produce a powdered product were soon established. The hot air/spray mixing was now found to be very good and no problems were encountered with the redesigned heater casing the newly constructed dosing pump made it possible to exercise very close control over the drying conditions. However, some powder continued to gather on the conical section of the dryer body. This accumulation, due to the rough nature of the mild steel surface, was expected to reach an equilibrium point after a long period of operation and could easily be cleaned off with a soft brush.

SECTION C: The Pilot Plant

1) Description of the process

The spray dryer is mounted in a specially constructed framework as seen in Fig. 14(see p.46), and the washing machine is mounted on a separate framework. The hammer mill used to grind the endod berries can be seen in the foreground. Hot water is supplied to the extractor by the water heater and the ground endod is weighed out and loaded into the machine by hand. The speed of the centrifuge can be observed on the speedometer and controlled by the rheostat. Power for the centrifuge is supplied from a 30V DC supply (next to dosing pump) and the car battery.

The extract flows by gravity to the rearmost of the two storage tanks shown in Fig. 13(p.46) . The dosing pump pumps the extract from this tank at accurately controlled flow rates through a length of clear plastic tubing to the spray nozzle. Air is supplied to the other storage tank from a small electric compressor, and the pressure in the tank is maintained at the desired nozzle operating pressure by a pressure release valve. Another length of plastic tubing supplies the compressed air to the nozzle.

The air which performs the drying flows into the air heater (behind the water heater), through the spray dryer, into the cyclone separator, and is exhausted out through the window by the vertically mounted blower. The switchboard controls the operation of all the electrical equipment and houses the thermocouple temperature measuring equipment. There are four temperature measuring points, and these can all be seen in Fig. 12 (p. 46).

1. A mercury/glass thermometer situated directly in the heater output stream to give the air inlet temperature.
2. Thermocouple at the top of the dryer.
3. Thermocouple at the middle of the dryer.
4. Thermocouple at the bottom of the dryer giving the exit temperature.

The powdered extract is collected in a plastic bottle that screws onto the bottom of the cyclone separator.

Before spraying is commenced the spray dryer is allowed to warm up fully (usually requiring 20 minutes) and then spraying can begin. The nozzle sprays onto the hot air stream emerging from the air distributor (Fig. 13 , p. 46). which is designed to induce strong turbulence and hence give good heat transfer between the spray droplets and the hot air stream. The interior of the dryer is illuminated to allow observation of the drying process to take place. The temperature distribution in the dryer can be read directly from the switchboard, and is continuously monitored when the dryer is in operation.

2) Evaluation of Pilot Plant Performance

In the past, endod extraction has been considered from the point of view of the toxicity of the extract that is produced. Any extract, since a specific molluscicide solvent is not yet available, will consist of two parts:

a) Molluscicide (toxic)
b) Non-active materual (non-toxic)

The toxicity of an extract could therefore be expressed as the ratio of the toxic material to the non-toxic, and is a function of the solvent that is used.

In evaluating the performance of the pilot plant the toxicity, which is basically a function of the solvent, is not so useful. What is required is an estimate of the total quantity of toxic material that has been extracted, and this is related not only to the toxicity, but also to the total quantity of extract produced, the productivity. It can be expressed most conveniently in a percentage, as the ratio of the volume of water the extract will treat, to the volume of water the crude endod will treat. Both factors can be determined from the respective productivities and toxicities of the extract and the crude. We therefore have three parameters with which to evaluate the pilot plant performance:

1) Productivity — Mass of extract (grams)/kg of endod extracted
2) Toxicity — Potency of extract in ppm
3) Molluscicide yield: $\frac{\text{Water volume treatable by extract}}{\text{Water volume treatable by crude}}$

For example, if the extraction of 1kg of crude endod gave 90gm of powder, active at 8ppm,

1) Productivity — 90gm
2) Toxicity — 8ppm

Drude endod is active at 25ppm, therefore:

25 of crude endod will treat 1,000 liters (or one million ml) of water ($1m^3$)

1,000gm of crude endod will treat $\frac{1,000}{25}$ 1 cu.m. of water

1kg of crude endod will treat 40 cu.m. of water

The extracted endod, the powder, is active at 8ppm:

8gm of powder will treat 1cu.m. of water

90gm of powder will treat $\frac{90}{8}$cu.m. of water

90gm of powder will treat 11.25 cu.m. of water

3) Molluscicide yield $\frac{11.25}{40} = 28.2\%$

For this process 28.2% of the molluscicide present in the crude endod is present in the powdered extract, and 71.8% of the molluscicide has been lost.

Furthermore

Mathematically the molluscicide yield can be shown to be equal to one fortieth of the productivity divided by the toxicity, provided the toxicity of the crude endod is invariant.

3) Molluscicide yield $\frac{90}{40 \times 8} = 0.282 = 28.2\%$

The molluscicide yield, which represents the overall efficiency of the pilot plant, should therefore be as high as possible. For the molluscicide yield to be 100%, the extraction process must remove all of the molluscicide from the endod berries and there must be no detoxicification of the liquid extract in the drying process. If we assume the latter we can extract the crude endod and obtain an extract, which can be subdivided into toxic and non-toxic components, and an insoluble residue. Water extractable material is known to form 50% by weight of the crude endod and so we obtain, for complete extraction:

	Crude	=	Extract (toxic)	+	Extract (non-toxic)	+	Residue
MASS	1000gm	=	500gm			+	500gm
MOLLUSCICIDE YIELD	100%	=	100%			+	0%

This gives 1) 500gm
2) 12 1/2 ppm (calculated from the above equation)
3) 100%

The toxicity of an extract is a function of the solvent used, and in the limiting case of complete extraction the toxicity of the water extract is therefore 12 1/2 ppm. If the rates at which the toxic and non-toxic components of the extract pass into the solution were always the same, it can be seen that using water as the extraction solvent it would not be possible to produce an extract more toxic than 12 1/2 ppm. If, however, the toxic material is more soluble than the non-toxic material and complete extraction is not carried out the extract will contain a higher ratio of toxic to non-toxic, and will consequently have higher toxicity than the extract obtained by complete extraction. One batch of water extract has been found to be toxic at 6-8ppm, it follows that the molluscicide yield will not be 100%, and the efficiency of extraction is effectively being sacrificed for toxicity of the extract.

In evaluating the performance of the pilot plant this inter-relation between the molluscicide yield and the toxicity of the extract has to be taken into account. For this reason, the use of solvents other than water which would preferentially extract only the toxic component, leaving behind all the non-toxic water extractable material and the residue, is now being sought.

3) Pilot Plant Performance

When the first spray dryer, and the original modifications to the washing machine were completed, attempts were made to produce extract as a dry powder. The first attempts were for the most part unsuccessful for the reason previously outlined and a second spray dryer body was constructed. This new design overcame the previous problems and produced a very finely powdered, light brown extract.

Test T1: 1kg of ground endod was extracted using 3 liters (L) of boiling water to produce 1,520cc of liquid extract. The spray dryer was heated up using the full 9Kw, and the temperature distribution in the dryer was observed as the extract was sprayed at 0.76cc/sec with a nozzle pressure of 1 atmosphere (11.7psi). (0.76cc/sec was the experimentally predetermined maximum spray rate before wetness appeared on the dryer walls).

Spraying time (mins)		0	5	10	15	20	25	30
Temperature (°C)	@1	290	290	285	288	282	286	286
	@2	134	104	105	108	114	116	119
	@3	116	88	86	88	94	96	99
	@4	140	103	96	92	93	94	95

Results 1) 97gm
2) 50ppm
3) 4.9%

From the discussion on the evaluation of the pilot plant performance it can be seen that the water extract, however produced, should have a toxicity of around 10-15ppm, and so the figure of 50ppm immediately indicates that considerable de-toxification of the extract has taken place.

Boiling water has no effect on endod, so there can be no de-toxification in the extraction process. The de-toxification must therefore occur in the drying process and could be due to either of two factors:

a) The output temperature of 95-100°C could be sufficient to de-activate the powder.
b) The high inlet temperature could be responsible.

Heating Tests 1, 2 and 3: Three samples of stock butanol extract active at 3ppm were taken. An electric oven was heated to 100°C, and a small metal tray, preheated to 100°C was used to heat the three samples for periods of one, two and three minutes respectively. Subsequent snail tests revealed no significant change in the toxicity of the extracts, as a result of the heating, and so de-activation was not due to the high exit temperature.

Test T4: This test was identical to T1 in all respects except that only 6Kw of heating power was used and consequently the spray rate was slightly reduced at 0.73cc/sec.

Spraying time (mins)		0	5	10	(Spraying discontinued)
Temperature (°C)	@1	230	228	228	
	@2	113	90	91	
	@3	111	81	81	
	@4	114	75	71	

Results 1) 95gm
2) 25ppm
3) 9.5%

A new blower was therefore constructed with approximately double the capacity of the original to enable the full capacity of the plant tc be utilized, and still employ a low inlet temperature. When installed in the dryer it was discovered that the air velocity was now too high in the cyclone, and that dried particles would not separate from the air stream, so a larger cyclone was constructed. The dynamotor-driven centrifuge was now ready and a final preliminary test was carried out.

Test T5: 1kg of ground endod was soaked in the centrifuge at a speed of 80rpm with 1L of water for five minutes and then run up to 400rpm and extracted twice, each time with 1L of boiling water, and 1310cc of liquid extract were obtained. This extract was then sprayed at a rate of 0.98cc/sec with a nozzle pressure of 1 atmosphere.

Spraying time (mins)		0	5	10	15	20
Temperature (°C)	@1	109	111	112	115	114
	@2	88	76	76	77	75
	@3	107	94	91	92	91
	@4	96	82	80	79	81

Results 1) 113gm
2) 15ppm
3) 18.8%

The tests that have so far been carried out have served to identify the faults that were present in the design of the pilot plant. With the completion of the variable speed drive for the extractor unit, and the obtaining of a dry product using an inlet temperature of only 115°C, the modifications that were necessary to the design of the pilot plant have now been completed.

At present the following factors are not properly understood:
Extraction: The relative solubility of the water soluble constituents of endod, and what bearing these have on the efficient operation of a centrifugal type extractor.

Drying: The mechanism involved in the temperature detoxification of the molluscicide.

A comprehensive series of tests and experiments are now being planned to provide sufficient operating experience and technical information to enable the desired toxicity of 6-8ppm to be obtained at the highest possible efficiency (molluscicide yield).

d) Future Plans

a) To complete the testing and commissioning of the pilot plant along the lines laid out in the previous section. When this has been completed production of the molluscicide and its preparation in different formulations for evaluation and eventual use for treatment of schistosomiasis is affected areas in Ethiopia will be commenced.

b) To investigate the possibility of developing a process for isolating the molluscicide in its pure form, using the water extract produced by the pilot plant as the raw material, will also be undertaken. Such pure material will be used for tests to determine the usefulness of endod as a foaming agent in light weight concrete preparation, in detergent preparations, as a dispersant in cosmetic factories, as a fungicide, and as a spermicide for possible use in antifertility efforts.

Unpublished Research Note No. 14

STUDIES ON THE LARVICIDAL PROPERTIES OF ENDOD (PHYTOLACCA DODECANDRA)

Aklilu Lemma (Sc.D.), Milton B. Flemings (Ph.D.),
Getachew Assefa (M.Sc.), and Tesfa Michael Tesfa Yohanes (Ph.D.)

Institute of Pathobiology, 1975

Original Objectives

The original objectives of the project, as approved by the African American Scholars Council, were:

1. To undertake a systematic and quantitative screening of the effect of endod against carefully selected representative species and life-stages of medically and agriculturally important insects.
2. To undertake some field evaluation of the activities of endod against mosquito and Simulium larvae under natural conditions in the field.
3. To undertaken some histopathological studies to determine the mode of action of the active principle in endod against mosquito larvae; and
4. To undertake some chemical studies to determine the structure specificity of the active ingredient against mosquito larvae.

The project was a collaborative undertaking between the Institute of Pathobiology, Addis Ababa University, and C.W. Post College, Long Island University. The first two objectives of the project were to be undertaken in Ethiopia under the supervision of Dr. Aklilu Lemma and the last two in the United States under the supervision of Professor Milton B. Flemings.

In accordance with the first objective of the project, a series of experiments were conducted to screen the larvicidal properties of the crude ground endod berries on different species of laboratory-reared mosquito larvae. Also attempts were made to develop techniques for the collection and maintenance of cockroaches, houseflies, army worms and Simulium larvae for similar tests.

In all of the screening tests, three different preparations of the endod berries were to be used: the crude ground berries, the water extract, and the butanol extract; prepared according to the procedures described by Lemma et al. (1972). During the early stages of our investigations there was some indication that some parts of the endod plant other than the berries, may also have some toxicity to insects. Therefore, an attempt was made to determine the relative toxicities of the stem, bark, root, mature and young leaves (shoots), and the ripe berries of the endod plant against mosquito larvae and cockroaches. Since there is some difference in the chemical composition of the female and the male plant, this experiment will be done with both sexes separately.

Materials and Methods

1. Mosquito Larvae

It took quite a bit of effort to try and establish adequate colonies of A. aegyptei, C. fatigans and A. gambiae. A. gambiae, the local vector of malaria in Ethiopia and other East African regions, was the most difficult of all the mosquitoes we tried to rear in our laboratory. Even to date we have not been able to get a good and continuous colony going.

This species is very well known for its fastidiousness in regard to its feeding and egg laying habits when in captivity. However, Dr. Tesfa Michael, our senior entomologist, is doing his best to develop appropriate techniques on how to rear and maintain large populations of this insect under our culture room conditions.

2. Test Procedures

All the procedures used in the larvicidal and insecticidal tests were as described by Spielman and Lemma (1973). The larvae of Aedes aegyptei, Anopheles gambiae and Culex fatigans were exposed to different concentrations of endod solution. In most of the cases, 1 gram of ground endod berries (bought from the market) was mixed with 999 cc of buffered distilled water to form 1000 parts per million (ppm) solution. This was then shaken for about 30 minutes to dissolve the active principle in the solvent. The solution was then serially diluted and distributed in 200 ml volumes in plastic cups. Twenty-five active larvae were then put in each cup containing the test solution. The larvae were left in such solution for 24 hours, after which time they were washed with clean water and left to recover for another 48 hours before recording the deaths in each cup.

3. Houseflies (Musca domestica)

These results are contained in the report by Getachew (1976).

4. Army worm (Spodoptera exepta)

In an attempt to rear the army worm in our laboratory, some batches of eggs and 3 pupae of Spodotera exepta were obtained from the International Insect Physiology and Ecology (ICIPE) in Kenya. The pupae and the eggs were kept in the humidified insectary. Most of the eggs hatched the same day they were received, but due to lack of experience in handling them, they all died. However, from 3 pupae we obtained 3 adults, one male and two females. These were given maximum attention and we were able to get from them some eggs, larvae and finally some adult moths. We got 13 adults, almost all of which were in excellent conditions. Unfortunately, all the 13 adult moths so obtained were females with no male to fertilize them. Thus, although they laid batches of eggs, none hatched out because they were not fertilized. However, the following information was obtained from our trials:

a. Duration from egg to larvae 2 - 3 days
b. Duration from larvae to pupae 12 -13 days
c. Duration from pupae to adult 7 -12 days
d. Duration from egg to adult 21 -28 days

Having these background information at hand, we now plan to have colonies of different species of army worm to test their susceptibility to endod under our laboratory conditions.

5. Cockroaches (Blatella germanica)

Cockroaches, supplied by NAMRU-5 in Addis Ababa, were reared in our laboratory for two generations in order to get enough population of different ages for use in our insecticidal trials. Rearing of cockroaches was very simple. About 10-15 cockroaches were placed in a 500 ml capacity glass beaker. Pieces of paper were dropped into the beaker to provide enough resting places and also to create the necessary niches. Water was provided through wet cotton placed inside the beaker and the cockroaches

were fed with dog chow food which was also kindly supplied by NAMRU-5. The beakers were covered with nylon cloth firmly held by rubber bands. Cockroaches of different ages were used in the preliminary trial for determination of their susceptibility to endod.

Results

1. Effect of different parts of the endod plant on Aedes aegyptei larvae

As mentioned above, early observations on the larvicidal properties of the endod plant indicated the possibility that besides the berries, other parts of the plant may also have insecticidal activities. Therefore, a series of tests were conducted to determine the relative toxicities of different parts of the female endod plant to the 2nd and 3rd stages of Aedes aegyptei larvae. The results are given below:

Parts of the endod plant	LC_{50} in ppm	LC_{90} in ppm
stem	1000	1000
bark	150	1000
root	150	600
matured leaves	130	600
young leaves	100	400
crude berries	40	100

The LC50 and LC90 determinations are all approximate. However, they are based on an average of 5 different tests for each experiment. In each test 25 larvae were exposed to each of the following different concentrations: 1000, 250, 100, 50, 25 and 0 (control) ppm endod in buffered didstilled water. The control was only buffered saline, and there were no ead larvae found in such a control. The test larvae were exposed to the endod solutions for 24 hours, after which time they were washed and allowed to recover for 48 hours in buffered distilled water. During exposure to the test solution, the larvae were not given any food, but during the 48 hours recovery time they were fed and treated as normal. All tests were performed at room temperature and water pH of about 7.0.

From the above data it appears that the crude berries of endod are more toxic than all other parts of the plant. However, the toxicity level of the leaves was also very interesting, particularly because the larvicide could be extracted in bulk from large quantities of such leaves.

2. Effect of endod on different larval stages (instars) of Aedes aegyptei

After establishing that the berries were the most potent part of the endod plant, the susceptibility of different stages of the larvae of A. aegyptei to the crude ground berries of endod was determined. The preliminary results are given below.

Larval stages (instar)	LC_{50} in ppm	LC_{90} in ppm
1st instar	35	105
2nd instar	40	120
3rd instar	60	180
4th instar	60	180

Although this experiment needs to be repeated and the LC50 and LC90 calculations are approximate, the data indicates that 1st and 2nd instar are more susceptible to endod than 3rd and 4th larvae. The reason for this may be due to the feeding habits of the different stages of the larvae; the 1st and 2nd stages are more voracious eaters than the 3rd and 4th stage larvae (Spielman and Lemma, 1973).

3. Effect of endod on Anopheles gambiae

As mentioned earlier, Anopheles gambiae was difficult to rear in the laboratory. However, some preliminary results of tests have shown that the larvae of this species are more susceptible to endod than those of A. aegyptei and C. fatigans

Preliminary experiments conducted to determine the toxicity of the crude endod berries on different stages of Anopheles gambiae larvae, revealed that the LC50 for the 1st instar larvae, after 24 hours exposure and 48 hours recovery time, was 4.8 ppm, while the LC90 was 11.0 ppm. These data were calculated from an average of three tests in each case, i.e. the experiment has been repeated three times and an average of the three tests was used for calculation. Due to lack of sufficient larvae populations only two tests have been conducted with the 2nd stage larvae so no LC50 or LC90 calculations have been made on these yet. This will be done as soon as more tests on these 2nd stage larvae are conducted.

The LC50 and LC90 for the 3rd stage larvae was 6.2 and 24 ppm, respectively. For the 4th stage larvae, the LC50 was 13 ppm and the LC90 was 52 ppm.

Stage	LC_{50}	LC_{90}
1st instar	4.8 ppm	11.0 ppm
2nd instar	-	-
3rd instar	6.2 ppm	24.0 ppm
4th instar	13.0 ppm	52.0 ppm

4. Effect of endod on Culex fatigans

The most ubiquitos and hardy larvae of Culex fatigans were easily reared in the laboratory. However, the larvae of this species have been found to be the most resistant to endod, requiring above 1000 ppm for LC90. This species is generally known to be resistant to other insecticides as well.

For preliminary screening of the susceptibility of C. fatigans to endod, the following concentrations were used: 1000, 500, 250, 100 and 50 ppm. The results showed that the LC90 for the 1st and 2nd stage larvae was greater than 100 ppm, the highest concentration tested, while the LC50 for the 1st and 2nd stage larvae were about 250-275 ppm. Tests are now in progress to determine the susceptibilities of the 3rd and 4th stage larvae.

Stage	LC_{50}	LC_{90}
1st instar	275 ppm	1000 ppm
2nd instar	260 ppm	1000 ppm
3rd instar	not yet determined	not yet determined
4th instar	not yet determined	not yet determined

It is evident from these results that C. fatigans is highly resistant to endod.

5. Effect of endod on cockroaches (Blatella germanica)

The susceptibility of cockroaches to different parts of the endod plant such as the leaves, bark, berries and root was determined. Each of these different parts of the plant was dried and ground to form a powder. This was then mixed with dog food in different proportions, i.e. 100% endod only, 75% endod + 25% dog food, 50% endod + 50% dog food, and 25% endod + 75% dog food. We also had a control where only dog food was used.

All cockroaches were starved for 5 days before use. In each test, 5 cockroaches were kept for 10 days in Erlenmeyer flasks containing the different proportions of endod and dog food. The results showed no significant mortality that can be attributed to endod. The few deaths observed were believed to have been due to starvation rather than the effect of endod. The hygroscopic nature of the powdered endod berries along with the sugar mixture resulted in the rapid molding of the bait. Such molds repelled the cockroaches from eating the powder. The powder from the leaves, bark and root did not have this property and it therefore remained clean and looked fresh during the entire experimental period. However, the cockroaches did not feed at all on these preparations. It is possible that these different parts of the endod plant may contain something which is not palatable to the cockroaches.

References

Lemma, A., Brody, G., Newell, G.W., Parkhurst, R.M. and Skinner, W.A. (1972). Endod (Phytolacca dodecandra), a natural product molluscicide: increased potency with butanol extraction. J. of Parasit. 58, 104-107.

Spielman, A. and Lemma, A. (1973). Endod extract, a plant-derived molluscicide: toxicity for mosquitoes. Am. J. Trop. Med. Hyg. 22, 802-804.

EFFECTS OF ENDOD ON THE PERISPIRACULAR GLANDS IN LARVAE OF MOSQUITOES

M.B. Flemings (Ph.D.)
C.W. Post College, Long Island University, Brockville, N.Y.
1975

INTRODUCTION

Objectives

To examine the ultrastructure of perispiracular glands within the larvae of mosquitoes from one species that are exposed to Phytolacca dodecandra, a plant which is commonly known as "endod" or "soapberry".

During recent years this plant, which is native to East Africa, has proven to be a selective molluscicide with insecticidal potentials (16).

Background and Rationale

Induced deterioration and/or morphological changes of specialized tissues such as perispiracular mosquito glands may be demonstrated by electron microscopy; and, therefore, may suggest a "mode of action" by an insecticide.

Ultrastructure of cuticular layers surrounding perispiracular glands within mosquito larvae has been demonstrated by Neville (11) and Weis-Fogh (17). According to Keilen and coworkers (8) and Keilen (9), the perispiracular glands of dipterous larvae secrete an oily substance which is responsible for hydrofuge properties of the spiracles and peritremes which are integral parts of the siphon tube (air intake tube). This tube also is necessary for larval suspension at the water's surface; allowed by a film created from surface tension. Thus, the larvae may acquire air without intake of water and subsequently drowning. Functional interference with these glands would therefore lead to drowning due to its inability to secrete oil and prevent "wetting" of the spiracular valves and peritremes.

Porter (13) demonstrated endoplasmic reticula from the perispiracular gland to be responsible for synthesis of lipid droplets. Additionally, Hackenbrock(3) concluded that mitochondrial "swelling" depended upon increased oxygen supply and electron flux. In order for energy rich ATP to be produced, there must be a steady input of electrons and an adequate supply of oxygen to accept these electrons at the end of the respiratory chain.

Apropos, "endod" contact with the siphon tubes when larvae emerge for air, could neutralize lipid substances that are secreted by the glands, thereby adversely affecting hydrofuge properties of the valves.

Another aspect of "drowning" could be the interaction of endod with the cuticle layer of the siphonal horn. Pal (12) and Holdgate (6) found that contact of a chemical monolayer film with the epicuticle resulted in a change of the cuticular surface which modified its physical characteristics and altered its hydrofuge properties. The specific cuticular sublayer most likely affected was the cuticulin which is composed of a thin layer of wax and a layer of cement that covers the wax. The latter consists of long chained hydrocarbons and esters of fatty acids and alcohols. The wax molecules adjacent to the cuticulin sublayer are strongly compact and oriented at an angle that normally renders them inpermeable to water.

According to Beament (2) and Randall (14), these oriented molecules

also present a row of aliphatic groups to the outside; and these also are partly responsible for the hydrofuge properties of the siphonal cuticle.

MATERIALS AND FACILITIES

Culex pipiens pipiens was the mosquito chosen for this study due to its easy availability and adaptability for laboratory rearing. Larvicidal activities were limited to 4th instar. Other materials consisted of:

Culex pipiens pipiens larvae
standard flinstone trays
endod (Phytolacca dodecandra)
mortar
distilled water (pH 7.2)
triple beam balance
1,000 ml Erlemyer flask
stirring apparatus
paraffin wrap
forceps
stop watch
Pasteur pipettes
screw cap tubes
3% glutaraldehyde
50% ethyl alcohol
70% ethyl alcohol
85% ethyl alcohol
95% ethyl alcohol
100% ethyl alcohol
propylene oxide
Epon 812 kit
Beem polyethylene capsules
drying oven
xylene
uranyl acetate
lead citrate
NaOH pellets
8 cc syringe
glass knives
razor blades
binocular light microscope
400 mesh formvar grids
monochromatic stains
glass slides
cover slips
Sorrensen's phosphate buffer
osmium tetroxide

METHOD

A LD-50 was confirmed for 1000, 2000 and 3000 ppm; however, the 2000 ppm was utilized for testing. Due to the non-availability of endod extract, dried berries were manually ground into a fine powder and weighed. The amount was added to appropriate volumes of distilled water (pH 7.2) in flintstone trays. These replicate test trays and appropriate controls, each consisting of 25 fourth instar larvae were utilized. Constant room temperature of 27°C prevailed; and mortality was recorded at invervals of 18, 24, 48 and 72 hours. Siphons from 48-hour controls were excised and processed for electron microscopy as follows:

Fixation in Sorrensen's phosphate buffer with 3% glutaraldehyde. The siphons were then washed in 3 changes of Sorrensen's phosphate buffer, 5 minutes for each change and left overnight at 4°C. Post fixation was accomplished with 1% osmium tetroxide for 2 hours at 4°C. Three buffer washes were conducted, 5 minutes for each change. An increasing ethanol series (50,70,85,95,100%) were used for dehydration, 10 minutes for each concentration. Three 10 minute washes in propylene oxide were used for clearing the tissue. To the third wash, an equal volume of Epon was added, stirred and allowed to infiltrate for 1 hour at room temperature. Following this, another equal volume of Epon was added, stirred and remained overnight at room temperature for further infiltration. The siphons were then embedded in Epon and polymerized at 70°C for 24 hours.

After embedding and polymerization, the hardened capsules were trimmed with a sharp razor blade. Thick and thin sections were cut by glass knives on a Reichert OM-U2 ultramicrotome. Thick sections were

deposited on Corning glass slides, stained with 1.0% toluidine blue and examined under a binocular light microscope. Thin sections were collected on 400 mesh formvar carbon coated grids, stained in uranyl acetate and counterstained in lead citrate. Micrographs were taken on a Hitachi HU-11a electron microscope and enlarged photographically.

The procedure for tissue processing and preparation of electron microscope chemicals are those found in the works of Hayat (1970) and Harm (4).

RESULTS AND DISCUSSION

"Control" Specimen (Untreated Larvae)

Figure 1* is a photograph showing a fourth instar Culex pipiens pipiens larva in its normal position at the surface area of water. Note the long siphon and its tip (distal end) in the process of obtaining oxygen from the water-air interphase.

Figures 2-6 are cross-sections of the siphonal felt chamber at its center and of the perispiracular glands which are located within the tip of the respiratory horn.

Figure 2 is a micrograph of an entire cross section of the siphonal tube at region of felt chamber. At reduced magnification, all significant structures are displayed.

Figures 3 and 4 show specific sections of perispiracular gland cells, each include the nucleus, nucleolus, mitochondria and other cytoplasmic inclusions. Directly opposite and contiguous with the gland cells are tracheal lumen (felt chamber) showing highly magnified microvilli that protrude into the felt chamber. These are confirmed notations from earlier publications by Keilen and coworkers (8) and Keilen (9), Badalamenta and coworkers (1973). Surrounding the nuclei of the figures are vacuolated protoplasm and numerous clefts.

These microvilli suggest avenues of lipid transport into the felt chamber and thusly, serve as hydrofuge properties within the respiratory horn.

Figures 5 and 6 display perispiracular gland cells, highly vacuolated protoplasm with dispersed lipid droplets and mitochrondria. Note the thickened cuticle lining. The lipid droplets are surrounded by dense granules which Smith (15) labelled as secretory droplets.

Observation of lipid substances utilizing light microscopy and Flemming's fixative as recommended by Keilen, Tate and Vincent (8) was unsuccessful. Similarly, the solutions and method as recommended by Humason (7) was also unsuccessful due to continued overstaining and consequently the obscuring of lipid materials.

"Test" Specimen (Treated Larvae)

Figures 7 and 8 are micrographs of perispiracular glands showing hypertrophied mitochondria and reduced microvilli. Hackbrock (3) attributed this abnormal "swelling" of mitochondria to the dependence on increased oxygen supply and electron flux; the mode of action being its cytotoxic effects. Lehninger (10) attributed this type of mitochondrial swelling to ATP inhibition caused by concentration of atractyloside in rat liver. Atractyloside is a toxic plant glycoside which is similar in its chemical structure to the active components of endod. Therefore, the inhibition of a specific mitochondrial transport system via endod suspension is a probability. Of course, additional detailed study is necessary.

---

* no figures have been included in this paper

Lemma (personal communication) has labelled endod as a long chain hydrocarbon and a triterpenoid saponin consisting of oleanolic acid glucoside. Thus, endod in an aqueous solution resembles that of a soap. It is possible, therefore, that endod, like soap, lowers the surface tension of water and simultaneously interferes with suspension of the mosquito larvae at the air-water interphase. Decreased surface tension also permits the formation of a permanent "lather" (a fine suspension of endod in water) which may act as a barrier to prevent the excape of oxygen, thus excess accumulation in the perispiracular glands, and thusly, the inducement of mitochondrial "swelling".

The rest of the paper is not available

LITERATURE CITED

1. Badalamenta, M.A., C.L. Kozenko and M.B. Flemings, .975. "Ultrastructural Observations of the Respiratory Siphon of the Fourth Instar Culex pipiens pipiens Larva Subjected to Chemical Monolayers," Annals of the Entomological Society of America, Volume 69, No. 1, pp. 114-116.
2. Beament, J.W.L., 1960. "Wetting Properties of Insect Cuticle," Nature, London, Volume 186, pp. 408-409.
3. Hackenbrock, C.R., 1966. "Ultrastructural Bases of Metabolically Linked Mechanical Activity in Mitochondria; I: Reversible Ultrastructural Changes with Change in Metabolic Steady State in Isolated Liver Mitochondria," Journal of Cellular Biology, Volume 30, pp. 269-297.
4. Harm, J., 1973. "The Effects of Some Chemical Monolayers on the Respiratory Horn of the Fourth Instar Culex pipiens," Master's Thesis. C. W. Post College, New York.
5. Hayat, M.A., 1970. Principles and Techniques of Electron Microscopy. Volume 1, Van Nostrand Reinhold Company, New York.
6. Holdgate, M.W., 1955. "The Wetting of Insect Cuticles by Water," Journal of Experimental Biology, Volume 32, pp. 591-617.
7. Humason, G.L., 1967. Animal Tissue Techniques, W.H. Freeman and Company, San Francisco, pp. 19-20.
8. Keilen, D., P. Tate, and M. Vincent, 1935. "The Perispiracular Glands of Mosquito Larvae," Parasitology, Volume 27, pp. 257-262.
9. Keilen, D., 1954. "Respiratory Systems and Respiratory Adaptations in Larvae and Papae of Diptera," Parasitology, Volume 36, pp. 1-66.
10. Lehniger, A.L., 1975. Biochemistry. Second Edition, Worth Publishers, New York, pp. 529-531.
11. Neville, A.D., 1969. "Cuticle Ultrastructure in Relation to the Whole Insect," Symposia of the Royal Entomological Society, Blackwell Scientific Publications, Oxford and Edinburgh, pp. 17-41.
12. Pal, R., 1951. "The Wetting of the Insect Cuticle," Bulletin of Entomological Research, Volume 51, pp. 121-139.
13. Porter, K.R., 1961. "The Sarcoplasmic Reticulum: Its Recent History and Present Status," Journal of Biophysics, Biochemistry and Cytology, No. 10, pp. 211-226.
14. Randall, K.M., 1963. "The Chitin/Protein Complexes of Insect Cuticle," Advances in Insect Physiology 1, pp. 257-311. (eds. Beament, J.W.L., J.E. Treherne and V.B. Wigglesworth), Academic Press, London.

15. Smith, D.S., 1968. Insect Cells: Their Structure and Function, Oliver and Boyd, Edinburgh.
16. Spielman, A. and Lemma, A., 1973. Endod extract, a plant-derived Molluscicide: Toxicity for Mosquitoes. Americal Journal of Tropical Medicine and Hygiene, Volume 22, pp. 802-804.
17. Weis-Fogh, 1969. "Structure and Formation of Insect Cuticle," Symposia of the Royal Entomological Society, Blackwell Scientific Publications, Oxford and Edinburgh, pp. 165-187.

Unpublished Research Note No. 16

PROGRESS REPORT: STUDIES OF THE INSECTICIDAL PROPERTY OF ENDOD
Getachew Assefa (M.Sc.), Institute of Pathobiology, 1976

One of the original objectives of this research was to evaluate the effect of endod on black fly (Simulium) larvae and houseflies (Musca domestica domestica). In accordance with the objective of the project, a series of experiments was conducted to screen the larvicidal properties of the crude ground berries on houseflies reared in the laboratory and black fly larvae collected directly from the field. Also attempts were made to rear black flies in the laboratory and techniques were developed to rear houseflies and to collect black fly larvae from the field.

Materials and Methods

In most of the black fly larvae tests, 4-5 different concentrations of endod, expressed in ppm (parts per million) were used. While in the case of adult and larvae of houseflies the concentration used was expressed in % (percent).

It took quite a bit of trial and error to successfully establish adequate colonies of houseflies (Musca domestica). Due to the confinement of Simulium ssp. larvae to streams 17 km away from the laboratory and lack of adequate laboratory equipment, it was beyond our ability to try techniques to maintain Simulium colonies in our laboratory.

Rearing Techniques of Houseflies (Musca domestica)

1. Early stage larvae were collected from the field and were transported to the laboratory.
2. In the laboratory the larvae were transferred to cups filled with malt molasses and kept in the humidified (70-80%) room temperature (26-27°C).
3. The larvae were fed with milk every other day.
4. The pupae allocate themselves on the upper dry layer of malt molasses in the cups.
5. Separation of the pupae from the malt was made by separating the upper layer of the malt and allowing it to a container half filled with water. All the pupae floated on the water.
6. After allowing the pupae to dry, the pupae were kept in a releasing case.
7. Adult flies start to emerge from the pupae after 2 days.
8. Adults were fed sugar.
9. Egg laying media (a cup which contains cotton soaked in milk) was kept together with the adult flies.
10. When the egg media contains sufficient number of eggs it was transferred to a container filled with malt molasses.

Duration from egg to adult in our laboratory condition was 11-14 days.
Duration from egg to larvae was 2-3 days.
Duration from larvae to pupae was 4-5 days.
Duration from pupae to adult was 5-6 days.

RESULTS

1. Effect of endod on houseflies

As mentioned in the previous progress report, crude endod leaves have indicated some insecticidal potency. Therefore, a series of tests has been conducted to determine the effect of crude endod berries and leaves against adult and their larvae; the results are shown in Table 1.

Table 1. Effect of endod on adult houseflies and their larvae

Conc. of endod (%)	Type of endod	Mortality in each test(%)	Stage of life	Average mortality(%)	Recovery period
100	Leaf juice	96,100, 98	Adult	98	48
75	"	100,100,100	"	100	"
50	"	100,100,100	"	100	"
25	"	100,67,82	"	83	"
0	"	0, 0,13.3	"	3.3	"
50	Crude	100,100,100,100	Larvae	100	24
40	"	100,100,100,100	"	100	"
30	"	96,96,88,96	"	94	"
20	"	88,80,80,88	"	84	"
10	"	76,76,60,68	"	70	"
0	"	0, 0, 0, 0	"	0	"
100	Crude	100,100,96	Adult	99	48
50	"	96,100,100,100	"	99.5	"
25	"	100,100	"	100	"
0	"	8, 0, 0, 0	"	1.6	

2. Effect of endod on Simulium larvae

From the previous observation on larvicidal properties of endod it appeared possible to control river blindness (onchocerciasis). Therefore a number of tests were conducted to determine the potency of endod against Simulium spp. larvae. A medium sized (2-3 mm in length) larvae was used during all tests. Different parts of the female endod plant (stem, bark, root, matured and young leaves as well as the crude berry) were tested. Since only the crude berry gave encouraging results, the data appearing here are for crude endod berry only.

The concentration we initially used was 50-1000 ppm. Since there was 100% kill in lower concentrations, we varied our concentrations from 200-0 ppm. A total of 16 groups of tests were conducted. For each group a series of 5 flasks containing 200 cc of solvent was used. In each flask we exposed a minimum of 25 larvae. They were aerated throughout the tests.

Results

The results are shown in Table 2.

Table 2. Effect of endod on *Simulium* larvae

Endod concentration in ppm	Mortality in each test (%)	Average mortality (%)
200	100,100,100,100	100
100	100,100,100,100,100,100	100
50	100,100,100,100	100
25	92,96,76,80,96,92	87
0	0, 8, 4, 0, 0, 4, 24	10

Conclusion

From the above table it appears that house flies and larvae, and *Simulium* larvae are highly susceptible to crude endod.

Unpublished Research Note No. 15

Report on the effect of endod on Culex fatigans and A. gambiae larvae

Tesfa Michael Tesfa Yohannes (Ph.D.), Institute of Pathobiology, 1976

1. Effect of endod on Culex fatigans larvae

Culex fatigans is the species of mosquitoes widely spread all over the world. This species is responsible for the transmission of the periodic form of Wuchereria bancrofti in India, Ceylon and Southeast Asia and it is also incriminated in the transmission of St. Louis encephalitis in Eastern U.S.A. It is a household mosquito and it causes great annoyance during the night either by sucking blood or by the buzzing sound it produces. This mosquito is not choosy in its breeding habitat. It was found breeding in foul water, in rain barrels, tubs, catch basins, faulty cesspools, ditches and ground pools.

Our colony of Culex fatigans originated from eggs of one female which was collected at the old airport, Addis Ababa, in 1974. Since then it was maintained in a laboratory with a temperature of 28°C and 80% relative humidity. Larvae were fed on combinations of dog food, fafa, nonfat milk, oats and meat meal in 5:5:1:1:1 proportions respectively. Adults were fed on human arm under darkened conditions. The average egg production per female was about 65.

Although there are many commercial insecticides to effectively control the infestation of surrounding areas, we thought it worthwhile to check the effect of endod on Culex fatigans larvae. For preliminary screening of the susceptibility of the different instars, the following concentrations were used: 1000, 500, 250, 100 and 50 ppm. However, when the Lcqo for the fourth instar was found to be higher than 100 ppm, it was decided to include 2000 in the series of concentrations. As shown in the table, the Lc50 and Lc90 is very high and more so of the fourth instar larvae. Culex fatigans is generally known to be resitant to other insecticides as well and it is evident that from these results that this species is highly resistant to endod. Having such high figures for the Lc50 and Lc90, the practical application of endod to control Culex fatigans needs careful thought. However, if there is enough endod to carry out a pilot project in the field, a small ditch or pool where the mosquitoes breed naturally could be selected somewhere in the periphery of Addis Ababa.

Stage	Lc_{50}	Lc_{90}
Egg	- 260 ppm	- 666 ppm
1st instar	- 150 "	- 600 "
2nd instar	- 250 "	-1000 "
3rd instar	- 145 "	- 833 "
4th instar	- 337 "	-2000 "

2. Effect of endod on Anopheles gambiae larvae

Anopheles gambiae is the most notorious mosquito in Africa south of the Sahara. It is very widely distributed, i.e. from sea level to about 2000 meters in equatorial regions but confined to lower altitudes further south. It breeds on open exposed ground pools of all sizes; brick pits, foot prints, tire ruts, etc. Very occasionally found in man-made containers such as wheelbarrows, mortar pans, etc. The adults feed on

on man (and animals) by night, mostly indoors. They transmit parasites such as plasmodia and filaria and viruses (i.e. O'njong-njong).

In our preliminary attempt to determine the effect of endod on Anopheles gambiae larvae, the first duty was to get enough larvae of different stages for tests to be conducted with different concentrations. With this in mind, we started a colony from eggs which were kindly supplied to us by NAMRU-5. The colony did very well for a short time and its larvae food was a combination of dog food and liver powder in a 20:1 porportion, respectively. Adults were fed on human arms.

However, after sometime due to unknown causes, egg production was reduced to about 20 eggs/female and its fertility was about 30%. A colony with such capacity was not able to provide all the needed larvae for the anticipated experiments. Then, we brought some eggs from Nazareth Training Centre for Malaria Eradication Service to start a fresh colony. We took extra care to maintain this colony and we fed the larvae according to the instruction given by the entomologists of the Malaria Eradication Service. The larvae food was a combination of dog food, fafa, non-fat milk, oats, and meat meal in 5:5:1:1:1 proportions, respectively. Blood meal to adults was offered from human beings as well as guinea pigs. Egg production was 80-100 per female and hatchability was about 90%. From this colony we were able to obtain enough larvae to conduct our experiments.

Tests were done on eggs and the four instars of larvae with different concentrations of endod, the highest being 100 ppm and the lowest 5 ppm. In each test, 10 specimens (eggs or larvae) were used and every experiment was repeated at least four times. The exposure time, i.e. the time the insects are immersed in endod solution was 24 hours. After that the insects were washed and transferred into distilled water for recovery and they remained there for 48 hours. All mortalities were recorded at the end of the recovery period.

Effect of endod on eggs. Eggs which were laid overnight were used in the morning, i.e. the eggs were less than 12 hours old. In each trial, 10 eggs were exposed in the known concentration for 24 hours and then 48 hours in distilled water for recovery. Failure of the eggs to hatch was taken as mortality. With such procedures, the LC50 for anopheles eggs was found to be 5.8 ppm and the LC90 was 24.2 ppm.

Effect of endod on larvae. The larvae of Anopheles gambiae were found to be more susceptible to the effect of endod than the larvae of the mosquitoes that had been tested in our laboratory. As it has been mentioned above, every experiment was repeated at least four times and this was done for each of the four instars. The first instar appears to be more susceptible than the other three instars because LC50 and LC90 were 4.6 and 11.0 ppm, respectively. The LC50 for the 2nd, 3rd and 4th instar larvae was very close to each other but it was almost double to the LC50 of the egg and first instar. By the same token, the LC90 for the egg, 2nd instar, 3rd instar were close to each other, i.e. about 24 ppm. However, it is a little bit difficult to give a logical reason for the lowness of the LC90 of the 1st instar which is 11.0 ppm and the extremely high LC90 for the 4th instar which is 48.9 ppm. For comparison between the lethal concentrations of 50 and 90% for the different instars, the table following is given.

stage	LC50	LC90
egg	- 5.8	- 24.2
1st instar	- 4.6	- 11.0
2nd instar	- 10.0	- 29.0
3rd instar	- 9.4	- 24.0
4th instar	- 9.3	- 48.9

Effect of endod on Anopheles gambiae larvae in the field. It is anticipated to carry out a pilot project for the determination of endod toxicity in a small oasis type area where A. gambiae breeds year round. This area is located 30 km north of Metahara village.

Results of the toxicity of crude endod on Anopheles gambiae larvae

Stage of larvae	No. of experiment	Lc50 in ppm	Lc90 in ppm
eggs	2	4.1	9.5
1st instar	5	4.6	11.0
2nd instar	5	10.0	29.0
3rd instar	6	9.4	48.9
4th instar	5	9.3	9.5

Results of the toxicity of water extract endod on Anopheles gambiae larvae

Stage of larvae	No. of experiments	Lc_{50} in ppm	Lc_{90} in ppm
1st	2	3.0	5.3
2nd	3	3.5	9.3

Unpublished Research Note No. 4

PROGRESS REPORT

I. Comparative Toxicity Studies of 3 Molluscicides (Bayluscide, Frescon and Endod) to Microflora (Phytoplankton sp.) and Microfauna (Zoo Plankton Crustacea sp. and Arthropoda Insects).
II. Toxicity study of Endod (Phytolacca dodecandra) to Fish and Snails

Maria Getaneh (M.Sc.) and Aklilu Lemma (Sc.D.), Institute of Pathobiology

April 1977

INTRODUCTION

The most important single method of controlling the increasing problem of schistosomiasis remains the descruction of the intermediate snail host. The most efficient means of doing this is by the application of molluscicides.

The toxicity of a molluscicide is of interest not only so far as snails are concerned, but also in regard to the freshwater fish which constitute a major part of the diet in many developing countries and serve as a major source of animal protein to the population. Various plankton organisms form basic parts of the food chain for fish populations and are essential for biological purification of water.

Concerning the effect of molluscicides on aquatic fauna other than snails and fish,systematic studies have been performed by Shiff and Garnett (9), showing that Bayer 73' at 1 ppm had no permanent unbalancing effect on the plankton occurring in biologically stable ponds.

The object of this study was to compare the toxicity of a) berries of Phytolacca dodecandra endod which have been found to act as a natural molluscicide (6) and b) Frescon(R) and Bayluscide(R) to freshwater microflora and fauna, various species and different sizes of fish, and mature snails.

MATERIALS AND METHODS

Some species of zooplankton-crustaceans, arthropoda insects, phytoplankton, tadpoles, leeches, fish and snails were tested.

Except the snails, all specimens were collected from the field. The snails used in this experiment were laboratory-reared Biomphalaria glabrata.

The experiment was conducted on immature fish of three species: Tilapia nilotica, Cyprinus carpio (carp) and Barbus sp.

Plankton, insects, leeches and tadpoles were tested in groups of 10 in 150 ml glass containers and fish in groups of 10 in 30 liters of molluscicidal solution in aerated aquaria.

The number of animals found dead after 24 hours exposure period in molluscicidal solution and 24 hours recovery period in dechlorinated tap water with food were recorded. The 24 hours LC90 (concentration that was lethal to 90% of animals exposed) was calculated. So far no sufficient quantity of the extract has been produced to allow evaluation of different formulation in this experiment. This is expected to be done during the next project period.

Berries of endod were purchased from local suppliers and grained before use. The Bayluscide (wettable powder) used in the present study was supplied by the Bayer Company in Ethiopia, the Frescon (emulsion concentrate) by the Shell Chemical Company in Ethiopia. The molluscicide solutions were prepared as described by Lemma (7) for testing snails.

TABLE 1

Table 1 shows the different stages of maturity, mean length and mean weight of fish used in the experiment.

Species	Stage of Maturity	Avg. length (cm.)	Avg. weight (gr.)
Tilapia nilotica (T.n.)	Embryo		
" "	Fry with yolk		
" "	-	4	1.2
" "	-	9	10
" "	-	16	100
Cyprinus carpio	-	7	9
Barbus sp.	-	11	9

RESULTS

TABLE 2

Table 2 shows comparative susceptibilities of various species of zooplankton, phytoplankton, insects, tadpoles, leeches and snails to the three molluscicides.

Species	Endod (crude ground berries)	Frescon	Bayluscide
Cladodera:			
Daphnia sp.	54	2.7	0.03
Moina sp.	56	2.7	0.04
Copepoda:			
Diaptomus sp.	56	2.0	0.05
Ostracoda sp.	320	7.0	8.0
Periphyton:			
Spirogyra sp.	110	1.2	0.4
Stigeoclonium sp.	115	7.0	0.3
Notonectidae:			
Notonecta sp.	110	3.0	7.0
Corixidae:			
Corixa sp.	110	2.8	6.8
Anopheles larva			
II instar	60	12	0.3
III instar	66	15	17
Anopheles adults	68	15	28
Leeches:			
Lymnalis nilotica	6	6	0.2
Tadpoles	19	3.0	0.4
Snails:			
Biomphalaria glabrata	23	0.1	0.2

LC90 (ppm) after 24 hours exposure and 24 hours recovery

ENDOD

This compound appears to be considerably less toxic to zoo- and phytoplankton and to insects than to snails. The LC90 (24 hours) was 54 ppm for Daphnia sp., 56 ppm for Moina sp. and Diaptomus sp., 320 ppm for Ostracoda, 110 ppm for Spirogyra sp., 115 ppm for Stigeoclonium sp., 110

ppm for Notonecta sp., 100 ppm for Corixa sp., 60 ppm for Anopheles larva (II instar) and 66 ppm for Anopheles larva (III instar), 19 ppm for tadpoles and 23 ppm for snails.

FRESCON

The 24-hour LC90 of this molluscicide was 2 ppm for Diaptomus sp., 2.7 ppm for Daphnia sp., 7 ppm for Stigeoclonium sp., 3 ppm for Notonecta sp., 2.8 ppm for Corixa sp., 3 ppm for tadpoles, 6 ppm for leeches, 12 ppm for II instar of Anopheles, 15 ppm for III instar of Anopheles larva and 0.1 ppm for snails.

BAYLUSCIDE

Bayluscide showed to be more toxic to some species of zoo- and phytoplankton than endod and Frescon. The LC90 (24 hours) was 0.03 ppm for Daphnia sp., 0.04 ppm for Moina sp., 0.05 ppm for Diaptomus sp., 80 ppm for Ostracoda, 0.4 ppm for Spirogyra sp., 0.3 ppm for Stigeoclonium sp., 70 ppm for Notonecta sp., 68 ppm for Corixa sp., 0.4 ppm for tadpoles, 0.2 ppm for leeches, 0.3 ppm for II instar of Anopheles larva, 17 ppm for III instar of Anopheles larva and 0.2 ppm for snails.

TABLE 3

Table 3 shows relative susceptibilities of different species and stages of maturity of fish to the crude ground berries of endod.

Species	Stage of maturity	Avg. wght (gm)	Avg. lngth (cm)	LC90 -24 hr (ppm)
Tilapia nilotica	Embryonic phase			3
" "	Fry with yolk			4
" "		1.2	4	6
" "		10	9	8
" "		100	16	10
Cyprinus carpio (carp)		9	7	19
Barbus sp.		9	11	11
Biomphalaria glabrata				23

As can be seen from this table, the fish are very sensitive to the piscicidal action of endod. The LC90 (24 hours) was 3 ppm for embryonic phase of Tilapia nilotica, 4 ppm for fry of Tilipia nilotica with yolk, 6 ppm for Tilapia nilotica 4 cm long, 8 ppm for Tilapia nilotica 9 cm long, 10 ppm for T. nilotica 16 cm, 19 ppm for carp, 11 ppm for Barbus and 23 ppm for snails.

DISCUSSION

The result of our experiment shows that all species of laboratory-tested zoo-plankton and phytoplankton are resistent to endod and Frescon at the snail killing concentration and susceptible to Bayluscide.

Shiff and Garnett (10) have shown that the application of 1 ppm of Bayer 73 in biologically stable ponds led to an immediate reduction of all plankton organisms. Following application of this molluscicide the biological equilibrium was almost completely restored during the follow-up period of one month.

Tadpoles appeared to be quite sensitive and insects quite resistant to all three molluscicides.

Endod, Frescon and Bayluscide showed less toxic effects to Anopheles

larva (II and III instar) than to snails. The only exception was II instar larva which were affected with Bayluscide in concentrations very similar to that for snails.

Leeches were very susceptible to endod and Bayluscide and less to Frescon.

The currently used molluscicides such as Bayluscide and Frescon are known to be toxic to fishes. This is especially evident during field trials where fish are regularly lost (12,15,9,2).

Our results show that different species of fish vary in their susceptibility to endod and there is no significant differenct in susceptibility of different sizes of Tilapia nilotica to this molluscicide.

Like other already known molluscicides endod in effective concentrations was found to be toxic to fish.

Lemma (7), and Lemma and Yau (8) have reported on toxicity of endod to fish. Although they used different species of fish from those we did, our results generally agree.

SUMMARY

The possible negative side-effects of endod, Frescon and Bayluscide were compared in the laboratory. The susceptibility of some representative species of microflora and microfauna to different concentrations of the molluscicides were determined and compared to the susceptibility of Biomphalaria glabrata snails under similar conditions.

The results of laboratory tests show that endod and Frescon do not lethally affect microflora and fauna at the snail-killing concentrations. In effective concentrations Bayluscide is toxic to Cladocera, Copepoda, Periphyton (Spirogyra sp. and Stigeoclonium sp.) leeches and tadpoles, but not to Arthropod insects. Endod was extremely toxic to leeches.

The susceptibility of Tilapia nilotica, Cyprinus carpio and Barbus sp. to endod were determined. Although different species and sizes of fish showed different susceptibility to endod, it was concluded that its toxicity for fish is very similar to that for snails.

Continuation of the Work in 1977/78

a) Continue studies on the comparative toxicity of different strains and preparations of the endod extracts, with the view of searching for new extraction and formulation procedures which would make the extract less toxic to non-target species.
b) Continue studies on the larvicidal, insecticidal, and insect repellent properties of the endod extract with particular emphasis on the development of procedures for possible use of this product in the control of mosquitoes and other harmful insects which breed in the same habitat as snails.
c) Continue studies on the toxicity and repellent action of endod to the common housefly, Musca domestica.
d) To investigate the short- and long-range effects of the endod extract on the aquatic biota during pre- and post-treatment surveys of the aquatic environment.
e) Collaborate with the Malaria Control Service in Ethiopia in conducting field evaluations on the larvicidal and insecticidal properties of endod. Our Institute is fortunate to have a senior and competent researcher, Dr. Assefa Tekle, to join our team with his assistant Mr. Teshome G. Michael, a B.Sc. graduate in the field of biology, to lead these investigations.

Unpublished Research Note No. 3

CHEMICAL ASSAY OF ENDOD (*P. DODECANDRA*): REPORT OF PRELIMINARY FIELD TRIALS IN TENSAE BERHAN AND CHUAHIT

Tesfaye Lemma (M.Sc.), Institute of Pathobiology, 1977

The chemical assay of endod was tried at Tensae Berhan (southern area) and at Chuahit (northern area) during the year 1976-77. In these two areas snail population, length, width and the flow rate of the rivers were different

1. Field Trials in Chuahit (near Gondar)

The length of the four rivers (Ambazina, Chelguaya, Chigaro and Derna) of Chuahit treated with fine powdered crude endod was about 5 km. Most of the snails at Chuahit were *Bulinus truncatus* and the flow rate of the rivers was very slow. In the 4 streams of Chuahit the population of the snail was very high during March-May. The pH of the water of the streams, before the application of endod, was between 7.5 and 8.0 and after the application of endod it fluctuated between 6.0 and 6.5. Because of the nature of terrain and the low flow rate of the stream, it was too difficult to calculate the amount of endod to be sprayed. So, a known quantity of endod was put in a given portion of the stream and after a period of 30 minutes the concentration of the active principle in endod was checked. This was done by preparing standard solutions of 125, 100, 75, 50 and 25 ppm with distilled water or by adjusting the pH of the river water to 7. The volume of the standard solutions in each test tube was 30 ml. To each of the test tubes containing standard solutions, 5 drops of alkaline picrate (freshly prepared) and bromothymol blue were added.

The determination of the color intensity of endod with thymol blue in the presence of alkaline picrate was carried out by means of visual color comparison in test tubes.

Bromothymol blue changes from yellow to blue depending on the hydrogen ions of the tested organic substance. Test solutions of bromothymol blue and alkaline picrate were prepared as follows:

a) 1% solution of bromothymol blue in 70% ethyl alcohol;
b) Freshly prepared alkaline picrate in the proportion of 1 (10%) NaOH: 5 (1.2%) picric acid.

Then fine powder of crude endod was dropped into the river; 30 ml were taken in a test tube; 5 drops of test solution was added and the color of this unknown concentration was compared with the standard solutions.

30 minutes after the application of endod, the potency was checked downstream from the first spot of application and the following results were registered:

Distance in meters from the first spot downstream	Concentration in ppm
10	75
20	50
30	25
40	traces
50	0

Three hours later the potency was again checked downstream from the first spot.

Distance in km	Concentration in ppm
0.5	75
1	75
1.5	50
2	50
2.5	25
3	25

Different concentration of 100, 75, 50, 25 and 8 ppm were prepared using stream water in jars. Then 5 snails were placed in each jar. After 24 hours exposure time the following results were observed:

Concentration in ppm	% dead
100	100
75	100
50	100
25	100
15	100
8	100
6	50
5	0

24 hours after application the potency was checked downstream all over the applied areas.

Distance in km	Concentration in ppm
0.01	traces
0.02	traces
0.04	traces
0.08	traces
0.10	25
0.2	50
0.5	75
1.0	100
1.5	100
2.0	100
2.5	100
3.0	100
3.5	100
4.0	100
4.5	100
5.0	100

2. Field Trials in Tensae Berhan

The length of the three rivers (Homba, Ferekassa and Arba Dima) of Tensae Berhan, treated with fine powdered crude endod, was about 2 km. Most of the snails at Tensae Berhan were B. pfeifferi and the flow rate of the rivers was higher than in Chuahit. Snail populations in the Arba Dima and Homba rivers were extremely large during March-May. The pH of the streams before the application of endod was 8.0-8.5.

The procedure which gave satisfactory results at Chuahit was also followed in Tensae Berhan. Concentrations of 150, 125, 100, 75, 50 and 25 ppm were prepared. The pH of the water was adjusted by adding 17 drops of 0.1 NaHCL to 1 liter of river water. At Tensae Berhan, some dye stuffs such as methyl orange, phenol red and curcumin were used, in addition to bromothymol blue. So far, good results were obtained by using phenol red and bromothymol blue. After 48 hours recovery time the

following results were observed:

Concentration in ppm	Percent dead
125	100
100	100
75	100
50	100
25	100
15	50
10	50
8	0
6	0

These data suggest that snails at Tensae Berhan have more resistance to endod than snails at Chuahit. At Tensae Berhan the speed traversed by the active ingredient within 24 hours was quite long in comparison with that of Chuahit.

24 hours after application the potency was checked downstream all over the applied areas.

Distance in km	Concentration in ppm
0.01	0
0.10	0
0.50	trace
1.00	50
1.50	100
2.00	100

Characteristic of the colors	Concentration in ppm
Dark blue	0 (blank)
Green	25 ppm
Light green	50
Light yellow	75
Yellow	100

Unpublished Research Note No. 1

NEW APPROACHES TO ENDOD (PHYTOLACCA DODECANDRA) EXTRACTION: A COMPARATIVE STUDY

Tesfaye Lemma (M.Sc.) and Aklilu Lemma (Sc.D.), Institute of Pathobiology

1977

INTRODUCTION

Schistosoma mansoni and S. haematobium are endemic in Ethiopia. Although the general distribution of the parasites is now fairly well known, no overall figure can be given for the total number of people affected in Ethiopia. With an ever increasing incidence of schistosomiasis the tendency is to cause major human diseases in this country (1-3).

The fruit of the endod shrub (P. dodecandra) contains approximately 27% by weight of triterpenoid saponins that are biologically active (4).

The saponins have been identified as triglycosides of oleanolic acid (5-7) and some of them have been found to be strongly molluscicidal (8-10), larvicidal (for the mosquito) (11), fungicidal, emetic (12) and spermicidal (13). In the town of Adwa, Ethiopia, Lemma (14) showed the value of crude endod as molluscicide in the control of schistosomiasis transmission.

Many studies have been carried out to determine the active ingredient of the endod berry, and much attention has been paid to the development of the extraction procedure. Even though procedures for development of an effective butanol extract of endod were developed (15), the results obtained could not be reproduced in Ethiopia, partly due to the fact that this involves relatively expensive procedures.

Water extracts, on the other hand, have been shown to be much less expensive but highly potent at concentrations of 10-15 ppm (8-10). Further work on improving this potency in comparison with the defatting technique is described in this paper.

Definitions:

1. Defatting technique (extraction with benzene): a method of obtaining a reliable series of endod extracts using expensive organic solvents.
2. Fermentation: a cheap, simple and practical method of extracting and concentrating the molluscicide material from the endod berries.

MATERIAL AND METHODS

1. Extraction by defatting

One hundred grams of crude endod berries collected from plants in the highlands of Ethiopia were weighed, powdered into fine particles and mixed with 500 ml water in a separatory funnel. Consequently 150 ml of cold pure benzene was added. The funnel was closed and shaken gently for 20 minutes and then left for soaking during 3 hours after which the solvent was drained off. This was followed by adding another 150 ml of pure benzene and constant vigorous shaking. The procedure was repeated 10 times until the fluid was colorless, i.e. until no green matter (chlorophyll) was present. The intensity of the removed color was checked by EEL type absorptiometer.

The defatted 100 gr of fine powdered crude endod was then placed on a plate and exposed to the air to dry. Two equal parts of the defatted crude endod and either 800 ml of cold distilled water (20-25°C) or 800 ml of warm distilled water (37°C) were added and left to stand for 1-8

days. Each test was repeated 6 times and the average was taken as the final results.

II. Fermentation method

One hundred gm of the sun-dried and fine-powdered (10 mm) crude endod berries was placed in a 1000 ml separatory funnel. Cold distilled water was added to the powder in order to produce different concentrations. The following series of concentrations were prepared: 0.2, 0.3, 0.4, 0.5, 1, 2, 3, 4, 8, 10, 15 and 20%. Incubation was carried out at 22°C, 30°C and 40°C. For each experiment the container was shaken vigorously and the precipitate was filtered through a piece of cotton or cheese cloth. Twenty-five ml of the filterate was air-dried. The residue obtained by this means is fresh extract. The acidity of this fresh extract was then checked. The same procedure was repeated every 3 days for one week and then every week until a decrease in potency was observed. The effect of antibiotics (1.5% tetracycline and 1.5% streptomycine), disinfectants (5% boric acid and normal saline), inhibitor (pure benzene) and pH was studied. The number of each replicates of each test was seven and the average of the seven replicates was taken as the final result.

For both methods toxicity tests were conducted according to the procedures recommended by the World Health Organization (16,17). The snails used for the bioassay were Biomphalaria glabrata. The snails were reared in dechlorinated tap water (pH 7.2) at 28°C and were fed with lettuce. Snails of uniform size (8 mm) were used. Three to 5 snails were exposed in 200 ml glass jars; 2 jars per test dilution in dechlorinated water prepared in twofold serial dilutions in all tests 24 hours exposure and 24 hours recovery period were used.

RESULTS

Results of the extraction by defatting and fermentation are presented below in figures 1-6 and tables 1-6. From these the following conclusions were made:

1. Increase in number of washes will remove all the green parts from the endod berries (Figure 1).
2. The water extract of the defatted endod berries is about 7 times more toxic to all snails tested, than the original crude endod berries (Figure 2).
3. A clear brown molluscicidal extract is obtained through extraction by the defatting.
4. It is shown that the defatting and the extraction processes of endod could be well enhanced by natural fermentation process.
5. Fermentation, which takes place due to the onset of microbial action, could be avoided by adding antibiotics, benzene, disinfectants and/or by adjusting the pH of the solution.
6. Water extract of fermented endod berries is about 7 times more toxic to all snails tested, than the original crude endod berries.
7. Increase of incubation temperature will create a favorable condition for fermentation (Figure 3).
8. In conjunction with the fermentation method it was observed that low concentrations of endod (10 ppm - 1%) lose their molluscicidal potency gradually, while high concentrations (10%) show an increase for some time and then decrease (figures 4 and 5).
9. During fermentation the pH of the endod solution will at first decrease up to pH 4 then increase and at last at pH 7 becomes constant. At this point the compound loses its molluscicidal property (Figure 6).

Table 1: Light Transmissions Characteristics of the Endod Filtrate

Pure benzene "blank" (in ml)	No. of filter	Defatted fluid benzene (in ml)	Absorbency	No. of washes
50	601	0	0	0
25	601	25	104	2
25	601	25	90	1
25	601	25	82	3
25	601	25	70	4
25	601	25	60	5
25	601	25	45	6
25	601	25	35	7
25	601	25	25	8
25	601	25	11	9
25	601	25	0	10

10. In fermentation method, unlike the extraction by defatting, a cloudy brown molluscicidal extract is obtained.

DISCUSSION

Crude endod contains various compounds which are toxic to snails. These compounds are purified by the extraction process. As these compounds are soluble in water we can extract them by soaking crude endod in excess water.

The original effort to concentrate the active principle in endod was based on butanol extraction. Because of unfavorable interaction between the "fat" and "glycoside" components of the endod berries while in aqueous solution, it became necessary to defat the berries before such an extraction. The defatting technique was improved after studying the extraction capabilities of different solvents such as benzol (pure benzene) gasoline, petroleum, ether and kerosene. The essential role of these solvents (especially benzol) in the defatting process was shown by Dr. Robert M. Parkhurst during his consultative services on endod at the Institute of Pathobiology in 1976. It was observed that the potency of defatted active endod decreases when butter is added to it, while in aqueous solution. The effect of butter was quickly traced to cholesterol, indicating that cholesterol and the active component of endod are able to mutually counteract the effects of each other. The increase in toxicity of endod after defatting could be explained by the existence of some physico-chemical linkage between the active surface of glycoside and the oils, lipids, carbohydrates, colloids, proteins and high molecular compounds such as cellulose, etc., which may block the active principles of the endod berry. Defatting method will definitely remove the linkage.

Table 2. Endod Potency Variation with Number of Washes

No. of Snails	Type of Endod	Concentration of Endod Expressed as LC 100 in ppm	No. of washes	% mortality of snails
5	crude *	25	0	100
	water extract **	15		
5	crude	25	1	100
	water extract	15		
5	crude	25	2	100
	water extract	15		
5	crude	25	3	100
	water extract	15		
5	crude	25	4	100
	water extract	15		
5	crude	20	5	100
	water extract	10		
5	crude	20	6	100
	water extract	8		
5	crude	15	7	100
	water extract	6		
5	crude	15	8	100
	water extract	6		
5	crude	15	9	100
	water extract	2		
5	crude	15	10	100
	water extract	4		

Defatted endod berries can resist fermentation as they produce no smell, and we observed no loss of activity during two weeks at room temperature. This could most probably be explained by the fact that washing the endod berries with an organic solvent (pure benzene) will break down enzymatic actions. Therefore inhibition of this action, combined with the removal of the linkage mentioned above, is thought to be responsible for the highly active extract which gives 100% mortality in Biomphalaria snails at 4 ppm, after defattation.

However, ways of getting an active extract of endod berries through defattation using the above mentioned solvents are very expensive for a country like Ethiopia. Recently a very simple, cheap and practical method of extracting and concentrating the molluscicides from the endod berries was discovered. Endod berries were soaked in water and left to stand for a few days, causing them to ferment rapidly and use up the free sugars and fats, leaving only the active principle to remain in solution for a relatively longer period. It was noted that an aqueous solution containing 10-100 ppm of the powdered berries lost molluscicidal potency in 3 to 4 days (15) primarily due to the enzymatic decomposition of the active principle of endod into its components, i.e. oleanolic acid and trisaccharides. Earlier it was found that once the endod solution smells, it is useless since it is no more toxic to snails.

* Plain ground berries subjected to repeated defatting with benzene.
** Water extract of berries subjected to repeated defatting with benzene.

Table 3: Effect of Antibiotics, Disinfectants and Inhibitors on Fermentation

Concentration of Endod Solution	Control	Effect of Additives on Fermentation				
	Toxicity 100% kill	1.5% Tetra-cycline 100% kill	1.5% strepto-mycine 100% kill	5% Boric acid 100% kill	Normal saline 100% kill	Pure ben-zene
Fresh 10% sol	12ppm	12 ppm	12 ppm	12 ppm	12 ppm	12ppm
1 day old 10% sol.	"	"	"	"	"	"
3 days old 10% sol.	10	"	"	"	"	"
5 days old 10% sol.	8	"	"	"	10	"
7 days old 10% sol.	6	"	"	8	6	"
10 days old 10% sol.	10	"	8	"	"	"
15 days old 10% sol.	25	"	6	10	10	6
20 days old 10% sol.	50	5	10	25	25	6
25 days old 10% sol.	100 does not kill	10	25	50	50	10
Fresh 0.1% sol.	12	12	12	12	12	12
3 days old 0.1% sol.	25	"	"	"	"	"
7 days old 0.1% sol.	50	"	"	15	25	"
15 days old 0.1% sol.	100 does not kill	15	"	50	50	20

Since microbial action in wet endod consumes the sugar molecules of the active glycoside very quickly, thus rendering it inactive, rapid extraction and drying of the endod was therefore originally regarded to be essential. However, the authors observed an opposite phenomenon to the one above stated. It was shown that with high concentrations of endod (above 10%) the potency increases in time. This phenomenon is shown colorimetrically and biologically. At present there is little scientific knowledge to explain the effect of microbial action on endod. However, it is suggested that the reasons for the increase of the concentration of the molluscicidal potency after what we call the "fermentation process" could be explained as follows:

1. Unlike the extraction by defatting, during this fermentation some of the sugars linked to the foaming compounds within the total saponin mixture are split off by enzymatic action, forming molluscicidal compounds

Table 4: Determination of Potency due to Fermentation with different Incubating Temperatures

Concentration of endod in %	Concentration of Endod Expressed as LC 100 in ppm 22°C	30°C	40°C	Time in weeks
10 - 20	12	12	12	0 crude
	10	10	8	1
	10	8	6	2
	8	8	4	3
	8	6	6	4
	8	4	10	5
	6	2	20	6
	4	2	60	7
	4	4	100	8
	2	8	300	10
	2	12	1000	12
	2	40		14
	2	90		16
	2	500		18
	8	1000		20
	50			22
	100			24
	1000			26
2 - 8	12	12	12	0
	10	10	10	1
	10	10	25	2
	15	75	100	3
	25	100	1000	4
	50	150		5
	100	1000		6
	1000			7
0.1 - 1	12	70	100	0
	25	125	500	1
	50	300	1000	2
	75	1000		3
	100			4
	125			5
	150			6
	300			7
	1000			8

the existence of which is already known (5-7).

2. As was shown in Figure 3, activation and/or detoxification of the extract will occur due to the removal of the so-called physico-chemical linkage and the degradation of the active substance into its components. In this way, two opposite processes, i.e. the release of the active principle (as was seen in the high concentrations of 10%) and the decomposition of the whole organic compounds (as was seen in the low concentrations - 1%), are taking place in the fermentation process.

SUMMARY

Chemical defatting and extraction processes of endod berries were

Table 5: Effect of the pH Media of the Endod Solution (10-20%) on Fermentation

Type of Endod	Concentration of Endod expressed as LC 100 in ppm at 25°C	Time in weeks	pH control	Buffered at pH = 5
Crude	12 ppm	0	5.1	12
"	10	1	4.8	"
Water extract	8	2	4.6	"
"	8	3	4.3	"
"	6	4	4.2	"
"	6	5	4.0	"
"	4	6	3.8	"
"	4	7	3.8	"
"	2	8	4.2	"
"	2	10	4.5	"
"	2	12	5.0	"
"	2	14	6.0	"
"	2	16	6.5	"
"	10	18	7.0	"
"	20	20	7.0	"
"	100	22	7.0	"
"	1000	24	7.0	"
"	1000	26	7.0	"

replaced by natural fermentation process. An old extract of endod soaked in water for more than five days was observed to be more active than a fresh one. The solution was separated and dried in a ventilated room. The extracts were shown to have molluscicidal potencies at 4 ppm, which can be compared with chemical extraction methods. Be it in the higher or lower concentration, endod has the advantage of being rapidly biodegradable. The biodegradability could be reduced and thereby the molluscicidal activity maintained over long periods through control by various agents as antibiotics, benzene, disinfectants and adjustment of the pH. From the experiment it was concluded that fermentation process compares favorably with the chemical defatting methods. In addition it is more economical, and the process is simpler.

The ultimate goal of our studies is to develop an integrated program of schistosomiasis control on a community self-help basis through the active participation of the broad masses. The experimental work presented here is a step toward developing a fermentation method which can be easily adopted locally.

ACKNOWLEDGEMENT

The work reported in this paper was supported by Addis Ababa University and by a research grant from the Office of Naval Research, Arlington, Virginia 22217, U.S.A.

Table 6: Detection of Deterioration of Concentration and Molluscicidal Potency of Crude Endod over Time

Concentration of Endod Expressed as LC 100 in ppm	Absorbency				% mortality			
	0 days	4 days	8 days	15 days	0 days	4 days	8 days	15 days
5	1.5	0.2	0	0	0	0	0	0
10	1.0	0.4	0.2	0	67	0	0	0
15	1.4	0.8	0.4	0	100	33	0	0
20	2.0	1.0	0.8	0	100	67	0	0
25	2.5	-	1.2	-	100	100	33	0
30	3.0	2.2	-	-	100	100	67	0
35	3.6	-	-	-	100	100	100	0
40	4.1	3.0	2.4	1.0	100	100	100	33
50	5.0	4.0	3.2	1.2	100	100	100	67
60	5.9	5.0	4.0	2.0	100	100	100	100
70	7.0	-	-	2.2	100	100	100	100
80	8.1	-	5.0	3.2	100	100	100	100
90	9.0	7.0	-	-	100	100	100	100
100	10.0	8.0	7.0	4.0	100	100	100	100

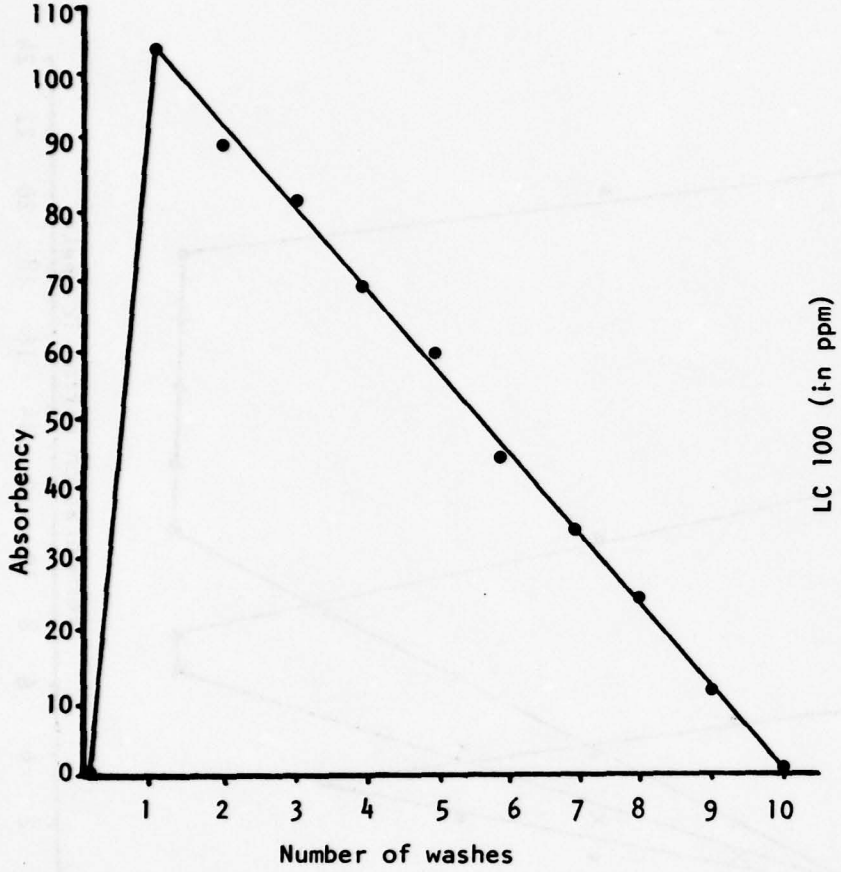


Fig.1. Light transmission characteristics of the filtrate

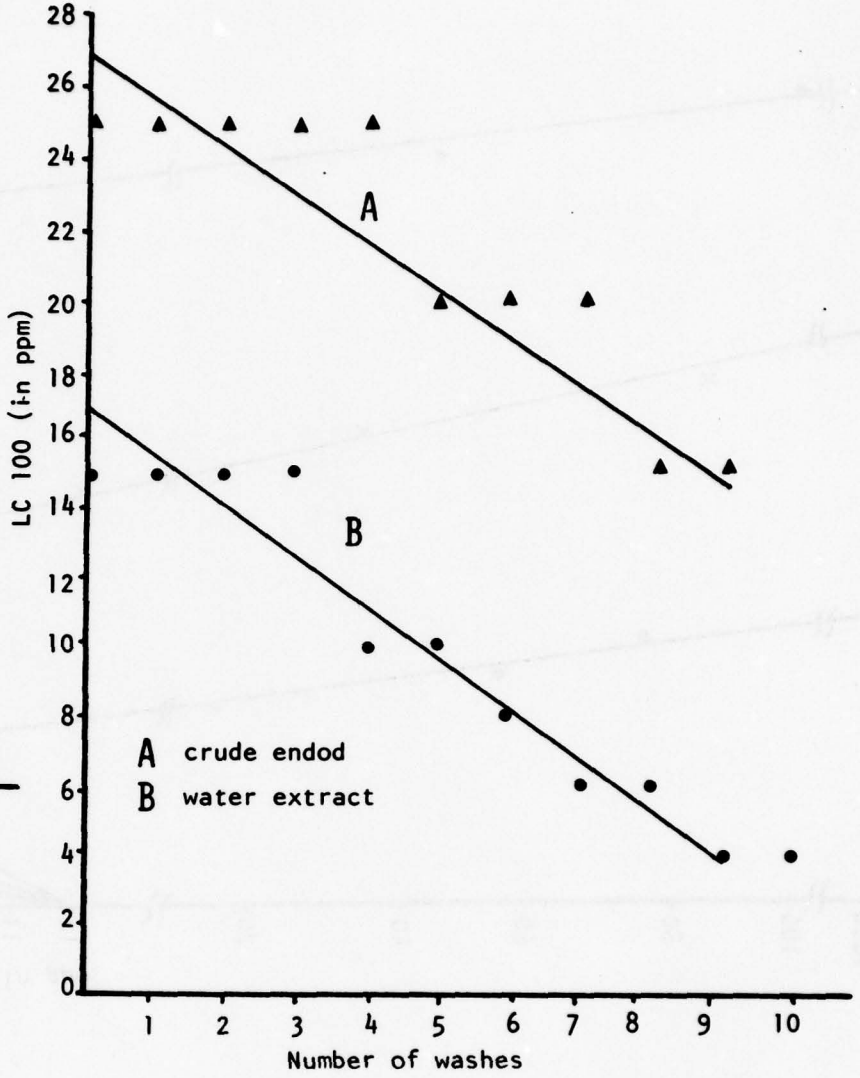


Fig.2. Potency variation of endod with number of washes

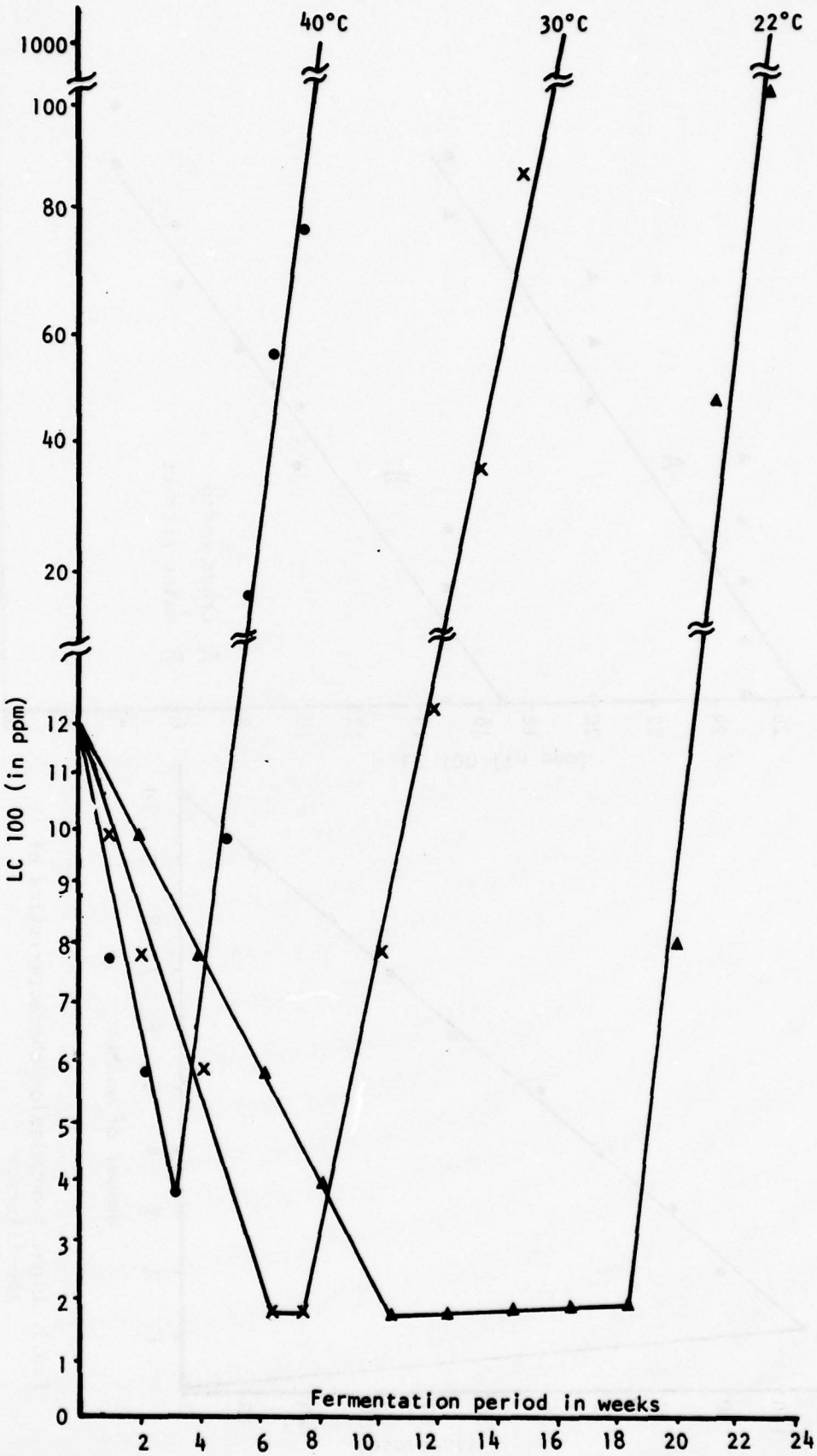


Fig.3. Increased concentration of active principle due to fermentation of crude endod berries with different incubation temperatures

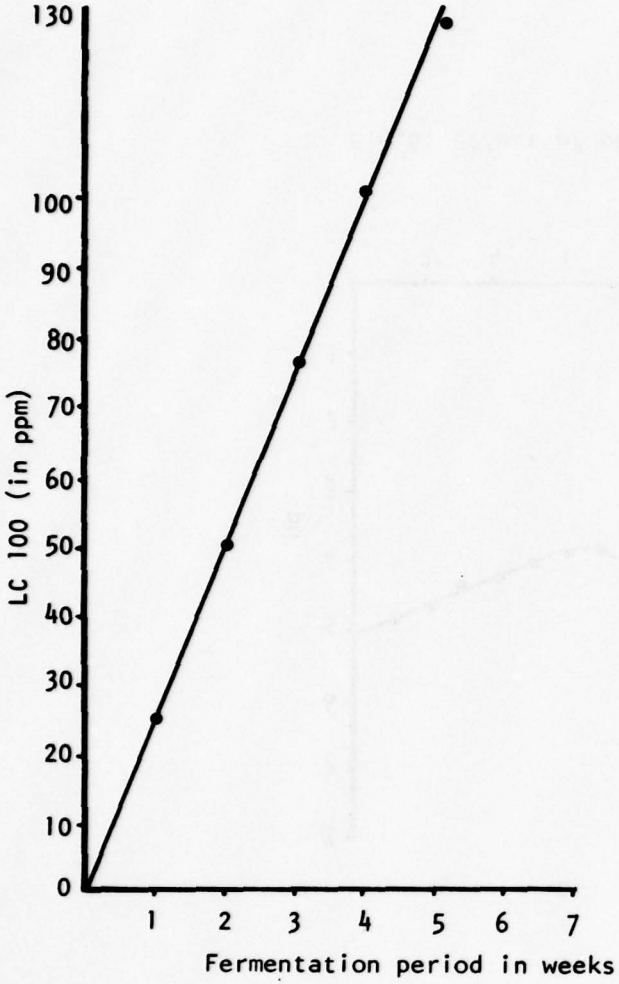


Fig.4. Decreased concentration of active principle due to fermentation of crude endod berries (0.1 - 1%)

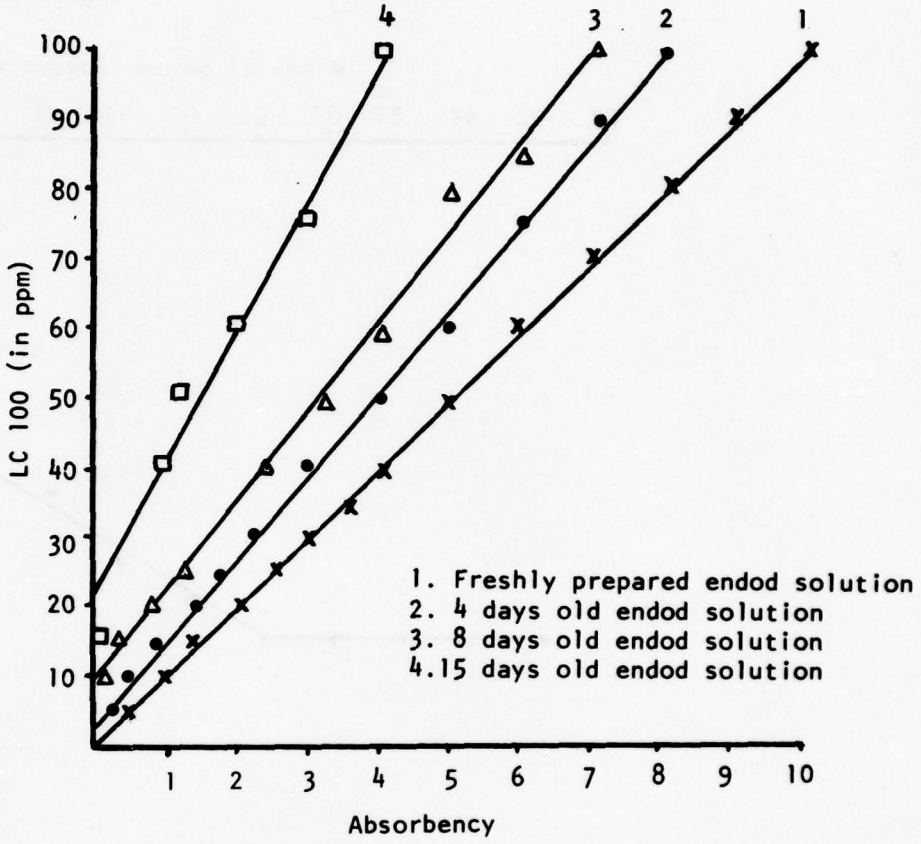


Fig.5. Deterioration of molluscicidal activity of crude endod over time

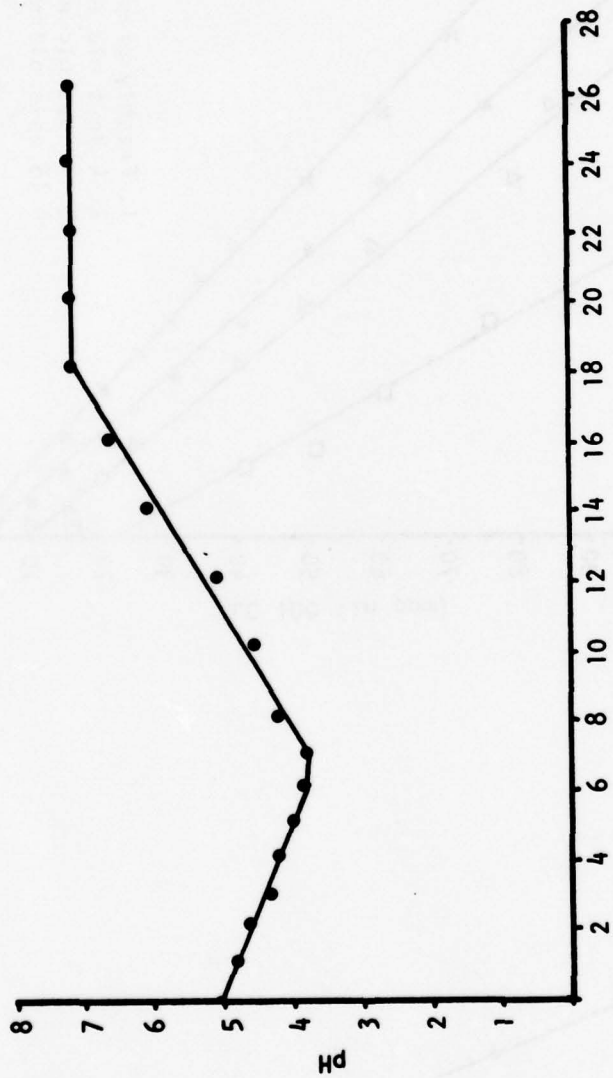


Fig.6. Effect of pH on fermentation

References

1. Kloos, H. and Lemma, A. Schistosomiasis in Irrigation Schemes in the Awash Valley, Ethiopia. Am. J. Trop. Med. Hyg. 26: 899-908 (1977).

2. Polderman, A.M. International Schistosomiasis North and West of Lake Tana, Ethiopia. Trop. Geogr. Med. 26: 170-177 (1974).

3. Lo, C.T., Flemings, M.D. and Lemma, A. Schistosomiasis in Harar, Alemaya and the Damota Valley, Ethiopia. Ethiop. Med. J. 11: 271 (1973).

4. Lemma, A. Studies on the Molluscicidal Properties of Endod (Phytolacca dodecandra). Final Report, Prospect LSU-8972, ONR, Contract N00014-71-C - 0123. Stanford Research Institute, Menlo Park, California (June 1972).

5. Parkhurst, R.M., Thomas, D.W., Skinner, W.A., and Cary, L.W. Molluscicidal Saponins of Phytolacca dodecandra: Oleanoglycotoxin-A. Phytochem. 12:1437 (1973).

6. Parkhurst, R.M., Thomas, D.W., Skinner, D.A. and Cary, L.W. Molluscicidal Saponins of Phytolacca dodecandra: Lemmatoxin. Canadian J. Chem. 52:702-705 (1974).

7. Parkhurst, R.M., Thomas, D.W., Skinner, D.A. and Cary, L.W. Molluscicidal Saponins of Phytolacca dodecandra: Lemmatoxin-c Indian J. Chem. 13: 757-758 (1975).

8. Lemma, A. Preliminary Report on the Molluscicidal Property of Endod (Phytolacca dodecandra). Ethiop. Med. J. 3: 187-190 (1965).

9. Lemma, A. Laboratory and Field Evaluations of the Molluscicidal Properties of Endod (P. dodecandra): Bull. World Hlth. Org. 42: 597-612 (1972).

10. Lemma, A. and Duncan, J. First Annual Report of Schistosomiasis Pilot Control Project in Adwa, Ethiopia. J. Parasit. 56: 438-439 (1970).

11. Spielman, A., and Lemma, A. Endod Extract. A Plant Derived Molluscicide: Toxicity for Mosquitoes. Am. J. Trop. Med. Hygiene 22: (6): 802 (1973).

12. Parkhurst, R.M. The Chemotaxonomy of Phytolacca Species. Indian J. Chem. 13 (7): 757 (1975).

13. Stolzenberg, S.J. and Parkhurst, R.M. Spermicidal Actions of Extracts and Compounds from Phytolacca dodecandra: Contraception 10: (2) 135 (1974).

14. Lemma, A., Goll, P.H., Duncan, J. and Mazengia, B. Control of Schistosomiasis with the Use of Endod in Adwa, Ethiopia. Results of a 5-year Study. Institute of Pathobiology Series No.2.

15. Lemma, A., Brody, G., Newell, G.W., Parkhurst, R.M., and Skinner, W.A. Studies on the molluscicidal Properties of Endod: (Phytolacca dodecandra): 1. Increased Potency with Butanol Extraction. J. Parasit. 56 (1): 104-107 (1972).

16. World Health Organization. Expert Committee on Bilharziasis. World Hlth. Org. Tech. Rept. Ser. No. 214 (1965).

17. World Health Organization. Snail Control in the Prevention of Bilharziasis. World Hlth. Org. Monogr. Ser. No. 50. (1965).

Unpublished Research Note No. 2

COLORIMETRIC DETERMINATION OF ENDOD

Tesfaye Lemma (M.Sc.) and Aklilu Lemma (Sc.D.), Institute of Pathobiology

1977

INTRODUCTION

Endod (Phytolacca dodecandra), being a natural produce and easily grown in suitable climatic conditions, could play an important role in the rural economy of Ethiopia. It is easy to teach farmers and other rural people how to use endod for the control of schistosomiasis on a self-help basis. Since endod grows in different parts of Ethiopia as a wild plant and is widely used as a soap for washing clothes as well as for different medical purposes, it is a well known and generally accepted plant in this country.

This paper describes colorimetric methods for the quantitative determination of oleanoglycotoxin (lemmatoxin), the active ingredient of the molluscicide Phytolacca dodecandra (endod), in low concentrations--up to 100 ppm.

MATERIALS AND METHODS

Two methods are presented here for determining endod in water samples at concentrations ranging from 5-100 ppm. The first method outlined is suitable for carrying out an exact determination of the active ingredient of endod in water samples using an absorptiometer (a type of an electrophotometer) in the laboratory, as this method is not suitable to use in the field. The second method has two variants, A and B. Method 2A provides only approximate values of the content of the molluscicide in the water, visually, but is very easy to apply and can be carried out in the field without any special technical equipment. Method 2B, on the other hand, employs a simple color-wedge colorimeter, which is not dependent upon a source of electric current for its operation and can thus be easily used in the field. The Zeiss Ikon Polytest Colorimeter proved to be most suitable for this purpose. This instrument, which is especially suitable for medical colorimetric analysis, is provided with interchangeable color wedges. The color wedge N°119, used for determination of haemoglobin, proved to be suited for measuring the color intensity of the molluscicide dye stuff complex. Measurements were carried out in glass vessels.

1. Reagents used

It was suggested to carry out laboratory work with some reagents, complex forming indicators or organic dye stuffs, such as Sudan 3, Eriochromo Black T and others. The main prerequisite for a dye stuff to be suitable for complex formation is that it should:

a. Form a complex that is more readily soluble in an organic solvent than in water, at least at certain pH values.

b. Possess a color that can be easily differentiated by the human eye, for example, blue or red, and must be obtainable at all times in the same quality.

c. Have very low "blank value"

Most of the complex forming indicators we used are Azo dyes. The most important of these Azo dyes, which gave reproducible results, are Sudan 3, Eriochromo Black T and Dinitrophenylhydrazine. These Azo dyes have the following structural formulas: Sudan 3--reddish brown powder,

insoluble in water, soluble in chloroform, glacial acedic acid and moderately soluble in alcohol, ether, acetone, petrol, etc. Eriochromo Black T--black powder, soluble in water, slightly soluble in alcohol and insoluble in almost all organic solvents. Because of the inductive effect of H^+, S^-, NO_2^- and OH^- groups, the alkalinity property of the dye stuff will increase and a dye stuff with such a property may increase the tendency of formation of a complex with endod.

Test solutions of Sudan 3 and Eriochromo Black T are prepared in the following manner: 0.0025 gm of Sudan 3 is accurately weighed on the analytical balance and dissolved in a) 50 ml of distilled water and b) 50 ml of acetone. In the case of Eriochromo Black T, 0.0025 gm is weighed and dissolved in 100 ml of distilled water. These solutions produce a concentration of 0.0025%. Because of the encouraging results of these organic dye stuffs, other complex-forming indicators were tried. From all indicators used the following gave positive results:

1) 0.0025% solution of Curcumin
2) 1.2% solution of freshly prepared Alkaline Picrate
3) 0.04% solution of Dinitrophenylhydrazine
4) 0.0025-1% solution of Bromothymol Blue
5) Congo Red
6) B.D.G. (Bromoclesol Green)
7) B.C.Y. (Bromoclesol Yellow)
8) Diphenylamine
9) HHSNN
10) Diphenyline

2. Test procedures

a. Experiments on color reaction of water extract of endod are carried out for the first time by using distilled water. To prepare a stock solution of 200 ppm, 50 mg of water extract endod is dissolved in 250 ml of warm distilled water with a vigorous and constant shaking for 30 minutes. A series of standard solutions of 0.8-100 ppm are prepared from the above stock solution.

E.g. To prepare a 100 ml volume with a concentration of 100 ppm from the stock solution of 200 ppm, ppm=mg/ml, i.e. 100 ppm=100 gm/ml. Therefore, the volume of endod stock solution to be diluted will be: 100/200 x 100 = 50 ml. Fifty ml of solution is taken from the standard solution and distilled water is added up to 100 ml.

Every time 25 ml of standard solution is taken from each series in a 100 ml test tube. In order to obtain reproducible results, it is essential to have a "blank" which is prepared parallel with the test solution. In both test solutions and the "blank", 6 drops of 0.0025% solution of Sudan 3 and Eriochromo Black T are added. The mixture is shaken very gently for a few seconds (normally 2 seconds).

The absorptiometer readings represent relative concentrations only. For quantitative analysis, calibration graphs must be prepared for each time of determination, by plotting the concentrations of series of known standard solutions against the reading obtained from them. Readings are taken through the absorptiometer by bringing the cell carriage to its forward position. For this, one cell is filled with "blank" solution and the other cell with standard or test solutions. See Tables 1 and 2.

b. After it was known that procedure could produce positive results, experiments were carried out with fine-powdered crude endod. To standardize conditions for field testing, one gram of fine powdered crude endod is dissolved in 1000 ml of cold distilled water, to produce

a stock solution of 1000 ppm. Results are given in tables 3 and 4.
c. This procedure is the same as described above except that river water is used, which was brought from the Laaga Taffo River. The pH of this water is 8.7. Therefore, when visual or wedge colorimeter are used the pH must be adjusted as described in Table 5. Results are given in tables 6 and 7.

The determination of the color intensity of endod with Bromothymol Blue in the presence of Alkaline Picrate is carried out by means of visual color comparison in test tubes (Method 2A) or by means of a simple compensation colorimeter which is not dependent upon a source of electric current (Method 2B). As for Method 2A, standard solutions of endod are prepared (100 ppm, 90 ppm, 80 ppm, 70 ppm, 60 ppm, 50 ppm, 40 ppm, 30 ppm, 25 ppm, 20 ppm, 10 ppm, 5 ppm, and the blank). See Table 8.

Test solutions of Bromothymol Blue and Alkaline Picrate are prepared as follows: 1. 1% solution of Bromothymol Blue. 2. Freshly prepared Alkaline Picrate in the proportion of 1 (10%NaOH):5 (1.2% Picric acid).

EXPERIMENTAL RESULTS

From 1000 ppm stock solution different working dilutions were prepared and the intensity was checked through the absorptiometer.

TABLE 1

Concentration of endod	Indicator Sudan 3 (0.1%)	Filter no.	Readings blank	sample
100 ppm	6 drops	601	0	26
50 "	" "	"	"	16
25 "	" "	"	"	8
12.5 "	" "	"	"	5
6.25 "	" "	"	"	3
3.2 "	" "	"	"	1.5
0.8 "	" "	"	"	0.8
0 "	" "	"	"	0

TABLE 2

Concentration of endod	Indicator Eriochromo Black T 0.1%	Filter no.	Readings Blank	Sample
100 ppm	6 drops	601	0	28
50 "	" "	"	0	13
25 "	" "	"	0	7
12.5 "	" "	"	0	4
6.25 "	" "	"	0	3
3.2 "	" "	"	0	1.5
1.6 "	" "	"	0	0.7
0.8 "	" "	"	0	0.4
0 "	" "	"	0	0

As is seen from the graph (Fig. 1), when using crude and extracted endod prepared with river and distilled water, the absorptiometer reading is proportional to the concentration of endod.

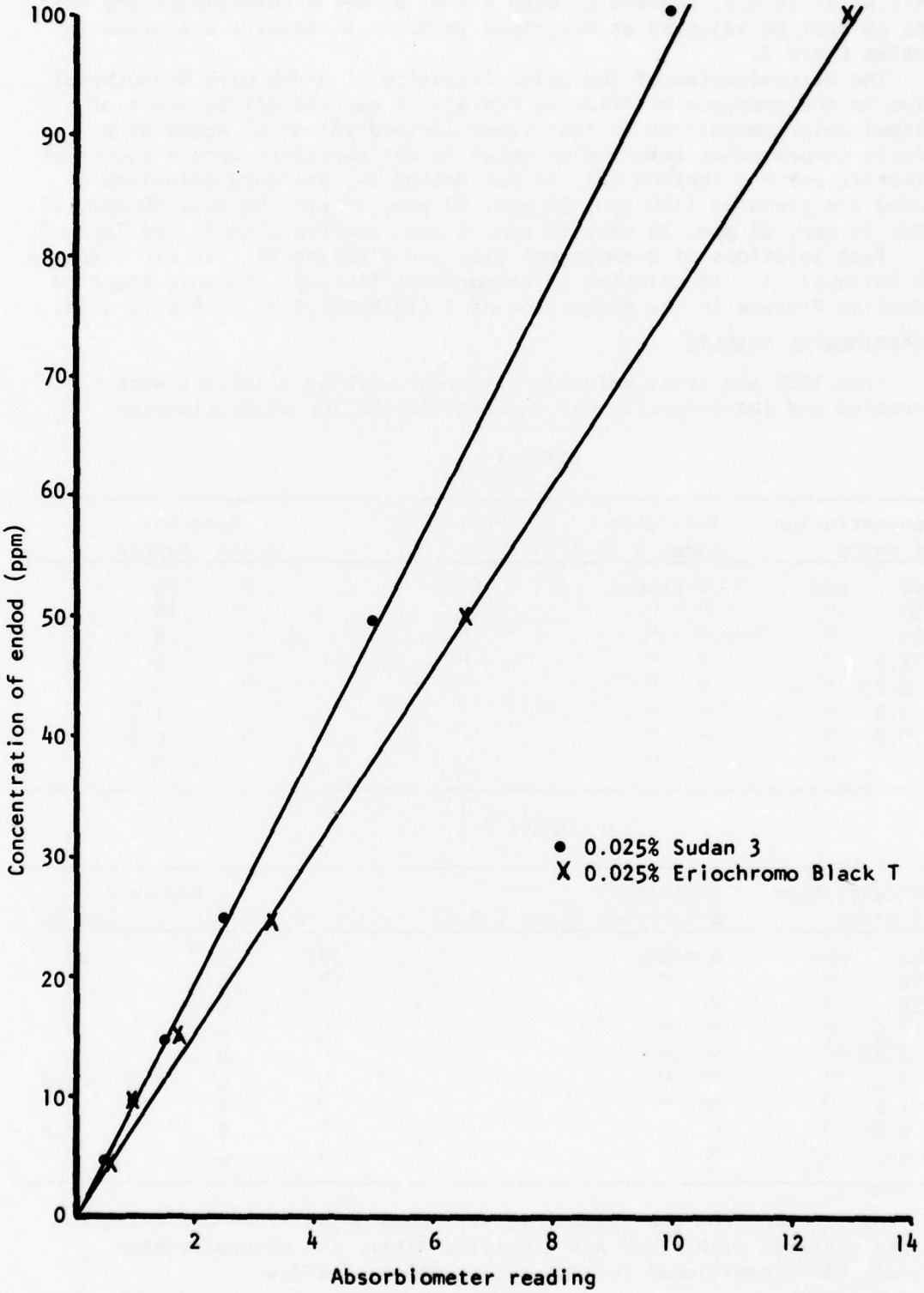


Fig.1. Relationship between concentration of endod and absorbtiometer reading

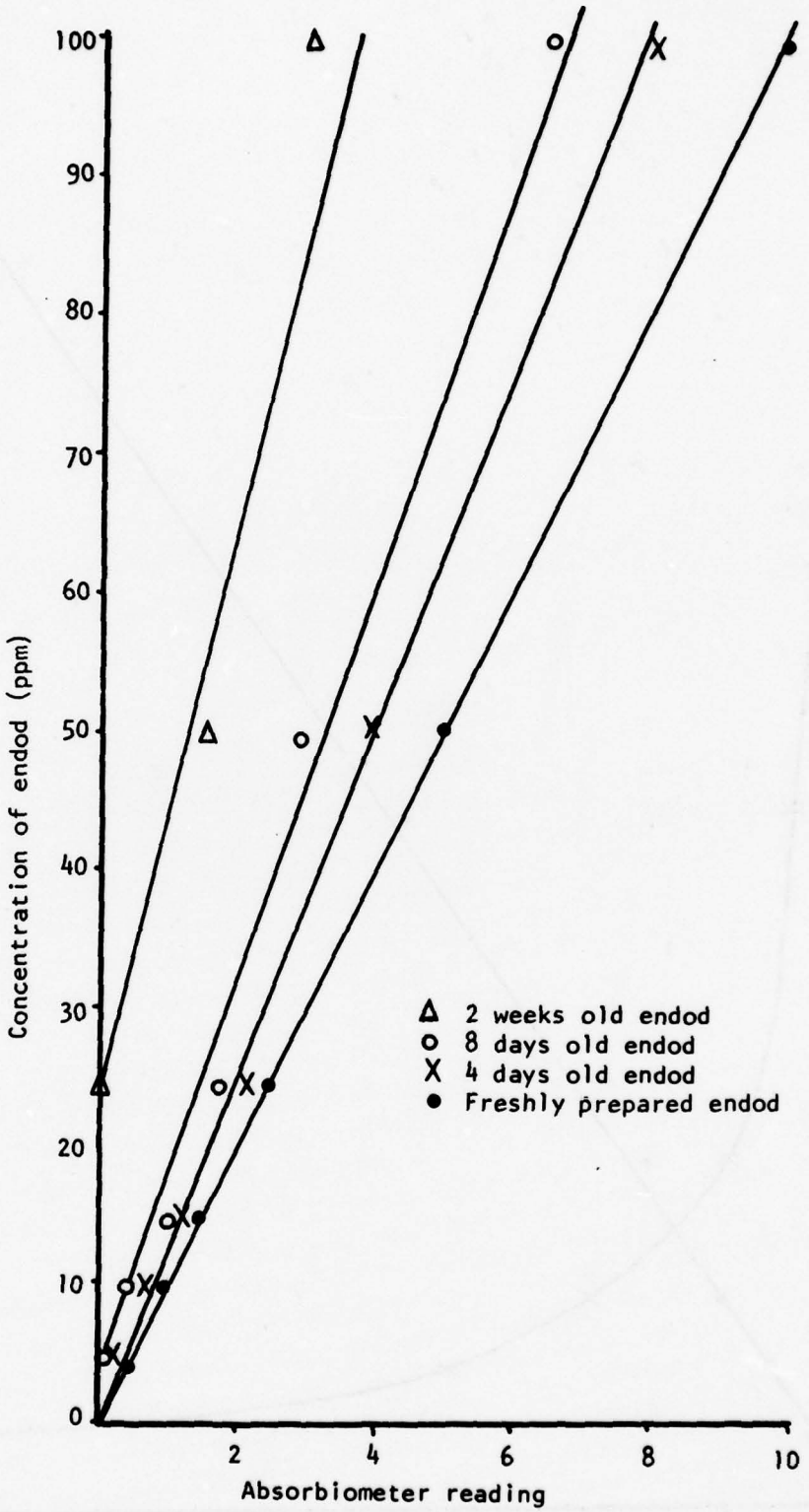


Fig. 2. Relationship between concentration of endod and absorbtiometer reading in the presence of Sudan 3 (0.025%)

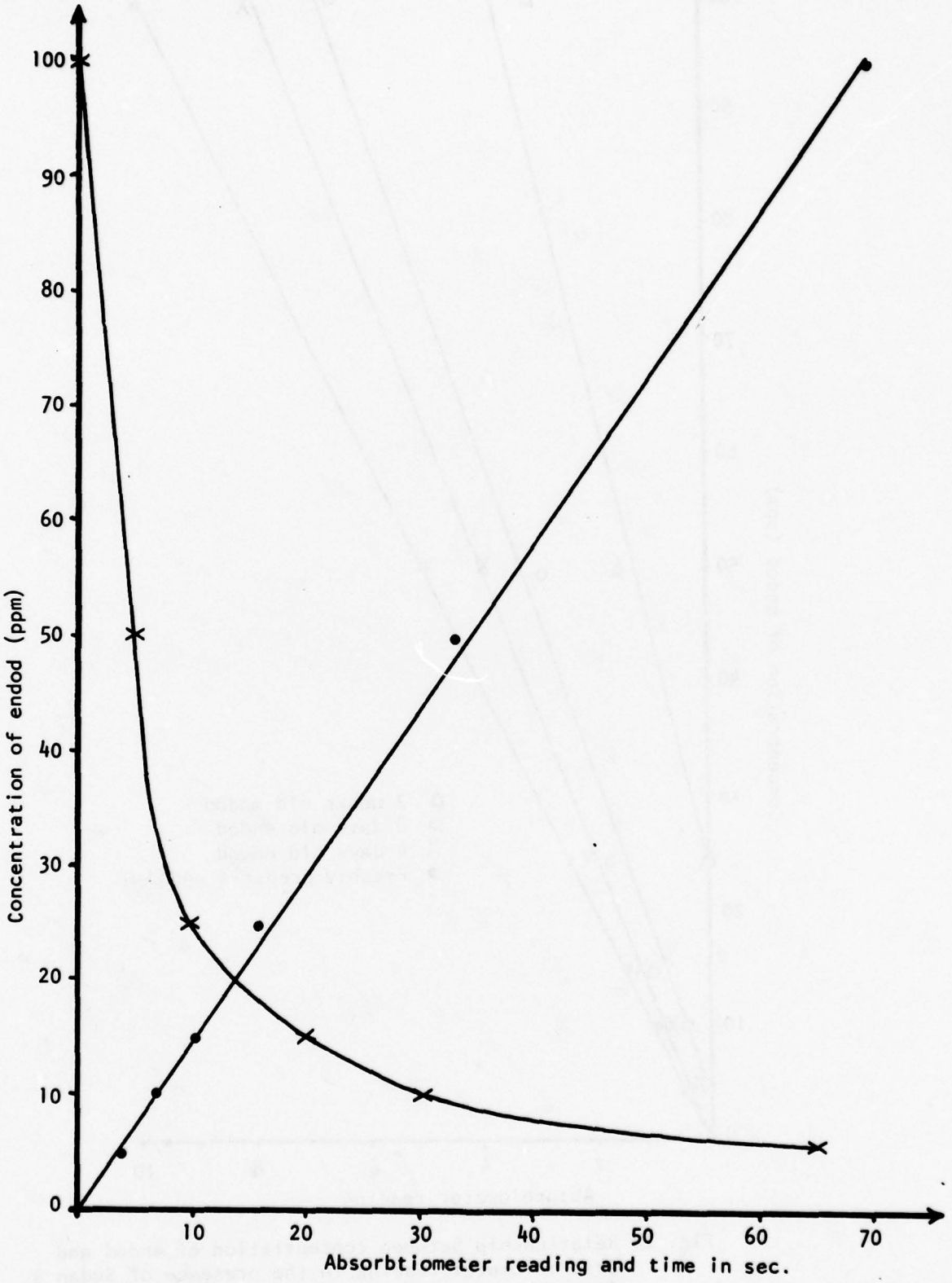


Fig.3. Relationship between light transmission, time of color development and concentration of endod

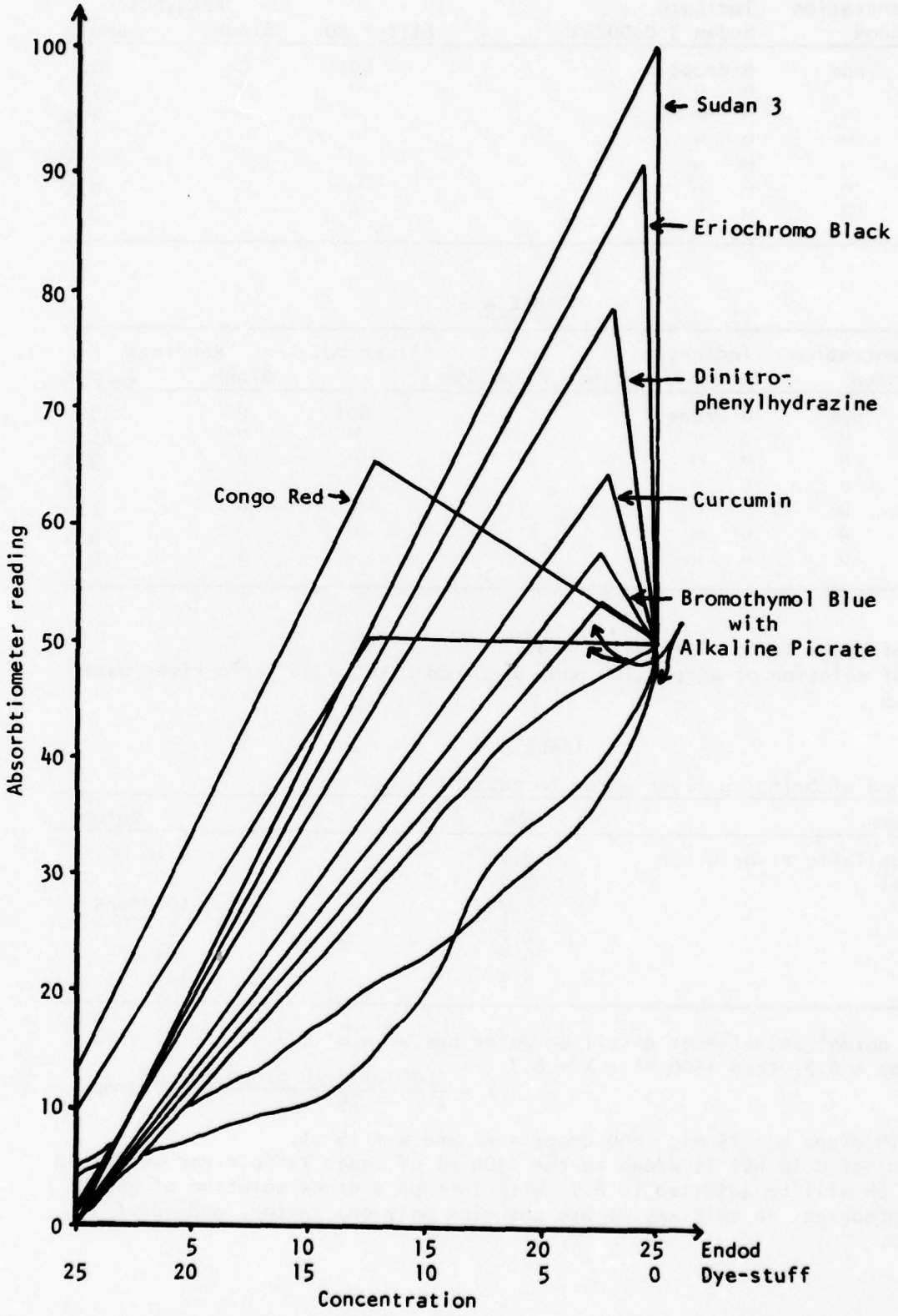


Fig.4. Relationship between absorbtiometer reading and concentration of endod and dye stuffs

TABLE 3

Concentration of Endod	Indicator Sudan 3 0.0025%	Filter No.	Readings Blank	Sample
100 ppm	6 drops	601	0	10.
50 "	" "	"	"	5.
25 "	" "	"	"	2.5
15 "	" "	"	"	1.5
10 "	" "	"	"	1.01
5 "	" "	"	"	0.5
0 "	" "	"	"	0

TABLE 4

Concentration of Endod	Indicator Eriochrome Black T 0.0025%	Filter No.	Readings Blank	Sample
100 ppm	6 drops	601	0	13
50 "	" "	"	"	65
25 "	" "	"	"	3.3
15 "	" "	"	"	1.7
10 "	" "	"	"	1.0
5 "	" "	"	"	0.5
0 "	" "	"	"	0

pH of Laaga Taffo river water = 8.7
pH of solution of endod (100 ppm) prepared with Laaga Taffo river water = 7.5

TABLE 5

Method of bringing river water to neutral

Volume	pH	Volume
Laaga Taffo river water	8.7	0.1N HCl
25 ml	8.7	0
" "	6.7	10 drops
" "	7.5	9 "
" "	6.9	9½ "
" "	6.5	11 "

The normal solution or distilled water has a pH of 6.7. If 25 ml + 10 drops = 6.7, then 1500 ml + X = 6.7

$$X = \frac{1500 \text{ ml} + 10 \text{ drops}}{X \qquad 25} = 600 \text{ drops}$$

If 10 drops = 1.25 ml, 600 drops = x and x = 15 ml.
15 ml of 0.1N HCl is added to the 1500 ml of Laago Taffo river water and the pH will be adjusted to 6.7. With this pH a stock solution of endod is prepared. In this way we are changing only one factor, adding of an acid.

TABLE 6

Concentration of endod	Indicator Sudan 3	Filter no.	Readings blank	sample
100 ppm	6 drops	601	0	9.6
50 ppm	"	"	"	4.3
25 ppm	"	"	"	2.1
15 ppm	"	"	"	1.2
10 ppm	"	"	"	1.0
5 ppm	"	"	"	0.5
0 ppm	"	"	"	0

TABLE 7

Concentration of endod	Indicator Eriochromo Black T	Filter no.	Readings blank	sample
100 ppm	6 drops	601	0	10
50 ppm	"	"	"	5
25 ppm	"	"	"	2.5
15 ppm	"	"	"	1.5
10 ppm	"	"	"	1.0
5 ppm	"	"	"	0.5
0 ppm	"	"	"	0

3. Factors affecting colorimetric method

The pH, time, temperature and nature of the water (turbidity, silt, etc.) play an important role in the determination of the molluscicidal potency of endod. It is known that pH ranging from 5 to 9 has no effect on the potency of endod. But if the solution of endod to be determined is too acidic or too alkaline, this method does not work. This is perhaps due to the decomposition of the structural formulas of the dye stuff molecules into its constituents at a lower or higher pH media. As it is shown in Table 8 and Fig. 2, endod at a lower concentration (100 ppm) decomposes or the potent form of endod is destroyed in time. As far as Bromothymol Blue is concerned, the color difference between all the concentrations (5-100 ppm) will match after a certain period (1-2 hours). Temperatures (4°40°C) have no effect on the determination of the potency of endod. It is not possible to determine the concentration or potency of endod if the temperature exceeds 40°C. This is, most probably, because of the:

a. Destruction of the structural formulas of the dye stuffs.
b. Destruction of the structural formulas of endod.

If the solution, being examined, becomes turbid it will absorb the color and decrease the intensity of the color. Therefore, turbidity may hinder the determination of the concentration of endod.

4. Determination of the molluscicity of endod

The molluscicity of endod is determined through the absorptiometer. Light transmitted through the solution is directly proportional to the concentration of endod (see Table 8 and Fig. 3).

TABLE 8

Freshly prepared Endod		After 4 days		After 8 days		After 2 weeks	
Conc. of Endod	Reading	Con.	Reading	Con.	Reading	Con.	Reading
100 ppm	10	100	8.0	100	6.5	100	3
50 "	5	"	4.0	"	3	"	1.5
25 "	2.5	"	2.2	"	1.8	"	0
15 "	1.5	"	1.4	"	1.0	"	0
10 "	1.1	"	0.8	"	0.5	"	0
5 "	0.5	"	0.4	"	0	"	0

It is seen from Table 8 that due to the dilution of endod (5-100 ppm) the potency of endod deteriorates. This is also shown biologically through snail killing tests.

5. Light-transmission characteristics of endod with various dye stuffs

It was observed that different dye stuffs behave differently with endod in the formation of complexes. Of the 30 dye stuffs thus studied 12 gave positive results and only 4 were acceptable as complex-forming agents in the colorimetric determination of endod concentration.

The requirements for an acceptable dye stuff are that:

a) No or low reading (0-5 on the absorptiometer scale of 100) without endod.
b) Maximum (peak) reading of absorptiometer at very low volume of the dye stuff.

The dye stuffs which have fulfilled the above requirements are Sudan 3, Eriochromo Black T, Dinitrophenydrazine (DNPH), and curcumin. As it is indicated in Figure 4, the addition of 6 drops of either Sudan 3 or Eriochromo Black T into 25 ml of 1000 ppm endod solution, the maximum peak of 100 and 90 respectively were obtained.

All light transmission determinations were made with filter number 601.

CONCLUSIONS

1. At low concentrations (up to 100 ppm) water-soaked endod decomposes into oleanolic acid and trisaccarides on standing for 3 or more days. Thus its potency deteriorates. But the opposite is true with high concentrations (above 10%), where potency increases over time. This is shown colorimetrically and biologically through the absorptiometer and snail killing tests respectively.
2. As it has been observed through the above method, the rate of decomposition is higher with the lower concentrations of endod.
3. All the dye stuffs mentioned in this paper form complex or colloids with endod. The form of these complexes and colloids have to be analyzed chromatographically.
4. The calibration curve of wave length versus absorptiometer reading of different concentrations of endod showed that the higher the concentration of endod, the higher the intensity of light is transmitted through the solution.
5. a) Visual colorimetric determination of endod is accurate at a deviation concentration of ± 10 ppm. The color of the dye stuff used

in this procedure varies from yellow to blue. It is possible to produce a distinguishable color by using a mixture of Bromothymol Blue and a solution of Alkaline Picrate. This method is developed on Laga Taffo river water. The pH of the river water is brought to neutral by adding 0.1 HCl.

Endod forms a yellow complex or colloid which gradually changes to blue with decreasing endod concentration, with Bromothymol Blue in the presence of Alkaline Picrate at a pH of 5.3-6.5. And this variation of color with the variation of concentration of endod in water depends on the pH of the solution. The control or blank produced a deep blue color which does not change over time. The color differences between different concentrations of endod match after 50-90 minutes. This is most probably due to: a) the turbidity of the test solution being examined. b) the shift of the pH from the higher to the lower. However, it is possible to shift the pH to alkaline and produce the same different colors as before by adding 1/2 - 1 drop of 0.1N NaOH. This method should be modified and improved during future field tests.

b) Upon shaking, foams of endod in the dye stuff formed. The height of the foam (or the thickness of the foam) changed with the concentration of endod. Therefore, it is easy to determine the concentration of endod by measuring:

i) the thickness of the foam
ii) the lifetime of the foam

The foam of the blank solution, which was produced by adding the dye stuff in water, disappears immediately (1/10 second).

Unpublished Research Note No. 8

SELECTED STAGE FOR ENDOD BERRY HARVESTING

Tesfaye Lemma (M.Sc.) and Legesse Wolde Yohannes (Ph.D.)

Institute of Pathobiology, 1977

INTRODUCTION

Endod berries can be grown and harvested locally and with proper genetic studies, strains with increased molluscicidal potency can be selected and cultivated for large-scale production and use. The investigation presented here is to determine the formation of the active ingredient in endod during the growing period and development of the generative part of the plant.

Endod is a perennial plant and berry production can be maintained in the field for several years. It is dioecious, its male flowers not yielding fruits. Planting in the production field should therefore use female plants. Propagation with cuttings from female (pistillates) plants will produce fruit and only a few male (pollination) plants are needed for fertilization.

Molluscicidal activity of the fruit and other plant parts can be determined using bioassay techniques. Lemma (1970) used various snail species and found some evidence of differences in susceptibility among species. Although the soapberry plant endod is found growing in various parts of Ethiopia, particularly in highland regions, there is virtually nothing known about its cultivation. One of the major objectives at the Institute of Pathobiology is to "domesticate" the endod plant and select strains for large-scale production, like all other cash crops, by developing strains that are high-yielding and have molluscicidal potencies.

This study shows that endod berries should be harvested during their green stage. Thus the generative phase of endod is prolonged and harvesting is more economical and time-saving. While endod is damaged by birds and other animals during maturity, the green berry stage, recommended here as appropriate for harvesting, is not associated with pests.

1. Materials and Methods

Complete molluscicidal testing was done for the following states of female endod:

root	buds/flower	green (III)
stem	early flowering	mature green (IV)
branch	stage	semi-ripened
leaves	flowering stage	ripened
completely closed	semi-green (I)	ripened II
flower buds	early green (II)	over ripened

All these samples were collected from a female endod bush grown behind the Institute of Pathobiology. Samples for the root, stem, branch and leaf were taken within two years in the manner of:

a. Two weeks before the flowering buds were coming out.

b. Two weeks after the berries were collected. These samples were dried in a room temperature and 100 gm were powdered into fine particles (1/10 mm) and placed in a conical flask of 1000 ml with 500 ml of distilled water. The flask was stoppered and shaken 10 times, 20 minutes each shake and left to soak overnight. This was filtered through a piece of cotton

and the filtrate was dried either in a ventilated room with 24 hours operating fan or with a spray drier operating at 120-140°C.

From the green and ripened berries of endod samples were taken every week until maturation and collection. Green and ripe berries of endod were crushed for juice. Both types of berries were defatted using benzene as a solvent and were fermented. The water content and dry matter were calculated. For each and every sample mentioned above, yield and molluscicidal yield were determined. Toxicity tests were conducted according to procedures recommended by the World Health Organization.

The snails used for the bioassay for all samples were Biomphalaria glabrata. The snails were reared in dechlorinated tap water (pH 7.2) at 28°C and were fed with lettuce. Snails of uniform size (8 mm) were used. Three to five snails were exposed in 200 ml glass jars; two jars per test dilution on dechlorinated water prepared in two-fold serial dilution. In all tests 24 hours exposure and 24 hours recovery period was used. This final result is the average of six repeated experiments (tables 1-3).

Table 1

Molluscicidal potencies of endod for different parts of plant at flowering and mature stages

Endod plant part	ppm	Before flowering- % mortality	After maturation of fruit - % mortality
root	1000	0	0
stem	1000	100	67
"	500	50	0
"	250	0	0
branch	1000	100	100
"	500	100	40
"	250	60	0
"	125	0	0
leaves	1000	100	100
"	500	100	100
"	250	40	100
"	125	0	40
"	100	0	0
flower buds	250	100	
" "	100	67	
" "	50	0	

Table 2

Dry matter, water contact and yield of crude endod by growth stage of endod berry

Stage	% dry matter	% water content	Yield
buds	10	90	20
buds/flower	11	89	26
early flower	15	85	30
flower	15	85	34
flower/green	18	82	38
early green	20	80	42

Table 2 (continued)

Stage	% Dry matter	% Water content	Yield
green	24	76	47
matured green	22.5	77.5	52
green/ripe	27.5	72.5	48
ripe I	26.5	72.5	46
ripe II	30	70	44
over ripe	32	68	44

Table 3

Molluscicidal potency of different growth stages of endod berries at various concentrations

Stage	Conc. in ppm	% mortality
early green	25	100
" "	15	33
" "	10	0
green	15	100
"	10	33
"	8	0
matured green	10	100
" "	8	33
" "	6	0
juice of the green	25	100
" " " "	15	33
" " " "	10	0
green defatted	4	100
" "	3	67
" "	2	33
" "	1	0
buds/flower	100	100
" "	75	33
" "	50	0
early flower	100	100
" "	75	100
" "	50	33
" "	40	0
flowering stage	50	100
" "	30	22
" "	25	0
semi-green	50	100
" "	25	67
" "	15	33
" "	10	0
green fermented	4	100
" "	3	67
" "	2	33
" "	1	0
semi-ripe	10	100
" "	8	0
ripe (I)	15	100
"	10	67
"	8	0

Table 3 (continued)

Stage	Conc. in ppm	% mortality
ripe (II)	25	100
"	15	33
"	10	0
ripe (III)	75	100
"	50	100
ready for collection	25	33
" " "	15	0
juice of the ripe	100	100
" " " "	75	33
" " " "	50	0
ripe defatted	15	100
" "	10	33
" "	8	0
Ripe fermented	10	100
" "	8	33
" "	6	0

Discussion

Three terms are currently used to describe the result of an endod extraction.

a) Toxicity: This refers to the number of parts per million of the extract required to produce 100% mortality in a standardized exposure test.

b) Yield: This figure, given as a percentage, is the ratio of the quantity of endod, also in grams, that was extracted.

c) Molluscicidal Yield: The active ingredient which can be obtained through extraction by calc-lating the water that can be treated with it and then by comparing this volume with the volume of water that the crude material would have been able to treat.

As for example, if we take one kg of crude endod that is active at 25 ppm, 25g is sufficient to treat one cubic meter of water (at 25 ppm), 1 kg is sufficient to treat 1000/25 = 40 m^3 (25 ppm).

Therefore there is sufficient of this active principle present in the crude material to treat 40m^3 of water. In practice, however, the following results for water extract have been obtained from crude endod that is toxic at 255 ppm.

If we had extracted 1 kg of crude endod we would have obtained 350g of the extract and we could say that:

a) toxicity = 6 ppm

b) yield = 40%

ç) 6gm is sufficient to treat 1 m^3 of awater at 6ppm
350 gm is sufficient to treat 350/6=58.3 m^3 at 6ppm/

d) molluscicidal yield = 58.3 x 100 = 143%

The observation is that we have obtained 43% more active ingredient by extraction than was originally present. The toxicity, yield and molluscicidal yield of an extraction depends on the method of the extraction process that is used and the molluscicidal yield gives the efficiency of the extraction with that method.

Therefore molluscicidal yield is the ratio of treated water of the extract to that of the crude in grams expressed in percentage. Having in mind the above expressions, from the figures given it is possible to deduce the following:

a) The toxicity of the endod plant increases from the root to the leaves and from the buds to the green berries.
b) The molluscicidal yield of the green berries was comparatively higher than that of the ripened ones.
c) In the process of the endod plant development the moisture content was decreased and the dry matter increased from buds to the ripened berries.

So far endod berries are collected when ripened, i.e. when the berries are pinkish (in the case of the arabe strain) and yellow (ahiyo strain). The advantage of using ripe fruits after the long ripening period is that they are eaten by birds and other animals. This is most probably explained by the slight sweetness* of the ripe berries. The molluscicidal test showed ripe berries to be four times less potent LC(100) than the green berries. This can be seen from the following examples:

100 g of these green berries were used and water extraction was obtained which gave:
a) toxicity = 6 ppm
b) yield = 40%

The same 100 g fresh green berries were sun-dried. This was ground up and extracted with water to give:
a) toxicity = 6 ppm
b) yield = 40%

4 kg of fresh green berries = 1 kg of dried berries.

For the fresh green berries:

6gm is sufficient to treat $1m^3$ of water at 6 ppm;
4 kg x 40% = 1600 gm, to treat 1600/6 = $266m^3$ (6 ppm)

$$\text{m.y.} = \frac{266}{40} \times 100 = 665\%$$

For the dried berries 6 gm is sufficient to treat 1 m^3 of water (at 6 ppm).

1 kg x 40% = 400 gm is sufficient to treat 400/6 = $66.7m^3$/6 ppm
molluscicidal yield = 66.7/40 x 100 = 167%

Therefore from the fresh green berries a product that is 4 times as effective as that of dry berries was obtained.

Conclusion

Laboratory investigations of molluscicidal concentration and yield of endod grown at the Institute of Pathobiology indicate that berries should be harvested when green to semi-ripe, but different ecological varieties of endod should be studied before more conclusive statements can be made. Endod berries harvested at the green stage have the added advantage of saving time, eliminating damage by birds and other animals, high yield and potency and a longer harvesting time.

REFERENCES

Lemma, A. (1970). Laboratory and field evaluation of the molluscicidal properties of Phytolacca dodecendra. Bull. Wld. Hlth. Org. 42, 597-617.

*The ripened berries have a high content of sugar. But which of the non-foaming parts of sugars are attached to the saponin of P. dodecandra is still under study.

Unpublished Research Note No. 5

A SHORT NOTE ON TOXICITY STUDIES OF ENDOD (PHYTOLACCA DODECANDRA) ON SHEEP AND DOGS

Ephraim Mamo (D.V.M., Ph.D.), Institute of Pathobiology, 1977

MATERIALS AND METHODS

Ethiopian highland sheep and mongrel dogs were used in these studies. The two animal species were taken as representatives of the ruminants and monogastric large domestic and wild animals that might drink out of endod-treated bodies of water.

Two groups of 8 sheep were used for oral toxicity evaluations at the rate of 200 mg per kg and 1.0 gm per kg body weight. Observations were made over a period of 28 days and 4 days respectively. Pulse rate, respiration rate, temperature, and pack cell volume were recorded on a daily basis.

In the case of dogs both oral and systemic administration were performed at low, medium and high levels and records were taken as above. In addition kidney and liver function studies were performed.

RESULTS

The results so far showed that at a concentration of 200 mg per kg body weight, the water extract caused little or no change in the parameters measured.

A concentration of 1 gm water extract per kg body weight was lethal to all sheep in less than 96 hours.

In the case of dogs oral administration at the rate of 200 mg per kg body weight caused vomiting within minutes. No significant difference from control animals was shown during the observation period in the parameters measured. On the other hand intravenous administration at the rate of 50 mg per kg body weight was lethal in less than 24 hours while 8 mg per ml of blood did not show any significant changes.

COMMENTS

The study is continuing and full details of the findings will be presented in due course.

Unpublished Research Note No. 9

PRELIMINARY REPORT OF THE INVESTIGATION ON INSECT PESTS OF ENDOD (PHYTOLACCA DODECANDRA)

Teshome G. Michael (B.Sc.) and Legesse W. Yohannes (Ph.D.)

Institute of Pathobiology, 1977

INTRODUCTION

Lemma (2) first found the berries of endod (Phytolacca dodecandra) to be highly molluscicidal. As a natural product, endod is considered a cheap and effective means of controlling schistosomiasis. This disease is affecting the extensive tropical and subtropical zones of the globe, where an estimated 200 million people are infected (1). Schistosomiasis is often a disease of rural development. Irrigation canals are sources of infection, thus becoming an obstacle to economic development of various countries. The berries of P. dodecandra could be the least expensive and most suitable molluscicide in countries with a predominantly agricultural base, like Ethiopia. However, certain insects have been found to breed on the young shoots of this plant and commonly kill it, and have become a grave concern for mass-cultivation of P. dodecandra. The present investigation, both in the field and insectary on the biology of these insects was undertaken to obtain some of the background information needed to formulate recommendations for the effective control and management of these insect pests.

THE INSECT PESTS

Infected branches and stems of endod were collected from endod plantations at the Institute of Pathobiology (Addis Ababa) and Sabeta (25 km west of Addis Ababa). These branches and stems were put in cages and incubated in the insectary where the relative humidity was 70-80%. Other times all pupae were collected by splitting open the infected branches. These were put on wet cotton in a cup kept inside a cage and incubated in the same insectary. Both incubations were done repeatedly at different times of the year.

From these, always three different adults emerged. These were sent to Dr. L. Tsacas (Museum National, D'Histoire Naturelle, Entomologie Generale Et Appliquee, Paris, France) for identification.

One insect was identified as Gitona pauliani, first described in Madagascar from Phytolacca abyssinica L'Herit (synonym P. dodecandra) by Seguy (3). G. pauliani was found to occur both in Addis Ababa and nearby Sabeta.

The second type which is much similar to G. pauliani but otherwise bigger in size is apparently a new species belonging to the same genus, Gitona n.sp. This new species will take Dr. Tsacas about a year to name and publish. It has been found only in Addis Ababa but never in Sabeta.

The third type belongs to the order Hymenoptera which will also take Dr. Tsacas about a year to identify and publish. This insect occurs in both Addis Ababa and Sabeta.

Appearance of the two species of Gitona

a) Gitona pauliani

The adult varies from 2.7-3.6 mm in length and is 1.2-1.5 mm in size. When young, it is orange brown in color and becomes brown when mature.

The body is covered with hair, usually black.

At the early stage, the larva is cream-white which becomes yellowish when mature and measures up to 6 mm in length.

The brown pupa is about 4.0-4.7 mm long and 1.3-1.6 mm wide. It becomes dark brown when the adult is ready to emerge.

The egg is about 1000 m long and 380 m wide. It is boat-shaped with a wing-like structure on both sides which is membranous. A similar structure but shorter in length is also found running the middle line of the dorsal surface of the egg from the anterior to the posterior end. Between this and the wing-like structure on the sides is grooved usually dark in color. Ventrally it is convex shaped with a thin membrane covering the embryo inside. The egg is greyish white in color.

b) Gitona n.sp

The adult varies from 4.2-6.0 mm in length and is 1.8-2.0 mm wide. It is much similar to G. pauliani in color, tending to be a little darker brown when mature, however.

The appearance of the larva and pupa is also much similar to that of G. pauliani. The larva measures up to 7.5 mm in length when fully mature. The pupa is about 5.4-5.9 mm long and is 1.8-2.0 mm wide. It also becomes dark brown when the adult is ready to emerge.

The egg is much longer and wider than that of G. pauliani. It is about 1250 m long and 512 m wide. It is boat-shaped like G. pauliani with similar structures on both sides and on the dorsal surface. Ventrally it is also convex shaped with a thin membrane covering the embryo inside. However, it is grey.

OBSERVATIONS ON LIFE HISTORY IN THE LABORATORY

The adults of Gitona pauliani and G. n.sp. reared from infested branches were kept separately in cages of 70 x 50 x 35 cm and 45 x 45 x 45 cm respectively in the laboratory with room temperature of 20°C. They were fed on 20% sugar solution with cotton in vials. A potted endod plant about 30 cm long was introduced in each cage to observe how the insects actually infest the plant and to determine the life cycle of the insects.

It is observed that eggs of G. pauliani are oviposited and glued singly on the young shoots of the plant. They are oviposited on both the abaxial and adaxial surfaces of the leaves (mostly on the 1st, 2nd and 3rd leaves from the apex of the plant).

Eggs of Gitona n.sp. are also oviposited singly and glued, but on the stem at distances between 1-4 cm down from the apex of the plant or shoot. They are very rarely laid on the leaf.

It was interesting to observe here that flight activity of both species was always high in the afternoons, especially between 16-18 hours, but the activity of the females was much higher than that of the males, and oviposit occurred mostly between those hours.

The plants were then transferred to different cages to retain the adults when emerging. The eggs of both species hatch between 3-5 days after oviposition, but the eggs of Gitona n.sp. may take up to 7 days to hatch, depending on the condition of the environment and the suitability of the host plant. After about 10 days the plant starts to wilt beginning from the apex. As the larva tunnels further down, the plant also wilts more and more from above the point of the active larva. Then the leaves begin to die and finally fall off. The stem or branch above the active larva also dies which will lead to growing of new branches

from the base or just below from the point of pupa.

Adults of G. pauliani emerged after an average of 35 days, while those of Gitona n.sp. emerged after an average of 60 days.

FIELD OBSERVATIONS

It was said by Dr. Takle and Mr. Tekie (private discussion), both of the Institute of Pathobiology, that the adults never have been found in nature. Ghidey (4), however, wrote that the adults were observed hovering on endod flowers (mostly on male flowers). Although it is quite possible that they feed on nectar (since they have typical sponging type mouth parts), this investigator has never encountered these flies on endod or other flowers of the surrounding vegetation.

However, the oviposition, which occurred between 16-18 hours in the cage, indicated the possible time the adults would be found in nature. Between 16.30-17.30 hours, on March 30, 1977, 3 adults of G. pauliani and 4 adults of Gitona n.sp., and on April 7, 1977, 5 adults of G. pauliani and 5 adults of Gitona n.sp., and on April 28, 1977, 1 adult of G. pauliani and 3 adults of Gitona n.sp. were captured from the tips of young branches with many others escaping capture. Adults of Gitona n. sp. were captured from branches at the base which are in shade while G. pauliani were captured both from branches at the base and upper branches which were not in shade.

All were captured when flying from one branch tip to the other selecting sites of oviposition. All captured were females. Not a single male was captured in all trials. The habitat of these adults in nature, except during the oviposition hour, still remains unknown.

In the field, the larve of G. pauliani is both a leaf miner and a stem borer, while that of Gitona n.sp. is only a stem borer.

G. pauliani

In small branches or stems less than 4 mm in diameter, the larva tunnels linearly through the sapwood. One larva per such branch is the usual number. In branches with 4-10 mm diameter, the larva tunnels along the line of the pith, surfacing at regular intervals to produce a spiral appearance. Sometimes holes are made at surfaces from which frass is extruded. During the larval stage, it may tunnel up to 20 cm from the branch tip. When fully mature, the larva cuts an exit hole, measuring about 1-2 mm in diameter, leaving sometimes a thin epidermal layer covering. The larva pupates in between 0.5-2.0 cm down from the exit hole. Again, 1 larva per such branch is the usual number, with 2 or 3 larvae being occasionally found.

Gitona n.sp.

In the field, branches which arise from the base of the main stem and which are 5-10 mm in diameter are mostly infested with the larvae of this species. The tunnelling is also a regular spiral when one larva is involved. But, sometimes, up to 7 larvae (or pupae) per such branch may be involved in which case the tunnelling becomes highly irregular with most of the sapwood pith tunnelled. During the larval stage, it may tunnel up to 20-40 cm down from the branch tip depending on the length and the diameter of the branch. When mature, the larva cuts an exit hole measuring about 2 mm in diameter.

The hymenopteran insect when kept in a cage did not cause any visible damage to a potted endod plant. It is at this stage that this insect is not a pest itself, but probably a secondary infection. It is

assumed that they oviposit the eggs through holes made by the larvae of the two species of Gitona. However, this has never been observed happening in the field. This will have to be investigated further.

Damage

The first visible symptoms of infestation by the two species of Gitona are wilting of leaves, branches or stems. Progressively, the leaves turn brown and fall off. The stems of branches turn black when they die. Sometimes, stem branches do not show any sign of wilting or death even under heavy infestation. They give the impression of well-being to an observer. Symptoms of infestation in such branches or stems are exit holes and the extruded frass. If the branch or stem is split, the larval gallery is revealed. In branches with diameter 5-10 mm up to 7 pupae (Gitona n.sp.) may be found. The presence of pupae of both species of Gitona in a single branch was also not uncommon. On infested branches which were in a shade (arising from the base of the plant), survey was made from Institute of Pathobiology plants for four months, in 1977. The length of these branches varied from 10-70 cm (Table 1).

It is observed from the table that pupae of Gitona n.sp. are predominant in these branches which goes parallel with the capture of the adults from such branches in the shade.

A detailed survey of 79 endod plants (about 4 months since planted) was made on August 30, 1977 on the damage caused by the larvae. Up to 8 branches per plant were infested on 65% of the plants surveyed (Table 2).

Table 1

Gitona n.sp. and G. pauliani collected from branches of endod plants at the Institute of Pathobiology, May-August 1977

Month 1977	No. of branches with diameters between 5-10 mm	Total No. of pupae of Gitona n.sp.	Total No. of pupae of G. pauliani
May	85	137	22
June	44	98	14
July	59	145	8
August	28	76	8

Table 2

Damage caused on 79 endod plants at the Institute of Pathobiology Compound (August 1977)

No. of damaged branches per plant	No. of plants
8	1
7	9
6	3
5	1
4	3
3	3
2	17
1	23
Total	51 plants

FUTURE STUDY ON CONTROL OF THESE INSECTS

1. Since eggs of the two species of Gitona are oviposited on surfaces of stems, branches or leaves they are exposed to any predator there may be (entomophagous). This will have to be studied for possible presence of such predators.
2. It is also observed in the field that certain varieties of endod plants have smooth shoots. Others have hairy shoots. Those with hairy shoots occasionally have been observed to resist the attack of these insects. This is probably due to the uncomfortable situation created on the shoot for the insects to oviposit. This also has to be studied in the future.
3. Finding a possible alternative host plant (if any) has to be studied also in the future.

REFERENCES

1. Barbosa, F.S. (1970) Symposium on Schistosomiasis. Health Bureau Publication 1. OAU, Addis Ababa, pp. 61-68.

2. Lemma, A. (1965) A Preliminary Report on the Molluscicidal Properties of Endod (P. dodecandra). Eth. Med. Journal 3, 187-189.

3. Seguy, E. (1951) Un Drosophilidae Phytophage Nouveau (Dipteria). Memoires, De L'Institut Scientifique De Madagascar Serie A, Tome VI-Fasciole 2:399-405.

4. Ghidey, G.E. (1971) The Life Cycle of Phytophagous Insect (Drosphillidae) of Ethiopia. Dept. of Biology, Science Faculty, Addis Ababa University.

ENDOD - WONDER WEED OF AFRICA

R.M. Parkhurst, Stanford Research Institute, 1977

As I peered from the small plane window out over the buff-colored Sahara sands that seemed to stretch into infinity, I was headed for home but I knew this would not be the end of the story about a strange weed called endod. While the story really starts sometime before the birth of Christ when native women of Ethiopia used the berries of the endod plant as soap to wash clothes in the headwaters of the Nile, a practice which persists even today, the major development came only ten years ago.

A Johns Hopkins trained Ethiopian scientist, Dr. Aklilu Lemma, was doing a study on the spread of a debilitating parasitic disease called bilharzia. Bilharzia is caused by a microscopic worm or schistosome. Unlike most diseases, bilharzia or schistosomiasis, as it is sometimes called, is not contracted by contact with another person. It is transmitted by water snails which breed rapidly in rivers and irrigation canals. The infected snails release large numbers of the disease organisms into the water. To become infected one only needs to be splashed with the infected water since the organisms are able to bore through human skin and take up residence in the blood stream of their victims. Here each pair of male and female worms will continue to live for perhaps the next 20 to 30 years and lay eggs at a rate of about 3000 per day. The eggs clog the flow of blood and finally break through the small blood vessels of the bladder or intestines where they find their way back to the stream and another water snail. The disease is only infective to another person after it has spent a short but necessary part of its bizarre life cycle in the water snail.

Bilharzia is becoming an increasingly more serious health problem, taking the place of malaria in some parts of the world. According to the World Health Organization (WHO) estimates, more than 200 million people are suffering from bilharzia and the number is rapidly increasing. In many developing tropical countries the spread of the disease parallels the development of irrigation canals, built with such good intentions but with what now seems such disastrous results.

As. Dr. Lemma tramped from village to village in Northern Ethiopia taking his tally of the spread of bilharzia and its vector snails, he made one dramatic observation - downstream from where native women washed with endod berries there were no live snails for sometimes several miles. He correctly speculated that the endod plant contained a powerful poison for the water snail and it took him only a few days to prove this conclusively in his laboratory at the University. He spread the word to the villages - "Crush the berries and throw them into the streams!"

It would be a tough fight because the process had to be repeated every few months to keep the few snails that had escaped in some isolated pool upstream from repopulating the stream again - but now there was hope - even the smallest isolated village had something they could do to help themselves in the fight against bilharzia.

In 1970 Dr. Lemma came to Stanford Research Institute to study further the potential of his discovery with the aid of biologists, chemists, toxicologists and other scientists in the U.S. I was one member of a team of chemists who would eventually isolate and identify several of the active substances from endod berries. The purified substance was so toxic that less than a millionth of an ounce would be enough to kill a water snail, yet a mouse could eat one tenth of its body weight of the material without ill effect.

Small doses were tested on higher animals, cats and dogs, and unlike the mouse even small doses caused immediate vomiting but no lasting effects. The stomach contents were emptied so completely by some local action still not completely understood, that endod was suggested as an antidote for poison particularly in cases of drug overdose. After sufficient experiments to prove its safety, dogs were given barbituates in amounts several times the amount that would ordinarily be lethal. Then they were given a small amount of endod. After a short bout of biolent vomiting all dogs came through unharmed. It was later discovered that a strong tea made from endod berries had been used as an Ethiopian folk antidote for poison.

The microbiology lab found that an extract of endod berries killed the fungus that causes athletes foot. A laboratory at Harvard, studying the extracts sent from Stanford, found that mosquito larva of the type that carry malaria were ten times more sensitive than the water snail. The larva could not develop into adult mosquitoes in the presence of even a trace of endod.

In the early part of 1972, Dr. Lemma returned to Ethiopia to continue his work but now laboratories all over the world were also working on endod. There was much to be learned about this week that had grown wild so abundantly but had never been cultivated. Much work was also required to build a small extraction plant to refine the active chemicals from the dried berries.

Work also continued at Stanford Research where extracts of endod were systematically screened for many kinds of other biological activity. The biology department found that the crude extracts had a powerful effect on human sperm. Sperm cells,when released into the female reproductive tract, must swim like pollywogs in order to find their way through the long fallopian tubes to cause fertilization of the egg. In the presence of even a trace amount of endod they are unable to complete their heroic swim. The endod extract was found to be about ten times as effective as the material used commercially in the U.S. as a vaginal contraceptive preparation. Further tests showed that introduction of small amounts of endod extract into the uterus of a pregnant rat caused immediate and clean abortion with minimum side effects. Again and again the word endod appeared, smothered in the unemotional jargon of the technical journals.

Late in 1975 I got a letter from Dr. Lemma - "Can you come over as soon as possible? We are having trouble with the extraction." I was delayed in coming by nearly a month and arrived in Addis Ababa only a week before Christmas.

There were almost no tourists in Ethiopia. This was probably due to the apprehension surrounding a visit to a country that has changed its government by revolution a little more than a year ago. I was prepared also for some kind of anti-American feeling. This proved however not to be the case. Addis Ababa, the capital city of about a million people, looks down on the rest of East Africa from a high plateau of 8,000 feet

above sea level. The days are hot and the nights are cold. Despite whatever problems may exist, its people have maintained a warm and friendly atmosphere.

At the Institute of Pathobiology, I joined with others already hard at work trying to solve the problems with the extraction. As it turned out, nature has provided the endod plant not only with a powerful poison for the water snail but also with the antidote for its own poison. If the extraction is not done exactly right, a produce is obtained that contains both the poison and its antidote and the product has little effect.

The clue to the nature of the antidote again came from the rich folklore about native Ethiopian medicinal plants: "When endod is used to induce vomiting, large amounts of butter can be taken to stop its action." We tried mixing the extracts of endod which were known to kill snails with small amounts of butter and found the snails now survived. The effect was quickly traced to cholesterol in the butter. It seems that cholesterol and the active components of endod are able to mutually counteract the effects of each other.

Although the endod plant does not contain cholesterol, it does contain some closely related materials that have the same effect if not completely removed during extraction. The production of active extract in large scale was now underway again. The endod producy is a light tan powder destined for test sites across the country.

The week before I left, Dr. Lemma came into the chem lab with eyes sparkling, "Come quickly, I want to show you something." In the next room we were told to look through the microscope into the microscopic world usually reserved for biologists. There we saw some microbes called trichomonas, that cause a less serious form of venerial disease, in their last struggle before death as a result of exposure to minute amounts of endod extract.

Before leaving Ethiopia, I had a chance to see endod plants growing for the first time. The small town of Debre Zeit is the location of the University Agricultural Experiment Station and only a few hours drive from Addis. It was a special treat for me to see a large field of endod growing well over my head and in full bloom with tiny white flowers and green berries. The berries will turn red when they are ripe and contain over 25% active material. The field was divided into obvious sections and subsections by poles and markers. This was not only to test different conditions of cultivation but also to separate the number of varieties of the plant that have now been found. The Agricultural Station at Debre Zeit made the first attempt only five years ago to cultivate the wild plants and to determine maximum yield conditions. Botanists are also now relooking at the endod plant and its supposed relatives that grow outside of East Africa and are now wondering if, indeed, the endod plant has any close relatives.

The drone of the airplane is broken by a somewhat inaudible announcement about passing over Aswan - and looking down I can see the long green ribbon on each side of the Nile. This is Egypt; it is totally dependent on the water that the Nile brings. As the plane passes over, the sun reflects sequentially in each of the irrigation canals and they look like silver swords stabbing their way through the green fields. These are two edged swords - carrying life-giving water but also bilharzia!

What a strange twist of nature. Egypt has always looked to the head-waters of the Nile for its life's blood which brings with it snails and

one of man's major infections. Now when irrigation is spreading schistosomiasis faster than ever, it is again the headwaters of the Nile that is the natural habitat of the plant that has the ability to control these snails.

Is endod the only answer to the control of bilharzia? Not by any means! There are other synthetic materials that also kill snails - each has its own advantages and disadvantages for particular applications. New drugs for treatment are being developed for those who already have bilharzia; each of these also has its own advantages and disadvantages. The development of general sanitary conditions, screening for new cases, early treatment, education, determination, time and ecological planning - all these things must be used to stop bilharzia. In the meantime endod will be another valuable tool in this fight and new and unrelated developments continue to appear including the use of endod extract in photographic emulsions and as an additive to concrete to change its setting time and properties. What next - this wonder weed of Africa!

Unpublished Research Note No. 6

THE ENDOD RESEARCH PROJECT: PROGRESS REPORT AND PLAN OF WORK

Yemanu Tekie (M.Sc.) and Legesse W. Yohannes (Ph.D.)

Institute of Pathobiology and Horticulture Department of the Debre Zeit Agriculture Experiment Station, Addis Ababa University, 1977

Progress Report

Since the establishment of the Project* in 1973 over 60 collections from seven administrative regions (provinces) were made. Methods of propagation both by seed and by cuttings were established. Although optimum planting distances are not yet determined, we now know some idea on the spacing of the plants. At this stage the plants can be divided into two groups based on their growth characteristics. The first group comprises of plants of semi-erect and erect shrubs which to a certain height can support themselves on erect stems. The second group includes the vining types that grow along the soil surface only. Cultural treatment, particularly with regard to spacing and training, for these two groups will be different and tests will be conducted separately.

It has been shown that endod can grow and produce berries at altitudes as low as 750 m (at Melka Worer) where it has been known not to grow naturally. But due to change of staff and lack of better follow-up of the work the feedback on the work was not satisfactory. The observation plot at Melkassa produced some interesting results as far as the killing potency of the berries is concerned. But the site is located on a narrow depression which might have its own microclimate and thus not be representative of the surrounding area. Future observation plots should be moved to the new site of the Institute of Agricultural Research's experimental station.

At Sebeta a nursery has been established to supply seedlings and rooted cuttings to establish the plantation there. In connection with this undertaking it has been our experience that establishing endod plants without irrigation is difficult though possible. Seedlings were grown in time to be transplanted at the beginning of the main rains but many of them did not have a high chance of survival during the dry season without adequate supplementary irrigation. Therefore, every rainy season the blank spaces had to be filled up with fresh seedlings until an even stand was achieved. But once the plants are established they can grow and produce berries without irrigation, i.e. at Sebeta and at places with similar rainfall patterns. An additional cause of plant loss at Sebeta was cattle, sheep and goats that used to graze around the plot and in the field. But now the plot is fenced and this problem is almost solved.

At Debre Zeit the 1976-77 endod harvests could not be properly recorded because: a) the plants became erratic in their production pattern. This may be attributed, at least in part, to the repeated damage by insect larvae which have been keeping the plants in check from

---

*As of March 1977 the Project has been incorporated into the Horticulture Department of the Debre Zeit Agricultural Experiment Station. Therefore endod will be considered as one of the horticultural crops and it will have its share of experimental work--subject to the limitations of the Department's budget.

normal development. b) Many plants have been drying, the exact cause of which is not known, but insect larvae (different from those that attack the shoots) were found in the dry roots. It was also reported from Melkassa that some plants were attacked by nematodes. Since plants were drying here too it is suspected that the cause can be either nematodes or insect pests or both.

Although the plants have survived an insect attack at their initial growing stage, the damage from the same insect larvae still reduces a considerable amount of vegetative part of the plant. The damage becomes more acute after the plants are pruned when nearly every shoot that emerges is attacked and the plants have to struggle for their survival. A few died as a result of this. Because of this problem pruning trials could not be carried on. The insect, Gitona pauliani, was recently identified, but two other species are also suspected to be attacking the plant. Until an effective control measure against these insect pests is devised, experiments on cultural methods and other tests cannot be successful. Therefore in future research work the pest control aspect will be given priority.

Parallel to the above investigation we have been studying under green house conditions the effect of different temperatures (low and high) and the effect of different nutrition as well as other environmental factors, on the biology and potency of the endod plant. The influence of nitrogen in the form of ammonium nitrate has been so far investigated favorably. For the coming year 1977/78 experimentation on other essential elements such as phosphorous and potassium are planned for similar investigation. The specific investigation on germination and growth characteristics on the botanical aspects will be continued. A Phytotron cabinet model is considered to give an effort for the studies.

Plan of Work

The following experiments and observations on endod will be carried out at the Debre Zeit Agricultural Experimental Station and in other stations:

1. Screening of various insecticides to find the most effective chemica- against the damaging insect pest(s).
2. Selection of erect and semi-erect shrubs from the existing plants and planting cuttings from these for the future cultivation without training. In regard to this experiment two types of planting systems will be developed: for the vining types that do not produce any upright stem training is essential. But the semi-erect bushy types may be grown, with appropriate pruning, without support. While this may be a preliminary basis for selection, other characteristics, including snail killing potency, will be used for further improvement of the plant.
3. Planting of several "types" of endod in observation plots in several climatically representative stations to test for their adaptability. This is a continuation of the current work but it will be pursued with a new setup.
4. Spacing trials to establish optimum plant population per unit area.
5. Establishing methods and frequency of pruning--subject to the prior solution of the insect problem.
6. Cufodontis recognizes three varieties in the Phytolacca dodecandra species in Ethiopia. Therefore, varieties that may exist within the present collection or fresh collections will be identified.

Unpublished Research Note No. 13

ANTIMICROBIAL PROPERTIES OF ENDOD

Chaltu G. Wossen (M.Sc.) and Aklilu Lemma (Sc.D.)
Institute of Pathobiology, 1977

Preliminary studies have been conducted to determine the antimicrobial activities of the butanol extract of endod against the following representative species:

(1) Trichomonas vaginalis
(2) Fungi of the genera Aspergillus, Penicillium and a dermatophyte most probably Trichophyton sp. isolated from children (ages 8-10) with ringworm infection (Tinea capitis)
(3) Bacteria:- Staphylococcus aureus, E. Coli
Salmonella typhimurium

A. Action of endod on T. vaginalis

Urine specimens from Trichomonas infected human volunteers (females) were collected at the gynecology hospital. The urine samples were centrifuged and the sediment inoculated into a CPLM medium (formula as recommended by Johnson & Trussell, 1943, 1945). Good growth was obtained between 24 to 48 hours.

A butanol extract of endod was dissolved in Locke's solution. Serial dilutions were made to achieve the required concentrations.

In vitro tests were conducted. Equal volumes (eg. 0.2 ml) of the endod solution and the culture were incubated at 37°C. All concentrations were final, i.e. after mixing with the culture. Samples were examined under the microscope at different time intervals (0, 5, 10, 15, 20, 30, 45, 60, 120 minutes). Concentration of endod µg/ml (40 ppm) and above, killed the cells within the first 5 minutes of incubation. 20 and 30 µg/ml kill 100% is incubated for about 30 minutes.

This was compared with KMNO4, 500 µg/ml didn't affect any within the first 5 minutes. A concentration of 800 ppm or more was needed to give more than 90% kill within the first 5 minutes.

Even though no detailed investigation was made on the mode of action, endod seems to affect the cell membrane. The cells get rounded, ruptured. The cell concents appear granular.

B. Action of endod on fungus strains

Endod was tried on the genera Aspergillus and Penicillium and also a dermatophyte isolate from a child with Tinea capitis.

In vitro tests were run in different ways.

1. Incorporating a known concentration of endod into a culture media and inoculating these media with the fungi.
2. Mixing the endod solution with the fungi, allowing a reaction time and transferring into a slant of Saboraud dextrose agar.
3. Using Reddish method of testing fungicides, i.e. flooding the solid culture of the fungi with a known concentration of endod and taking a cube of it after a known time interval, rinse and transfer to a growth media.

All concentrations tested including 1000 µg/ml did not inhibit the growth of the fungi tested no matter the method used. One may run more tests using a higher concentration before eliminating it from the picture; however, it is not a broad spectrum fungicide.

Action of endod on bacteria

Using both Reddish method and Klarman and Wright "semi-micromethod" of testing antiseptics, endod was tried on the following organisms:

a) Staphylococcus aureus (a gm+ bacteria)
b) E. coli (a gm- bacteria)
c) S. typhimurium (a gm- bacteria)

Concentrations up to and including 1000 mg/ml (with a maximum contact period of 60 minutes) did not kill bacteria.

Antiprotozoan properties of endod

Results of preliminary screening of endod for its antimicrobial property, showed that it is promixing as an antiprotozoan. In vitro tests conducted on Trichomonas vaginalis showed that concentration of endod as small as 20 to 30 mg/ml could kill the organism. The cells get rounded and the membrane ruptured and contents leaked out.

Endod was also tried on Trypanosoma congolense and seems to kill the parasite. However, the hemolytic action of endod on RBC has to be considered when tests involve blood parasites.

On the other hand some attempt has been made on fractionation of the endod to see the possibility of getting endod fraction(s) that could kill the parasite without being hemolytic. This is not concluded yet.

Detailed investigations along this line could be planned. Preliminary toxicity tests of endod on the vagina of cows have been done by our laboratory with the aid of a veterinarian in a nearby farm. There were no apparent ill effects detected. The possible usage of endod in treating T. foetus infections in cattle is being considered.

Study Plan

I. Trichomonas vaginalis
   1. Further in vitro tests of different varieties of endod on T. vaginalis
      a) using extracts as done previously
      b) using wet crushed berries (for possible local use)
   2. In vivo tests using experimental animals, most probably monkeys Infecting and treating the animals with endod
   3. Toxicity tests of the endod on the experimental animals before it could be recommended for human use.

II. Trichomonas foetus
   1. Prevalence survey of Trichomonas infections in cattle
   2. Culturing the Trichomonas foetus in the laboratory
   3. In vitro testing
   4. In vivo testing using experimentally infected calves
   5. Field trials

III. Trypanosoma congolense
   With the aid of the veterinary unit
   1. Continue fractionation of the endod for possible non-hemolytic and antitrypanosoma fractions
   2. In vivo testing using experimental animals, in this case, mice were used previously to maintain the parasites.

If the above fractions are obtained studies on other blood parasites could also be considered.

Unpublished Research Note No. 10

MEDICINAL PLANTS CHEMISTRY RESEARCH

Tesfay Lemma (M.Sc.), Intitute of Pathobiology, 1978

1. General Information

The effect of heating on the molluscicidal activity of endod is studied in our laboratory and the result is as follows: If endod beries are dehydrated at 105°C for about 6 hours, its potency is reduced. Similarly, if powdered endod is extracted with boiled water, it is deactivated. Besides this, it is planned and some work is already started to make a complete chemical assay on endod which would lead us to the study of many other important medicinal plants. Some results of the ongoing and completed experiments are:

a) Metallic contents in the endod berry
b) Total lipid content (5.71%)
c) Total protein contents (15.6%)
d) Water content (11.25%)
e) Total carbohydrate content, etc.

All these experiments have to be carried out before and after fermentation.

2. Endod extraction process

The original effort to concentrate the active principle in endod was based on butanol extraction (Lemma et al., 1972). Because of unfavorable interaction between the fat and glycoside components of endod berries while in aqueous solution, it has been necessary to defat the berries before such an extraction. The defatting technique was improved by comparing the extraction capabilities of different solvents such as benzol (pure benzene) gasoline, petroleum, ether, and kerosene. The essential role of these solvents in the defatting process was shown during the consultative services of Dr. Robert M. Parkhurst, the chemist who has done most of the chemical work on endod at Stanford Research Institute. Dr. Parkhurst showed that the potency of defatted active endod extract decreases when butter or cholesterol is added to it while in aqueous solution. Following the path of Dr. Parkhurst the antidote of active ingredient in endod is determined in collaboration with Professor Jovanovich using different additives.

Table 1. Antitode determination of endod

Concentration of crude endod in ppm	Concent. of additives in ppm		Percentage of dead snails after 24 hours	Percentage of dead snails after 48 hours
25	0	Control	100	100
10	0	(water)	100	100
8	0		100	100
6	0		0	0
0	100	Soap	0	0
75	75	(bar)	100	100
50	50		60	100
25	25		0	0

Table 1 (cont.)

Conc. of endod ppm	Conc. of additives ppm		Percent dead snails - 24 hrs	Perc. dead snails - 48 hrs
0	100	Soap	0	0
50	50	("Rol")	100	100
25	25		60	60
20	20		0	0
0	1000	NaOH	0	0
250	250		100	100
125	125		67	67
100	100		0	0
0	1000	NaOH + soap	0	0
250	250		100	100
125	125		67	67
100	100		0	0
0	100	NaHc03	0	0
50	50		100	100
25	25		0	0
0	100	NaCl	0	0
50	50		100	100
25	25		0	0

Adding sodium ions (Na+) to the glycoside decreases the potency of crude endod by 6-15 times. As it is seen from the above data, deactivation of the active principle in endod will occur when sodium ions (Na+) interact with the carboxylic ions (C) of the glycoside. So endod after saponification is no more potent to snails.

However, ways of getting an active extract of endod berries through defattation using the above mentioned solvents are very expensive for a country like Ethiopia. Fortunately we discovered another very cheap, practical and easily handled method in our laboratory. Endod berries are soaked in water and left to stand for a few days. It ferments rapidly. This solution can be easily separated and dried with a spray dried (120°-140°C) or using solar energy in the incubator. Such extracts have shown to have molluscicidal potencies (2 ppm) which are better than the butanol extract (4 ppm). The fermented endod is again extracted using chloroform. The chloroform extract kills 100% of the snails at 1.5 ppm concentration.

Using sephadex reagents the foaming part of endod is separated from that of the non-foaming one and it is proved that the foaming part is not toxic to snails. At this time quantitative experiments of collecting the foaming and non-foaming (toxic) part is going on. As soon as sufficient amount of these two parts are collected the sample will be sent to Dr. Parkhurst for further molecular investigation. Every cycle of water extract of endod (from freshly-fermented and up to loss of potency) is passed through the sephadex reagent. Before the fermentation takes place a lot of peak having different sizes (with only one exceptionally high) are observed. But after the fermentation is complete all peaks disappear except one. After the endod extract loses its potency because of further fermentation no peak is observed at all.

3. Development of Chemical Assay

A chemical assay which involves the use of organic dye-stuffs and their interaction with lemmatoxin (the active snail killing ingredient in endod) has been developed in our institute. Measurement of resulting color changes was determined by adsorptiometer and it correlated well with the concentration of the active ingredient in endod as determined by snail kill.

This process is now being repeated using a more sophisticated and more accurate apparatus (Beckman, Model 25 spectrophotometer). Some complexion and dye-stuff gave a reproducible results so far. The standardization of this chemical assay is nearing completion both in the laboratory and in the field in Tensae Berhan.

4. Industrial use of endod

In collaboration with the Indo-Ethiopian textile factory some experiments are being carried on. The determination of surfactant, wetting and soaping properties of crude and extract of endod is continuing. So far this factory is using very expensive imported surfactants, wetting and soaping agents from West Germany. As far as wetting surfactant properties are concerned, warmed (40-60°C) and decolorized endod extract mixed with charcoal $CaSO_4$ or ash seems comparable with that of imported surfactant and wetting agents.

5. Detergent property of endod

So far the work on the detergent property of endod is delayed. This is because our institute is not in a position to buy the expensive detergeometer. We are working with locally available materials. In cooperation with the National Soap Corporation a proposal in the study of the detergency properties of indigenous soap plants for the combined use as a soap and molluscicidal product was drafted. The corporation promised to start the work with making detergent bars of endod using its Asmara detergent bar factory. As of yet no practical work has been done.

6. Further studies on chemical assay

The chemical assay for the different active principles developed during the current investigation period (1976-77) will be further refined and standardized for routine use to monitor the application of different formulations of endod in the field. In this context we are working to develop endod for use as a practical and locally available molluscicide for the control of schistosomiasis on a community self-help basis in Tensae Berhan. An attempt will also be made to modify the chemical assay procedure with the view to making it suitable for determination of yields and potencies of different strains of the endod plant grown under different conditions.

REFERENCE

Lemma, A., Brody, G., Newell, G.W., Parkhurst, R.M. and Skinner, W.A. (1972). Studies on the molluscicidal properties of endod (Phytolacca dodecandra). I. Increased potency with butanol extraction. J. Paras. 58, 104.

Unpublished Research Note No. 7

PHYTOLACCA DODECANDRA (ENDOD) AS A MEANS TO CONTROL SCHISTOSOMIASIS TRANSMITTING SNAILS

Charles B. Lugt (Ph.D.), Institute of Pathobiology, January 1978

INTRODUCTION

With reference to my lecture on June 9, 1977, during which I offered the Institute of Pathobiology a Progress Report, I should like to present you now the results of the work done in the past year, with respect to selection and breeding for high berry production, high molluscicidal potency of the berries, and pest-resistance of Phytolacca dodecandra.

Successively I will review the research in regard to breeding and selection, pest resistance (stem borers), phytochemical investigation (haemolysis, chromatography, extraction of glycosides), and quantitative output figures for endod breeding on a large scale.

BREEDING AND SELECTION

This part of the work is, as far as selection is concerned, closely related with our pest-resistance investigations (see Chapter 2).

Breeding

The breeding part of the work as a whole has not been successful. Being a dioecious plant, P. dodecandra has to be propagated by means of cuttings. Slightly wooden parts of branches, 20 cm long, were taken, treated with b-indolic-acid (rooting powder), and put into the soil at an angle of 45°, leaving 5 cm of the cutting uncovered with soil. The emergence of the shoots seems to be fully dependent on the type and part of the plant that is taken. Cuttings from certain plants gave an emergence of 80%, while other gave none. It is not fully clear to what circumstances this could be described. One important point, however, is that the type of soil is of utmost importance.

Soil types like those near the Institute and in Sebeta are most unfavorable and those in Melkassa and Metahara do a lot better. At the end of last year an experiment was started with cuttings from green branches with leaves. After treatment with rooting powder, they were put into a mixture of forest soil and sand (1:1) and kept for 40 days under humid conditions. So far the results give hope for the future.

Selection

Apart from the pest-resistance selection aspects, in the past year, samples have been taken from a total of 22 different female endod plants. Investigations on their molluscicidal property have given positive results in the sense that some plants (berries) were found to possess very high molluscicidal potency of 5 ppm (provided they were harvested at the right stage, see Chapter 3).

Taking into consideration that this result has been obtained without any special selection, we must say that there is quite a possibility to go down well below this figure of 5 ppm.

PEST-RESISTANCE (STEM BORERS)

Together with Ato Teshome Gebre Michael a method has been developed to

investigate the possible existence of resistance within the species of P. dodecandra. It was observed that particularly the plants which possess felt-like or hairy stem tops, were less susceptible for an attack of Gitona spp., than the tops of plants which were smooth. On the other hand, we found that plants with smooth stem tops sometimes also possess a certain resistance. This resistance in general could be ascribed to inside causes, of which so far nothing became clear.

Selection for Pest-Resistance

Together with the work mentioned under Selection, plants which show great susceptibility to stem borers or other pests, like bacteria, were excluded from any further selection procedures. On the field in Sebeta, some plants (about 7) so far show a certain resistance. We are trying to multiply these plants by cuttings and are beginning to test them for their molluscicidal potency.

CHEMISTRY OF P. DODECANDRA GLYCOSIDES

It is well known that not all so-called saponins possess foaming capacity. As the concept of saponins comprises those compounds which have foaming capacity, it is therefore wise to speak of glycosides, which comprise foaming and non-foaming components of the biological active mixture.

Determination of the Best Harvesting Stage

Consequently, from five different stages of development of thirteen female P. dodecandra plants, racemes were taken at their bud, bud/flower, flower/fruit, unripe fruit, ripe fruit, overripe fruit stages. From these samples the haemolytic capacity, the molluscicidal potency and the percentage of dry weight was determined. Table I shows the average results of the 13 plants, as well as the ratio of dry weight and molluscicidal potency, thus giving the most suitable harvest stage for the racemes.

TABLE I: Haemolysis, molluscicidal potency, dry weight and calculation of the most appropriate harvest stage of the racemes.

	Haemolysis (1/x)	Molluscicidal*	Dry Weight (%)	Ratio
Bud	54	118	14.7	0.125
Bud/flower	77	108	14.3	0.131
Unripe fruit	352	16	22.1	1.381
Ripe fruit	270	43	27.3	0.640
Overripe fruit	30	113	30.3	0.268

*ppm giving 100% mortality. The values higher than 100 ppm are estimated because of their non-100% killing results.

It is shown that the most suitable harvest moment is the unripe stage. The ratio between dry weight and molluscicidal potency is then 1.381. We can also see that, during the development of the raceme from flower to fruit, the haemolytic and the molluscicidal capacity go parallel with each other, and that the development of the various components of the glycoside mixture from the unripe stage, is in favor of the haemolytic part of this mixture (regression curve haem. = $f(x) = Y = 1/x. 100 = 11.04 + 0.152x$; regression curve Moll. = $101.8 - 1.196x$).

Chromatography and Isolation

By means of thin-layer chromatography, 8 different components derived from a biological active powder could be determined. Separation, over a period of 7 hours, of the glycosides took place with a mixture of butanol and acetic anhydride (5:1), which was saturated with water. The 5 main components became visible after spraying with a mixture of acetic anhydride (5 ml), sulphuric acid (5 ml), and ethanol abs. (50 ml), of which the one with the lowest Rf value colored brown and the others from light to dark purple.

After isolation of the 8 components by means of elution with a mixture of chloroform emethenol (1:1), they all showed a clear but different pattern in snail killing potency. It varied between slightly and highly molluscicidal. For further selection on valuable P. dodecandra types, research should go also into this phytochemical direction.

QUANTITATIVE ASPECTS

During the past year, investigations have been undertaken, also with respect to the size of the endod bush, the quantity of racemes per bush, the berries per raceme, and the dry weight of each raceme. By this means it was possible to estimate the quantitative output per hectare. Table 2 shows the results from two different types of endod bushes--small and large bushes.

TABLE II: Estimation of P. dodecandra berry production per hectare:

	SMALL (n = 13)	LARGE (n = 9)
Racemes/bush	50 - 80	120 - 250
Berry weight/raceme	2.5 - 6 gm	1.5 - 3 gm
Planting distance	1 x 2 m	2 x 2.5 m
Plants/ha	5000	2000
Output/ha/year	625 - 2400 kg	360 - 150 kg

We see that small bushes have a more favorable output than big bushes. Besides this, for practical reasons it is advisable to breed small bushes, because of maintenance, insect treatment, and harvesting.

Final remarks

In order to show the possibility of a practical implementation of growing P. dodecandra (endod) for treatment of the water against schistosomiasis-transmitting snails, an example is given, based on the data presented in this report. Starting from the small bushes, we will calculate how many hectares of endod are needed to kill snails in an area of 1200 ha of sugar cane, for which 100,000 tons of irrigation water are required per year. Supposing that the water is treated twice a year and the endod berries have a molluscicidal potency of 15 ppm, we need 3000 kg of berries per year. At the present stage of endod-research, without any selection, for 1200 ha of sugar cane, we see that an average output of 1500 kg of berries can be obtained from an area of 2 ha of endod. If after selection to a more uniform population of plants, which procudes a quantity of berries lying near to the upper limit of 2400 kg, and after selection to types with a molluscicidal potency of 5 ppm (we already found plants with this figure), for 1200 ha of sugar cane an area of about 0.5 ha only is necessary to treat irrigation water twice a year.

SCHISTOSOMIASIS IN ADWA
A REPORT ON
AN ECOLOGICAL PILOT STUDY

Aklilu Lemma

Schistosomiasis is known to occur in undetermined degrees of prevalence in various localities in Ethiopia. The first few case reports on the occurrence of the disease in the country were written by Italian physicians during the second world war. In 1956 Ayad made the first schistosomiasis survey and reported that the disease was endemic in the Lake Tana region, in Harar and in Eritrea. The 1959 Ethiopian Nutrition survey team also reported a high incidence of *S. Mansoni* in the same areas. Some studies made in Gorgora (on the northern shore of Lake Tana) by Chang (1961) have shown that 22.8% of 202 schoolchildren, 5% of 100 military personnel and 1% of 342 policlinic patients were positive for *S. Mansoni*. Kubasta (1964) has recently shown that this disease is particularly endemic and has a high degree of prevalence (70% in 152 schoolboys) in the town of Harar. In all of these studies only *S. Mansoni* was encountered. The only *S. haematobium* infection reported was by Dr. H. Russel (1958) who found 48% of 189 urine specimens examined at Gawani in the Awash Valley positive for urinary bilharziasis.

Recently, provincial hospital records sent to the Ministry of Public Health, showed that some hospitals in Tigre Province, especially in Adwa and Makale areas, were reporting about 100 cases of schistosomiasis a week. This alarming news was brought to the attention of the Ministry of Public Health officials by Dr. H. Russell the former W.H.O. representative, and in immediate response to it, H.E. Ato Yohannes Tseghé, Vice-Minister of Public Health, requested Dr. Alfred Buck of the Johns Hopkins University, Dr. D. Spruyt, Miss M. Wade and Ato Asrat Deressa of the Demonstration and Evaluation Team of the A.I.D. project, and myself from the Medical Faculty of the University, to go down to these areas and make the necessary surveys to check if these hospital records were correct or not. Accordingly, Mr.Elder of the Demonstration and Evaluation Team was sent out to make a pre-survey tour and select an area of study for the team. After considering several localities where schistosomiasis is known to be endemic in Tigre Province, the town of Adwa was chosen as the most appropriate, from the logistic and endemic point of view, for the proposed study.

The team left Addis Ababa for Adwa on July 13th, 1964 and spent there a period of five days doing two kinds of studies.

1. *Prevalence rate determination* — Dr. Buck, Dr. Spruyt, Miss Wade and Ato Asrat determined the prevalence of the infection by skin testing 802 people and examining stool specimens (using Merthiolate-Iodine-Formaline fixative and ether concentration technique) from 459 persons of different age, sex and residential area. Tne results of their tests showed that 80% of the people

Reprinted from the Ethiopian Medical Journal, 3, 84-92, by permission.

were positive for the skin test and 60% of the stool specimens were positive for *S. mansoni* ova. The detailed studies on these tests are reported in a separate paper in this issue of the Journal.

2. *An ecological study* — I undertook the study of the ecological factors which enable the dissemination of the disease in the town of Adwa and its vicinity. This was especially studied with a view to applying control measures to reduce the infection rate in the town.

The approach used in the ecological study was a straightforward one involving a complete survey of the only two perennial rivers in the area. The survey consisted of (i) collection of snails and determination of whether they were naturally infected or not. (ii) notation of frequent human or other animal faecal deposition sites in or near the rivers. (iii) observations on the bath taking, clothes washing, drinking water collection, and other sites of human and animal contact areas along the two rivers.

The present paper deals with the findings of these studies and includes recommendations on how to control the disease in this particular town.

THE TOWN OF ADWA.

Adwa is a small town of about 10,000 to 15,000 inhabitants. It is located 984 kilometers north of Addis Ababa and 156 kilometers south of Asmara at an altitude of 2,230 metres (7,300 ft.) above sea level. The highways connecting the town with both Addis Ababa and Asmara are excellent.

As is seen on the map (Fig. 1) the houses in the town are built in clusters over a rather small area. About five kilometers out of the town there is the small village of Adi-Abuna where the only hospital in the vicinity is located. The majority of the people in the town are farmers, but there are a good number of clergymen, merchants, businessmen and soldiers. An elementary school and a junior high school, with a total of about 3,000 students are located in the middle of the town. There is a small clinic (dresser station) near the secondary school, a spacious market area above and across the river from the school, and a big army camp just outside the town.

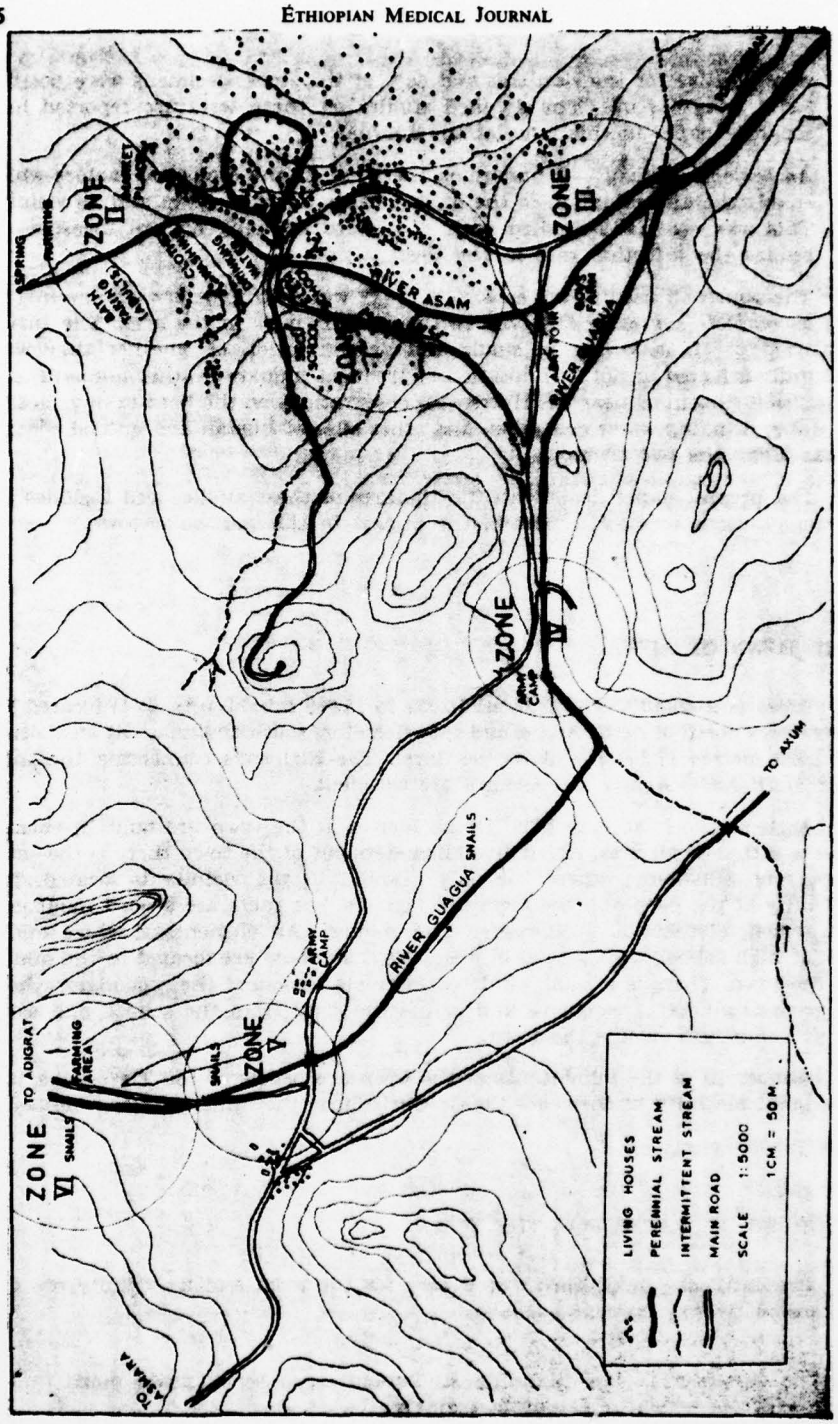
Almost all of the inhabitants of the town are people of the Tigre tribe, and the great majority of them are Coptic Christians (the minority being Moslems).

SOURCES OF WATER FOR THE TOWN.

Presently, the only sources of water* for the town and its vicinity are two perennial rivers: Asam and Guagua.

---

* The Governor is now planning on having clean water piped down to the town from a nearby mountain spring.


ZONE II
ZONE III
ZONE IV
ZONE V
ZONE VI
SNAILS
SPRING
BATH
WASHING
LAUNDRY
SCHOOL
RIVER ASAM
RIVER MAINA
ARMY CAMP
ARMY CAMP
FARMING AREA
SNAILS
ARMY CAMP
RIVER GUAGUA SNAILS
TO ADIGRAT
TO AXUM
TO ASMARA
LIVING HOUSES
PERENNIAL STREAM
INTERMITTENT STREAM
MAIN ROAD
SCALE 1:5000
1CM = 50M.

Asam River

Asam is a small river which starts in a spring about one kilometer above the market place and runs right across the town and joins Guagua River near the last bridge on the road to Tembien. The whole length of this river is about three to four kilometers. Practically everybody in the town uses this water for drinking, taking baths and washing clothes. There are only two bridges crossing this river, so the majority of the people in the town cross it by wading through it at various points along its course of flow. Animals also frequently utilize and come into contact with the river at various places along its flow.

Guagua River

Guagua also starts from a spring, but it comes from a much longer distance beyond the mountains. It is larger and faster than the Asam River. There is comparatively little human contact but much domestic animal contact with this river.

SNAIL SURVEYS AND NOTES ON HUMAN DEFAECATION AREAS.

Both Asam and Guagua rivers were surveyed for snails. The technique used in the survey was a five minute catch by 50 helpers who were getting instructions directly from me. The snails were looked for under stones or on the leaves of small bushes on the banks of the river. No quantitative determination of the number of snails per square meter of the area, or any other equivalent measurement, was done.

The snails collected from different regions of the river were separately put in special jars and carried to the hospital laboratory in Adi Abuna where they were exposed to sunlight or artificial light (microscope lamp) for about ten minutes and the water they were kept in was microscopically examined to determine whether there were any cercariae present or not. At the end of the survey period all the collected snails were kept alive and carried to Asmara where some of them were dissected and examined for the presence or absence of developing cercariae in their internal organs. Since all the snails were not dissected, their per cent positive rate could not be given, but in general, the great majority of the Biomphalaria species were found positive. Speciments of these species have now been sent to Dr. D.M. Blair of the Research Laboratory in Salisbury, Rhodesia, for identification.

Since the cercariae of the different human and domestic animal schistosomes are morphologically indistinguishable, an attempt was made to infect mice with these cercariae with the aim of getting the mature worms from these mice and specifically identify the species of schistosomes involved. Unfortunately, the infected mice died within a few days of infection, due to an unknown cause, and we were not able to recover any adult worms to make a conclusive diagnosis.

Along with the survey of snails, special notes were also taken whenever fresh human or other animal faecal deposits were encountered. The people

generally defaecate in or near the rivers, but sometimes their favourite areas have been observed to be open fields just above the streams. Several times during the survey, I have observed the rain washing faecal material from such areas down to the streams.

SURVEY OF ASAM RIVER.

The survey on Asam River started at the spring where it originates. The area around the spring is farm land and the stream flows through a heavily eroded gorge. Animals (cattle, goats, sheep and donkeys) frequently utilize the upper part of the stream. Several kinds of snails of which Biomphalaria and Bolinus species were the most predominant, were collected from this area, but none of these snails were observed shedding cercariae under examination in the laboratory.

About one kilometer down the stream from the spring, just outside the town and above the market place, is an area of epidemiological importance. As a traditional habit of the people in the vicinity, the male adults take their bath in a secluded area just outside the town. Sometimes these people defaecate and urinate in the nearby bushes before taking their bath. During the present survey, lots of snails were found in this area. Some of the Biomphalaria species collected were seen discharging active cercariae when observed in the laboratory. A few yards down stream in the same vicinity, people from the market place frequently come down to drink water, defaecate, urinate or wash themselves or their clothes. This area (Zone II on the map) is probably the second highest endemic foci for the infection.

The area behind the junior high school and elementary school unit in Zone I on the map, is probably the focus from which the majority of the people get their infections. The two schools have a combined number of about three thousand students and yet there are no latrine facilities built for them. The only latrine serving both compounds is a small one built for the use of the 37 teachers in the two schools. The children generally defaecate on the stones in the stream behind the elementary school or on the farm just above the stream. Large numbers of snails (mostly Biomphalaria species) were collected from under the stones where faeces have been deposited. Examination of these snails has revealed that the majority were discharging cercariae. The teachers in the school have informed me that the school children routinely wash, swim and play freely in this part of the river. Just a few yards below the defaecation area and play ground of the children, adults in the neighbouring area wash their clothes and drink from the same water. This area is no doubt the highest endemic focus for human infection in the whole town.

Zone III, the area next to the most important Zone I, has also some ecologically significant points. There is an abattoir near the river below the bridge on the road to Tambien. Although this place is not frequently used for slaughtering, however, when an animal is slaughtered there, all the stomach and intestinal contents and the blood are washed in the river. A few yards below this area, the Governor has a farm which he irrigates with the water from the river. Abundant Biomphalaria snails were collected from both the slaughter place and

the irrigation canals. Some human faeces have also been observed in this area. From this study of the environment and human contacts with the river, the Zone III area may be the third and least important endemic focus on Asam River.

SURVEY OF GUAGUA RIVER

Since the spring where this river originates is some distance from the town of Adwa, the present survey on Guagua River started from an area near Mount Adi-Wali on the road to Adigrat. The total length of the river surveyed, starting from the foot of the mountain to the point where the two rivers meet, was about ten kilometers.

In general, because of the concentration of the houses in the town along Asam River as shown on the map, there is comparatively little human contact with Guagua River. The early parts of the survey area, Zone VI on the map, is primarily farm land with a lot of domestic animals. Cattle, sheep, goats and donkeys were seen freely wading through, drinking from, urinating and dropping their faeces into the river. Some of the Biomphalaria species of snails collected from this area were shedding cercariae when examined in the laboratory. These cercariae were very similar to those of *S. mansoni* cercariae, however, since the cercariae of human and domestic animal schistosomes are morphologically indistinguishable, the observed cercariae may very well have been those of *Schistosoma bovis* or any other animal schistosome.

Microscopic examination of some droppings collected from three different cattle in this area have shown some schistosome ova resembling those of *S. bovis* (terminal spine). Also, a post-mortem examination of a cow which was said to have been raised in this area and was slaughtered for consumption by the people in the town of Adwa, has revealed that some of the small veins of the mesentery were loaded with mature and live *S. bovis*. In an attempt to determine the prevalence rate of schistosome infection in the animals of this region, about 100 animal droppings were collected and preserved in Merthiolate-Iodine fixative. These preserved specimens were sent to Captain F. Eliot, Chief Veterinarian of the Kagnew Station in Asmara, for proper diagnosis using the concentration technique. The results of this test are not yet available, but they will be made available on request to any interested party in the future.

Considering the limited possibilities of human contact with this river, Guagua seems to be by far less important for the transmission of human schistosomiasis than Asam River. The two possible endemic foci for human schistosomiasis on this river are the areas covered by Zones IV and V on the map. Although abundant snails were collected from other parts of the river basin as well, since these other areas are very rarely utilized by human beings, their epidemiological significance has been disregarded for the purposes of the present study.

Zone V is the area on the intersection of the roads coming from Asmara, Adigrat, Axum and Adwa. There are a few private houses and a small army camp in the vicinity. The people utilize the water of the river for washing clothes, taking baths and for drinking as well. Some fresh human faeces have

been seen deposited by the river. Abundant amounts of Biomphalaria species of snails were also collected from this area.

Zone IV is the area just outside the more heavily populated part of Adwa. There is a relatively big army camp and much washing, bathing and other activities utilizing the river take place in this area. The Mayor of the town told us that his administration had at one time advised the people of Adwa to avoid washing clothes in Asam River (because of the risk of getting schistosomiasis), and instead to use Guagua River. As a result of this advice, a lot of people come from other parts of Adwa to wash their clothes there. However, probably due to the high concentration of soap and "Endod" (the local herb used for washing clothes), there were comparatively few snails collected from this area during our survey.

CONCLUSIONS

In conclusion, from the above studies it seems that Asam River is the primary focus for the dissemination of human schistosomiasis (*S. mansoni*) and Guagua River may play a big role in the dissemination of animal schistosomiasis (*S. bovis* and possibly other species as well) in the town of Adwa and its vicinity.

It should especially be noted that the occurrence of animal schistosomiasis in this area may very well be affecting the transmission and even the diagnosis of the human schistosomiasis. For example, in Dr. Buck's study he found 80% of the 802 people positive for skin test whereas only 60% were positive in the stool examination using the highly sensitive M.I.F.C. technique. It is a well known fact that skin testing using adult *S. mansoni* antigen does cross react with other schistosoma species, therefore the large number of positive skin test reactions may possibly, at least in part, be explained as being due to a continual exposure of the people in the area to animal schistosomes. The epidemiological significance of this should be considered in future studies.

RECOMMENDATIONS ON POSSIBLE CONTROL MEASURES.

At the end of our studies H. H. Luel Dej. Menguesha Seyoum, the Governor-General of Tigre Province, asked us to prepare him a report on our findings and include specific recommendations on how to control the disease in this particular town. Thus, the recommendations* included in this text are the summaries of those reported to him as most feasible and applicable for the conditions in Adwa.

From a control programme point of view, the fact that Asam River is the major source of human infection responsible for the high prevalence of the disease in the town of Adwa, and that it starts in a spring just above the market place and extends down for only less than 4 kilometers, makes it very attractive to attempt control measures. In fact, it is believed that this may make an ideal situation to demonstrate a control programme of a communicable disease in a community of more than 10,000 people where 60 - 80% of them are known to be infected.

Any schistosomiasis control programme should involve three things. The first and most important one is *health education*, the second in *mass treatment*, and the third is the long and elagorate process of *eradication of snails*.

In the case of Adwa, it was thought that the most practical first step to take in the campaign against this disease was to establish a sub-health centre unit in that town. Such a sub-health centre should be headed by a research oriented health officer. The purpose of having this person would be: (i) To make him responsible for the general health education of the people, compaigning not only against schistosomiasis but also against all other similar communicable diseases. (ii) To make him be responsible for the building of adequate latrines in several localities (particularly in school compounds), and for finding sources of clean water for the people. (iii) To train him to conduct proper stool examinations and snail collecting for shipping to a central laboratory in Addis Ababa for identification.

The other important person needed for the project is a mallacologist to study the ecology, behaviour, comparative density and seasonal fluctuations in the number of the various species of snails present in the river. Such a mallacologist would also be of much use in determining the volume of water in the river, its acidity, salinity, mud content and other essential information needed in making the right choice of chemicals to kill the snails.

While health education is being carried on by the health officer, ecological studies by a mallacologist, and parasitological studies by a parasitologist, a mass treatment campaign can be started by a medical man.

The application of chemicals to kill the snails should be the last step in the project. A follow-up study by making a yearly parasitological and snail survey should then continue for several years.

SUMMARY

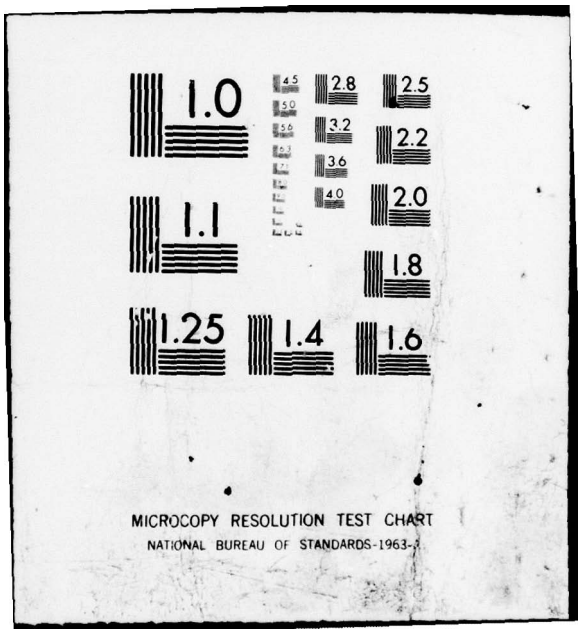
An ecological pilot study of schistosomiasis was conducted in the town of Adwa. During the survey of the only two perennal rivers (Asam and Guagua), the following studies were carried out:

1. Different species of snails were collected from various regions and out of these some of the *Biomphilaria* species were found infected and discharging active cercariae.
2. Observations on bathing, clothes washing, drinking water collection faecal deposition and other sites of human and animal contact areas along the two rivers, were made.

From the results of these studies it was concluded that Asam River is the primary focus for the dissemination of human schistosomiasis (*S. mansoni*) and that Guagua River may play a big role in the dissemination of animal schistosomiasis (*S. bovis* and possibly other species) in the environment. As a first step to control the disease in Adwa it was recommended that a sub-health centre, primarily to be responsible for giving health education to the people, be established. It was further recommended that a mass treatment campaign should follow and the application of chemicals to kill the snails be the last step to be done.

AD-A080 030 CALIFORNIA UNIV SAN FRANCISCO F/G 6/5
STUDIES ON MOLLUSCICIDAL AND OTHER PROPERTIES OF THE ENDOD PLAN--ETC(U)
JUN 79 A LEMMA, D HEYNEMAN, H KLOOS N00014-76-C-0218
UNCLASSIFIED NL

4 OF 6
AD
A080030


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-

RESUME

Une étude écologique et de santé publique sur la Bilharziose a été entreprise dans la ville d'Adua.

Durant l'enquête qui a porté sur les deux divières d'ASAM et GUAGUA les recherches suivantes furent entreprises:

1) Différentes espèces de mollusques furent recueillis dans des régions variées et parmi ceux-ci quelques uns de l'espèce Bromphilaria furent trouvés infectés de cercaires actifs.

2) Des observations furent faites sur les Baignades, le lavage du linge, les dépôts de matières fécales et sur les lieux de contact entre les humains et les animaux.

La conclusion des résultats de cette étude a mis en évidence que la rivière Rsam est le foyer de dissemination de la Bilharziose le plus important, et que la rivifire Guagua joue probablement un rôle important dans la transmission de la Bilharziose animale.

Comme première mesure de contrôle à instituer à Adua il est recommandé qu'un sous-centre soit établi pour assumer en premier lieu l'éducation sanitaire de la population.

En second lieu, une campagne de traitement en masse devra être entreprise suivie comme dernière mesure de l'emploi de produits de destruction pour faire disparaitre les coquillages.

REFERENCES

1. Ayad, N. Bilharziasis survey in British Somaliland, Eritrea, Ethiopia, Somaliland, the Sudan and Yemen. Bull. Wld. Org., 1956, 14, 1.
2. Chang, W. P. Report on epidemiological study of bilharziasis in Gorgora, north shore of Lake Tana, Ethiopia. Gondar Health Series. 1961, 1, 5.
3. Ethiopian Nutrition Survey, 1958. Interdepartmental Committee on Nutrition for National Defense, U.S.A., 1959.
4. Kubasta, M. Schistosomiasis Mansoni in the Harar Province. Eth. Med. J 1964. II, 260.
5. Russell H. B. L. Final Report. Pilot Mabile Health Team, Ethiopia W.H.O. document EM/PHA†62. Ethiopia 13, 1958.

Dr. Akilu Lemma
Haile Sellassie I University.
Box 1176, Addis Ababa.

Journal of Parasitology, vol. 56 (1970), 438-439

794. The use of Endod in the control of schistosomiasis in Adwa, Ethiopia.

AKLILU LEMMA and JOHN DUNCAN, Institute of Pathobiology, Haile Selassie I University, Addis Ababa, Ethiopia.

Adwa is a town in the province of Tigre in the northern part of Ethiopia. It has a population of about 17,000 and from previous investigations (Aklilu Lemma, 1965), it is known that approximately 60% of the people are infected with *Schistosoma mansoni*. For this reason and also since the town is relatively isolated and the people are very well enlightened, it was thought to be an ideal place to attempt a pilot project for the control of schistosomiasis using Endod (*Phytolacca dodecandra*), a plant which grows abundantly in and around the Adwa area, as a molluscicide (Aklilu Lemma, 1965 and 1970). Accordingly, a programme to control the snail population in Adwa over a five year period and to measure the impact of this on the incidence of the disease in the human population was drawn out and started in April 1969.

Adwa lies near a junction of two perennial streams, the Gua-gua and Assam. These rivers support sizable populations of different species of snails; *Biomphalaria pfeiferi* predominates everywhere. Initial reconnaissance revealed that human contact with the river was very frequent for such purposes as taking water, laundering, washing, recreation, defaecation and watering animals. In some places along the Assam river up to 60% of the *B. pfeiferi* were found naturally infected with *S. mansoni* cercariae.

In order to establish a base-line datum before the application of the molluscicide, the prevalence rate of the disease in 10% of randomly picked inhabitants was determined. This was achieved with the use of aerial photographs on which every house in Adwa was numbered and with random picked lots, houses were traced and stool specimens collected. About 2,000 stool specimens were examined with the formol ether concentration technique. About 70% of the total population had S. *mansoni* and 50% of children between the ages of 1 to 5 were infected. With a good snail control measure over a five year period, it is expected that the disease in this age group, would be considerably reduced, if not completely eliminated.

Highly concentrated solution of Endod was prepared by pouring water over the powdered berries and this was poured along the shores of the river with the use of buckets, or made to flow at constant rate from barrels put above the river. An attempt is also being made to compare the efficacy and cost of Frescon and Baylucide with that of Endod in comparable rivers in the Adwa area.

In addition to the use of Endod as a molluscicide, one of the main objectives of the pilot control project was to have community participation in the control programme. For this, the Governor-General of the Tigre Province, and the Mayor and the Municipality Council of the town, were consulted at an early stage and made to participate in the planning and execution of the project. Because of their strong support, thousands of people came out to help during the application of the molluscicide and to listen to the general health education talks given on the disease. The Municipality Council of Adwa assigned an adequate budget for the control project. The project is progressing well.

82

Proc. Afro-Asian Symp. Chemother. Schist. pp. 665-677 (1971)

Progress Report on Schistosomiasis (Bilharzia) Control in a Northern Ethiopian Community

M.B. FLEMINGS and P.H. GOLL

Institute of Pathobiology, Haile Selassie I University, Addis Ababa, Ethiopia

A five-year pilot project for the control of schistosomiasis in Adwa, Ethiopia commenced March 1969.

Interruption of the life-cycle of *Schistosoma mansoni* is being accomplished by control of the only snail intermediate host *Biomphalaria pfeifferi* via Endod (*Phytolacca dodecandra*). and two other reference chemicals. Other means utilized are prevention of water pollution by human population, public health education, and to a very small degree, definitive treatment of human patients.

At the half-way period, a human "stool" survey from entire population reveals a decrease in the disease *prevalence* from 63.1 to 15.7%. Among ages 1-6 and 1-3 respectively, the disease *incidence* decreased from 50.1 to 2.3%. No significant changes exist in the nearby reference control area.

At the first annual meeting of the Ethiopian Medical Association (May 1965), Dr. Aklilu Lemma reported bilharzia as an emerging problem of Ethiopia in the context of an expanding one in Africa as a whole and stressed that the effects of the disease on the human population were insidious, the initial infection passing unnoticed until clinical symptoms appear often only some years later.

In 1968, Lemma and Duncan reported an integrated epidemiological, malacological, parasitological and molluscicidal study of schistosomiasis in Ethiopia. As a result of this study, a five-year pilot project for the control of intestinal schistosomiasis in Adwa, Ethiopia, commenced in March 1969 under the direction of Dr. Aklilu Lemma and Dr. John Duncan, Institute of Pathobiology, Haile Selassie I University. The project is continued in 1970 under the direction of Dr. M.B. Flemings and Mr. P.H. Goll, Institute of Pathobiology.

Adwa is a northern Ethiopian town of 12,000 to 15,000 inhabitants and is located 6,630 feet (1,989 m) above sea level, it is easily accessible throughout the year by surface transportation. Two main rivers, Assem and Guagua, pass through

it, and three tributaries which flow into the Guagua, Enta, Shanna and Edaga, are within the outlying areas but included in the scope of this project, (see map of Adwa, Fig. 1). The river Assem is 4 kilometres long and runs through the centre of the town, while the river Guagua is much longer originating in the mountains to the north of the town. The rivers join to the west of Adwa and drain away to the south. According to studies of Akliku Lemma (1965), these bodies of water are the only possible sources of schistosomiasis infection in the Adwa area, Rivers Guagua and Assem are utilized daily by the population for bathing, washing and drinking, but no major agricultural irrigation project is present.

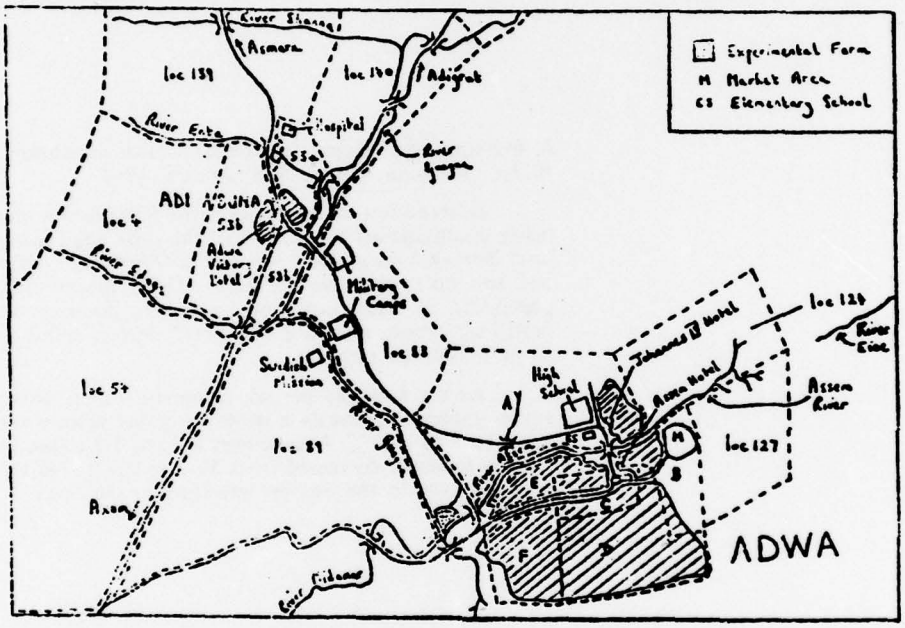


Figure 1 : Sketch map of Adwa and surrounding districts.

Three snail species are prevalent in the Adwa area : *Biomphalaria pfeifferi*, the only intermediate host of *Schistosoma mansoni* ; *Bullinus truncatus*, the intermediate host to *S. bovis* ; and *Lymnaea natalensis*, the intermediate host to *Fasciola gigantica*. *Schistosoma haematobium*, although prevalent in certain parts of Ethiopia (Lo, 1971), is not present in the Adwa area, perhaps due to altitude and/or lack of an efficient intermediate host.

Both intestinal and urinary schistosomiasis are known to occur in undetermined degrees of prevalence in various localities of Ethiopia as Ayad (1956) ; the Ethiopian Nutrition Survey Team (1958) ; Chang (1961) ; Kubasta (1964) ; Lemma (1964) ; and Buck (1965) have confirmed. Lemma and Buck both found intestinal schistosomiasis to be highly endemic in Tigre Province in which the town of Adwa lies.

In general, it appears that intestinal schistosomiasis is restricted to, or predominantly found in the highlands, whereas the urinary form is found in the warm and arid lowlands (Russell, 1958 and others). Brown (1964) in his study of fresh water gastropods in Ethiopia, showed that *Biomphalaria* and *Bulinus* species, the important intermediate hosts, are widely and abundantly distributed in areas that are very high above sea level. These genera are present in many of the lakes that are used as resort areas, such as Lake Tana, Alemaya and the Rift Valley lakes, as well as in areas presently being developed for agricultural purposes. A special situation may exist in the Lake Alemaya area, where according to current surveys (Lo, Aram, Goll, 1971 — personal communication) breeding of the *Biomphalaria* host probably does not occur in the lake itself, its presence there dependent on passive transfer from affluent streams.

The pilot control project at Adwa is important as it will be utilized as a "guide" for control of schistosomiasis in all of Ethiopia, as the disease, although relatively new in this country, is spreading to virgin areas especially those of river basin development and other agricultural schemes. Further, this control project is being utilized for field testing of endod (*Phytolacca dodecandra*) (also known as soap-berry) as a potential molluscicide. In 1965 and 1970, Aklilu Lemma reported on its molluscicide properties and laboratory field evaluations. Adwa was the first place where the molluscicidal activity of endod was observed.

Procedures

In order to assess the degree of success or failure of such a control scheme, certain preliminary data were required on the susceptibility of the intermediate snail host to different molluscicides ; the area, was aerially mapped (Fig. 1) ; buildings listed and numbered ; demographic and climate data were obtained ; and a reference area (Intitcho) selected.

Baseline data concerning snail population density and distribution were obtained by weekly surveys using a time restricted search method of sampling, before the start of the project, and then continued throughout the period under review (except for June to September each year during the main rains). Simultaneous determination of the infection rate of *Biomphalaria* was also made in order to delineate potential transmission sites.

Complementary to the information on the intermediate host was an initial prevalence and incidence survey of the disease among the human population. This was obtained by a 10% stratified random stool sampling programme with respect to age structure of the whole population, the analysis of the samples being by Ritchie's Formol-Ether Concentration technique, skin and microfluorescent antibody test (MFA). Questionnaire sheets were completed showing age, sex, religion, occupation, individual habits on disposal of faeces, places where clothes washing and bathing occurred, places for drinking water, etc., in order to gain some idea of epidemiological factors involved. This information definitely incriminated certain spots along the River Assem and Guagua as the foci of human *Schistosoma* infection.

Control

Disruption of the life-cycle of the parasite as a means of controlling the disease can be accomplished by (a) elimination of the intermediate snail host, (b) prevention or reduction of contact between human population and water sources, and (c) elimination of parasite by chemotherapy.

Snail Control, Physical and Chemical

Improved sanitation of the water in both rivers and their three tributaries with a view to making them as unsuitable as possible for snail life is being accomplished by removing excess debris and aquatic vegetation from the streams and improving their contour for increased water flow.

Because of the nature and extent of the habitats of snails and their prolific breeding potential, molluscicides are being utilized as emergency and temporary measures for interrupting the life cycle of *Schistosoma mansoni* as well as for control of other trematodes, by effectively reducing and/or maintaining the snail population at a minimum level. "Radius control" (treatment of all snail infected water streams within a 1300-3000 metre radius from the periphery of human settlements) was chosen because during a period of 5 years, the problem of re-infestation is not yet solved and because the application of molluscicides must be repetitive.

Initially, three molluscicides were chosen for snail control :

1. *Endod* (*Phytolacca dodecandra*) for treatment of Assem River. The preparation consisted of crushing ended berries to powder, dispersing in water and applying at first from 50 gallon drums by "dripping" for 8 hours, but this was ineffective so subsequently by "sprinkling" from buckets to margins of the streams at a concentration of 80 ppm. Three "treatments" of each river were administered during May, November and February according to the seasonal fluctuations of the snail populations. Due to the non-ovicidal nature of ended when applied at low and or practical concentrations, each "treatment" was divided into two applications at 2-4 weeks intervals, depending of course upon the hatching of eggs as revealed by post treatment survey. Between 500 and 800 kilos of ended were utilized for each treatment. The endod plant fruits only during January and July, therefore harvesting of the berries for adequate supplies must be planned and administered in advance.

2. *Frescon* (R)* (N-*tri*tylmorpholine), a synthetic chemical, supplied as a 16.5% EC, was initially applied to the Guagua river and its three tributaries Enta, Shanna and Edaga, from a specially constructed dispenser at a concentration of 0.025 ppm. Due to excess dilution of the chemical in "seepage" areas and the tendency of the chemical to become diluted as it diffused to river edges, the dispenser method of treatment was backed up by spraying the margins of streams using a "Hudson" malaria spray tank.

(*) Frescon (R) Registered Trade Mark - Shell Chemical Co.

Two applications per treatment were again necessary since it is also non-lethal to eggs, and due to the slow-flowing streams, it required 4-6 days for the chemical to cover the entire distance. Although some effects of the chemical could be seen in subsequent snail surveys, the concentration reached was insufficient by this method of dispensing and the use of Frescon was discontinued.

3. *Bayluscide* (R)* (niclosamide), which is ovicidal when applied at low concentrations was used for treatment of River Guagua and its three tributaries upon discontinuance of Frescon, and will be continued for the duration of the project. Bayluscide is applied from a Hudson sprayer the 70% wettable powder being dissolved in water at the rate of 100 g per 8 litres per 150 metres. Emphasis was placed upon the necessity of spraying the river banks or edges as well as thorough treatment of seepage areas.

Prevention of Pollution and Human Contact

A preliminary study of human contact with local water sources demonstrated that certain sites which were easily accessible along the rivers were frequented by human and animal populations for daily crossings, defaecation, washing, etc. Some of these "critical" areas were "fenced off" for prevention of such contact, while other critical areas were patrolled during the day, early mornings and night by hired guards. Towns people cleaned the streams of debris and cleared the river edges of excrement.

518 latrines consisting of 1 × 3 metre pits covered with wooden platforms and 4 large pits for refuse disposal, were constructed in heavily human populated areas adjacent to the rivers.

Health education films and public meetings involving schistosomiasis prevention were arranged and presented to the public at Elementary and High Schools.

Definitive Treatment of Infected Persons

Mass treatment of all diagnosed cases of bilharzia was an appealing thought for control of schistosomiasis in Adwa, but in actual practice, therapeutic measures are not practical in Ethiopia for a number of reasons. A very few individuals, however, did receive therapeutic treatment during this 2½ year period when Ambilhar (R)** was used.

Results

Prevalence and Incidence of S. mansoni

Two and three years after initiation of this pilot control programme, stool surveys were accomplished for determination of progress made.

---

* Bayluscide (R) Registered Trade Mark — Bayerfabriken A.G.

** Ambilhar (R) Registered Trade Mark — Ciba-Geigy A.G.

Table 1 shows results of the initial parasitological survey by which base-line data were obtained for Adwa (the treated area) and Intitcho (reference area) respectively. This 10% sample of the population in Adwa represented by 1695 people of both sexes and all ages, showed 63.1% of the total examined to be initially infected with *Schistosoma mansoni*. Other intestinal parasites ranged in frequency from 42.2% for *Ascaris*; 16.1% for *Entamoeba spp.* and 9.5% for *Trichuris trichuris*; while *Giardia sp., Strongyloides spp., Oxyuris sp., Ancylostoma sp., Fasciola sp., Hymenolepis sp.*, and *Taenia saginata* were also present in a small degree.

The survey revealed that the age prevalence of bilharzia was as follows:

50.4% among ages 1 thru 6; 83.8% among ages 7 thru 15; 73.0% among ages 16 thru 25; 45.7% among ages 26 thru 35; 34.3% among ages 36 thru 45; 44.5% among ages 46 thru 55; and 31.7% among ages 56 and up. These figures demonstrate the normal age/frequency pattern seen in endemic communities, the increase of the infection rate among ages 1-15 probably being associated with the increasing number of individuals being exposed each year to schistosome infested water (streams and rivers) while the "tailing off" of the infection rate among ages 16 and above may be attributed to acquired immunity and/or self limitation of the disease due to less frequent contact with schistosome infested water sources.

As a mean for assessing the results of our control efforts in Adwa, the village of Intitcho was selected as a reference area (one where no efforts would be made to control the disease). The stool survey of Intitcho which was made simultaneously with that of Adwa, revealed that 22% of the total population was infected with *S. mansoni* (Table 1). For reasons presently unexplained, the survey revealed no infections among ages 1 thru 6, in contrast to Adwa, and a lower infection rate among the total population, phenomena which may be attributed to the smaller size and greater distances of the rivers from the village. These factors perhaps limit the young and a majority of the total population from contact with the water supply.

Initially it had been hoped that determination of prevalence among 1-6 year old children would suffice to assess the effect of control measures, and so a survey was made at the end of two years. However the apparent fall in prevalence in this age group was so dramatic that confirmation was required and a further stool survey was made across the entire community after 3 years.

Results of these prevalence and "incidence" surveys are contained in Tables 2(a) and 2(b). The data show a significant drop in prevalence in the three years from 63.6 to 43.3%, while prevalence among children ("incidence") fell from 50.4 to 12-15%. The decline in this estimate varies between 15 and 40% there being no significant difference between the estimates for 1971 and 1972. However, there has been a mean reduction in the number of infected children in the order of 75%.

TABLE 1. Prevalence of some intestinal parasites recorded by stool examination grouped according to age (figures in parenthesis are percentage), in 1969

Adwa	Age Group Tested							Total
	1-6 yrs.	7-15	16-25	26-35	36-45	46-55	56-	
Total No.examined	401	659	252	173	105	72	63	1695
+ve for *Schistosoma*	202(50.4)	527(83.8)	184(73.0)	79(45.7)	36(34.3)	32(44.5)	20(31.7)	1080(63.1)
+ve for *Ascaris*	172(42.9)	270(42.8)	96(38.0)	85(49.1)	38(36.2)	33(45.8)	21(28.8)	715(42.2)
+ve for *Entamoeba*	56(14.0)	90(14.3)	45(17.9)	27(15.6)	22(21.0)	17(23.6)	15(20.6)	272(16.1)
+ve for *Trichuris*	40 (10)	59 (6.2)	22 (8.7)	31(17.9)	17(16.2)	7 (9.7)	6 (8.2)	162 (9.5)
Intichio								
Total No.examined	27	34	15	15	8	5	5	109
+ve for *Schistosoma*	—	12(35.3)	3(20)	6(40)	3(37.5)	—	—	24(22.0)
+ve for *Ascaris*	10(37.0)	11(32.3)	3(20)	3(20)	1(12.5)	1(20)	2(40)	31(28.4)
+ve for *Entamoeba*	1 (3.7)	3 (8.8)	4(26.7)	—	—	—	—	8(7.3)
+ve for *Trichuris*	1 (3.7)	1 (2.9)	1 (6.7)	—	—	—	—	3(1.8)

TABLE 2 (a). Comparison of the prevalence of *S. mansoni* recorded for individual locations within Adwa, 1969-1972

Location	1969			1972			% reduction of infected population
	No. examined	No. +ve	% +ve	No. examined	No. +ve	% +ve	
Zone A	169	112	67.0	131	60	45.8	31.3
Zone B	265	174	65.7	101	44	43.6	33.6
Zone C	264	167	63.3	116	56	48.3	23.7
Zone D	120	78	65.0	117	51	43.5	33.1
Zone E	147	104	70.8	—	—	—	—
Adi Abun	201	117	58.2	108	37	34.2	41.2
Military Camp	112	61	55.0	—	—	—	—
Total	1278	813	63.6	573	248	43.3	32.6

The data contained in the above table consists only of that which is strictly comparable between the surveys.

$X^2 = 8.29$, with $P = <0.01$ indicating that the fall in prevalence was significant.

TABLE 2 (b). Comparison of the prevalence of *S. mansoni* among 1-6 year old children for individual locations in Adwa, 1969-1972.

Location	1969			1971			1972		
	No.examined	No. +ve	% +ve	No.examined	No. +ve	% +ve	No.examined	No. +ve	% +ve
Zone A	—	—	—	78	12	15.4	11	1	9.1
Zone B	—	—	—	72	15	20.8	10	2	20.0
Zone C	—	—	—	25	7	28.0	13	2	15.4
Zone D	—	—	—	45	8	17.8	19	3	15.8
Zone E	—	—	—	22	8	36.5	—	—	—
Adi Abun	—	—	—	100	14	14.0	10	0	0
Military Camp	—	—	—	193	21	10.9	—	—	—
Total	401	202	50.4	535	85	15.9	63	8	12.7

It is also apparent from the data in Tables 2(a) and 2(b) that the reduction in bilharzia occurred throughout the community although with some variation between the different localities, the percentage reductions ranging from 24% to 40% with a mean of 32%.

Intermediate Snail Host Surveys

1. Population Density Fluctuations

By means of the weekly surveys it was possible to judge the most appropriate timing for molluscicide application, depending on the various streams, either following the rains, when the populations are decimated, or following a treatment. The survey data also enable the pattern of repopulation and distribution of *Biomphalaria* to be mapped, and display those areas favoured by actively breeding snails or those places where snails can survive adverse conditions or avoid molluscicide treatment. The surveys also monitor the success or otherwise of an application of molluscicide.

TABLE 3. Summary of prevalence and incidence of *S. mansoni* in Adwa and Intitcho in 1969, 1971 and 1972

Location		1969	1971	1972
Adwa	Prevalence	63.6%	—	43.3%
	Incidence	50.4%	15.9%	12.7%
Intitcho	Prevalence	22.0%	35.0%	—
	Incidence	—	—	—

Each stream must be surveyed separately, each having its own characteristics resulting in snail densities increasing at different rates. Because of the number of streams it was possible both to test the efficacy of endod and to compare it with an established synthetic molluscicide, in this case Bayluscide. Thus the Assem river was assigned endod while the Guagua and its tributaries were treated with Bayluscide.

Endod. There have now been 5 complete split-dose treatments with endod at a projected concentration of 80 ppm with the population density of *Biomphalaria* at the time of application ranging from means of 12.5 to 48.2 snails/sample, see Table 4. There was no obvious correlation between the density and the time of application and the time required for repopulation after the second dose, this interval generally being 3-4 weeks. However complete, albeit temporary, elimination of snails was obtained for approximately 6-7 weleks for each treatment.

Bayluscide. There have been eight treatments with Bayluscide in the various rivers where an active concentration of 0.25 ppm for 8 hours was aimed at, with

TABLE 4. Details of Endod applications to the River Assem including the time taken for repopulation by *Biomphalaria pfeifferi*

Date	Weight of Endod (kg).	Mean No. of *Biomphalaria*/ sample at start of treatment	Time taken for repopulation	Time taken for ×—5.0
10. 5.69	540	48.2	3 weeks	4 weeks
26. 5.69	540			
21.10.69	800	12.5	3 weeks	15 weeks
20.11.69	800			
12. 5.70	550	19.6	3 weeks	3 weeks
29. 5.70	530			
16.11.70	560	39.6	5 weeks	6 weeks
7.12.70	413			
3. 4.71	600	34.2	2 weeks	(rains)
26. 4.71	620			

snail-free intervals ranging from 3-9 weeks, and again there was no obvious correlation with the initial snail density. The average snail free interval for each treatment was again in the region of 6-7 weeks (see Table 5).

TABLE 5. Details of Bayluscide applications to Adwa rivers including the time taken for repopulation by *Biomphalaria pfeifferi*

River	Date of Application	Wt. of Bayluscide (kg)	Mean No. of *Biomphalaria*/ sample at start	Time taken for repopulation	Time taken for ×—5.0
Guagua	14.12.70	2.2	11.9	3 weeks	7 weeks
	14. 1.71	1.8	8.1	6 weeks	4 weeks
Edaga	14. 5.70	1.092	15.4	5 weeks	(rains)
	15.12.70	0.7	23.4	10 weeks	12 weeks
Enta	19. 5.70	2.106	11.2	5 weeks	(rains)
	16.12.70	1.8	51.5	10 weeks	11 weeks
Shanna	23. 5.70	2.457	25.7	4 weeks	(rains)
	17.12.70	2.5	40.3	9 weeks	14 weeks

2. Cercarial Infection Rate

During the snail surveys, periodic samples from each river were returned to the laboratory and examined for the shedding of schistosome cercariae. This enables the timing of molluscicide application to be determined and to define those areas requiring closer attention — infected snails are aggregated in the same way that normal snails are distributed and so the distribution pattern denotes potential transmission sites. The measured infection rates of *Biomphalaria* have however shown a drop since the start of the programme with exception of two sections which were either unpoliced or poorly policed and much used every day by people as their only source of water. Such areas are subject to "spot" treatment.

Discussion

Dr. Aklilu Lemma selected Adwa as a very suitable site for testing the efficacy of endod, in the hope that it would provide an inexpensive and "self-help" means for controlling the host snails of bilharzia and possibly other trematode diseases. It was also convenient that its activity could be compared with a commercial product.

Results so far indicate that endod when applied as a crude crushed powder of the dried berries is in fact as efficacious as Bayluscide in that both temporarily reduce the population of *Biomphalaria* to undetectable levels until repopulation starts 6-7 weeks later. Application of endod is somewhat laborious since it cannot be sprayed (although this it is hoped will be rectified on receipt of a new type of sprayer from U.K.), but labour is readily available and inexpensive. Even so, we could not overlook the "self-help" value and the import substitute nature of endod, it being an indigenous molluscicide source. The other drawback with endod, and shared with Frescon, is the necessity to apply as a split dose 20 days apart, this being obviated with Babyluscide which is ovicidal.

The most gratifying aspect of the programme to date is the considerable reduction in prevalence "incidence" of the disease in the 1-6 year old age group which is itself a good indicator of a general reduction of transmission in the community. Study of the human contact made with the various water sources in the town, combined with the schistosome infection rate of snails, would imply that most transmission was occurring in the Assem. Thus, it might be concluded that a major part of the reduction of prevalence is attributable to the use of endod.

The wealth of data being accumulated concerning the population density and distribution of the host snails and their cercarial infection rate should eventually make it possible to define a direct relationship between two of the parameters (density and infection rate), and so enable timing of the molluscicide applications to be made more critically.

The project is of necessity circumscribed by economic considerations and therefore measurements of all the parameters recommended for sophisticated control programmes have not been possible ; but in spite of lack of data on some of

the ecological aspects, the fact remains that a fairly dramatic reduction in active transmission has been made possible, and that in the absence of a significant amount of chemotherapy. However, because of this latter fact, constant vigilance will be required in future years with regard to snail control since the parasite itself will be retained in the community for many years to come.

Regarding the prevention or limitation of human contact with the infected water sources, the various measures adopted were not strictly maintained and were of doubtful significance.

Conclusion

The introduction of a molluscicide programme in the town of Adwa, Tigre Province, Ethiopia, utilizing both endod (*Phytolacca dodecandra*) and Bayluscide (R), has substantiated the high molluscicide value of endod. With its further development (studies in progress by Dr. Aklilu Lemma) one may expect in the future an inexpensive and "self-help" means for controlling bilharzia by elimination of the intermediate snail host making it comparable economically to Bayluscide or Frescon.

During the first two years of a five-year control programme there has been a reduction of "incidence" from 50% to 15% in Adwa compared with a rise of some 10% in the control village of Intitcho, contrasting with the absence of corresponding changes in other intestinal parasites from both the treated and reference areas.

Bibliography

1. Aram, Lo and Goll, Schistosomiasis in Alemaya, Hararge Province. (Personal Communication).
2. Ayad, N., Bilharziasis Survey in British Somaliland, Eritrea-Ethiopia, Somaliland, the Sudan and Yemen. Bulletin of the World Health Organisation, **14**, 1.
3. Brown, D.S. and Lemma, A., The malacology of the Awash River, Ethiopia, in relation to the transmission of schistosomiasis. *Annals of Tropical Medicine and Parasitology* (in Press) (1970).
4. Buck, *et al.*, Schistosomiasis in Adwa. *Ethiopian Medical Journal*, **3**, 93 (1965).
5. Chang, W.P., Report on Epidemiological Study on Bilharziasis in Gorgora, North Shore of Lake Tana, Ethiopia. *Gondar Health Series*, **1**, 5 (1961).
6. Ethiopian Nutrition Survey, Interdepartmental Committee on Nutrition for National Defence, U.S.A. (1958-1959).
7. Kubasta, M., Schistosomiasis mansoni in the Harar Province, *Ethiopian Medical Journal*, **2**, 260 (1964).
8. Lemma, A., Schistosomiasis — an emerging problem in Ethiopia. Presented at the First Annual Meeting of the Ethiopian Medical Association, May 1965 Addis Ababa (1965).
9. Lemma, A., A preliminary report on the molluscicidal property of Endod (*Phytolacca dodecandra*) *Ethiopian Medical Journal*, **2** (4), 187 (1965).

10. **Lemma, A. and Duncan, J.**, An integrated epidemiological, malacological, parasitological, pathological and clinical study of leishmaniasis in Ethiopia. Institute of Pathobiology Monograph, Haile Selassie I University, Addis Ababa (1968).

11. **Lemma, A.**, Bilharziasis in the Awash Valley. I. An epidemiological study with special emphasis on its possibie future economic and public health importance. *Ethiopian Medical Journal*, 7, 147 (1969).

12. **Lemma, A.**, Laboratory and field evaluations of the molluscicidal properties of Endod (*Phytolacca dodecandra*), *Bulletin of World Health Organisation*, 42, 597 (1970).

13. **Lemma, A. and Duncan, J.**, First annuai report of schistosomiasis pilot control project in Adwa, Ethiopia. Institute of Pathobiology Monograph, presented at the Second International Congress of Parasitology, September 6 to 12, 1970, Washington D.C.; abstract published in *Journals of Parasitology*, 56, 438 (1970).

14. **Lemma, A.**, Present status of Endod as a molluscicide for the control of Schistosomiasis. Proceedings of the OAU Symp. on Bilharzia in Africa (1970) in press.

15. **Lo, C.T.**, Schistosomiasis in Ethiopia. Proceedings of the OAU Symp. on Bilharzia in Africa. (1970) in press.

16. **Russell, H.B.L.**, Unpublished WHO EM/PHF/62. Ethiopia 13.

Proc. Int. Conf. Schisto., 415 — 436 (1978)

CONTROL OF SCHISTOSOMIASIS BY THE USE OF ENDOD IN ADWA, ETHIOPIA : RESULTS OF A 5-YEAR STUDY

Akliilu Lemma, Peter Goll, John Duncan and Bahta Masengia

Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia

Both intestinal and urinary schistosomiasis occur with different prevalences in various parts of Ethiopia. In general, intestinal schistosomiasis is predominant in the highlands, whereas urinary schistosomiasis is largely restricted to the warm and arid lowlands (Russel, 1958 ; Lemma, 1969).

Biomphalaria pfeifferi, the predominant transmitter of *Schistosoma mansoni,* occur in small natural streams in isolated foci in the highlands. *Biomphalaria sudanica* has been reported to transmit *S. mansoni* in some of the Rift Valley lakes. *Bulinus abyssinicus* (and as yet unidentifield *Bulinus* spp.) transmit *Schistosoma haematobium* in the Awash and Wabi Shebelle valleys, and the Genale river basin. In the lowlands, where swamps and traditional flood irrigation practices are being rapidly replaced by modern irrigation canals, *Bulinus* snails are flourishing more than ever before. This situation, coupled with the rapid movement of infected and uninfected people in and out of the same area is becoming so serious that it now causes a potential threat to the agricultural development efforts of the country. The problem of schistosomiasis in the major agricultural development areas of Ethiopia may yet lead to what has been encountered in the Gezira project of the Sudan and the irrigated land of Egypt.

Intestinal schistosomiasis due to *S. mansoni* has long been known in the highland areas of the northern province of Tigre, particularly in the town of Adwa. In this and other highland areas where *S. mansoni* is prevalent, it is transmitted in isolated foci in natural streams upon which people depend for their water supply. Adwa was particularly well known for schistosomiasis. About 10 years ago, there was an unusually large number of cases reported from this area, which eventually led to the current epidemiological and control studies (Lemma, 1965 ; Buck et al., 1965).

The molluscicidal properties of the native Ethiopian plant *Phytolacca dodecandra,* known locally as Endod, was discovered in 1964 during epidemiological and ecological studies of schistosomiasis in Adwa (Lemma, 1965). This plant has been used in Ethiopia for centuries as a soap, particularly in the highlands. Its molluscicidal properties were discovered by observating that in areas immediately downstream of where people were washing clothes with Endod more dead snails were found than in areas upstream or elsewhere. Many studies have since been done on the molluscicidal properties of this plant and highly promising results were obtained (Lemma, 1970 ; Lemma et al., 1972 ; Parkhurst et al., in press).

In 1969, when the potential of Endod for the control of schistosomiasis was apparent, a long-term plan was drawn up to test the plant under natural conditions in the field. The town of Adwa was chosen as it was the place where the molluscicidal properties of Endod were originally discovered and the seriousness of the disease established.

This study involved pre-control baseline data collection on disease prevalence, snail population studies, the proper orientation and education of the people, and involvement of local authorities on an organized campaign against the disease. The program was planned to run for six years. The first year was devoted to pretreatment baseline data collection and encouragement of cooperation by the people. This was followed by a 5-year continuous effort to control the snail population with the use of locally obtained and quantitatively applied Endod berries.

The effectiveness of the control program was to be measured by the degree of reduction in prevalence rate before and after the control program, with particular emphasis on children between the ages of 1 and 5 years. In an ideal control program where transmission could be completely stopped for a 5-year period, prevalence among children born during that period should be zero. Comparison of prevalence in children of the same age group before and after the 5-year control period should give a measure of degree of success of the program. Results of our effort to control intestinal schistosomiasis chiefly by use of Endod on a community self-help basis in Adwa from 1969 to 1974 are reported here.

Study Area

Adwa

Adwa, a former provincial capital of Tigre province, lies about 1000 km north of Addis Ababa and 150 km south of Asmara on an all-weathered road.

At an altitude of about 1800 m, Adwa lies in a cradle of mountains, enclosed on 3 sides but open to the west. It is the seat of the Awraja (District) governor, the governorate consisting of 11 weredas or such districts. The area of the study also includes Adi Abun, a suburb of Adwa, where there is a military camp, a 50-bed general hospital, and a regional office for the Malaria Eradication Service.

The 5 churches and one mosque in different parts of the town and the weekly market held on Saturdays, together with the Awraja government activities bring a large amount of human traffic through the town.

The population of the town increased from an estimated 15,800 in 1969 to 17,300 in 1974. About 98% of the people are indigenous Tigreyans, 1% are Amharas and the rest are from various parts of the country. About 85% of the people are of the Ethiopian Orthodox Coptic religion, 10% are Muslims and the rest belong to other religions.

Adwa is provided with electricity from a diesel generation plant in Axum, and with a piped water supply from near the source of the Shanna. The main outlet of the piped water supply is in the center of the town, close to the Assem Hotel on the shore of Assem river.

The amount of water available from this source fluctuates considerably, and it never meets the entire water needs of the town. It dries completely during the dry season and the people then depend on the Assem river.

The rivers

There are two main streams in Adwa : Mai (river) Assem, and Mai

Guagua. Mai Assem starts from a small spring to the east of the town in a deeply eroded area of sedimentary deposits. It flows through the center of the town with 2 road bridges over it and finally joins the Mai Guagua 4 km from its source. Mai Guagua originates many kilometers away from Adwa in the mountains along the road from Adi Abun to Adigrat. In the vicinity of Adi Abun, Mai Guagua is joined by 3 small tributaries : Mai Edaga, Mai Enta, and Mai Shanna (See Fig. 1).

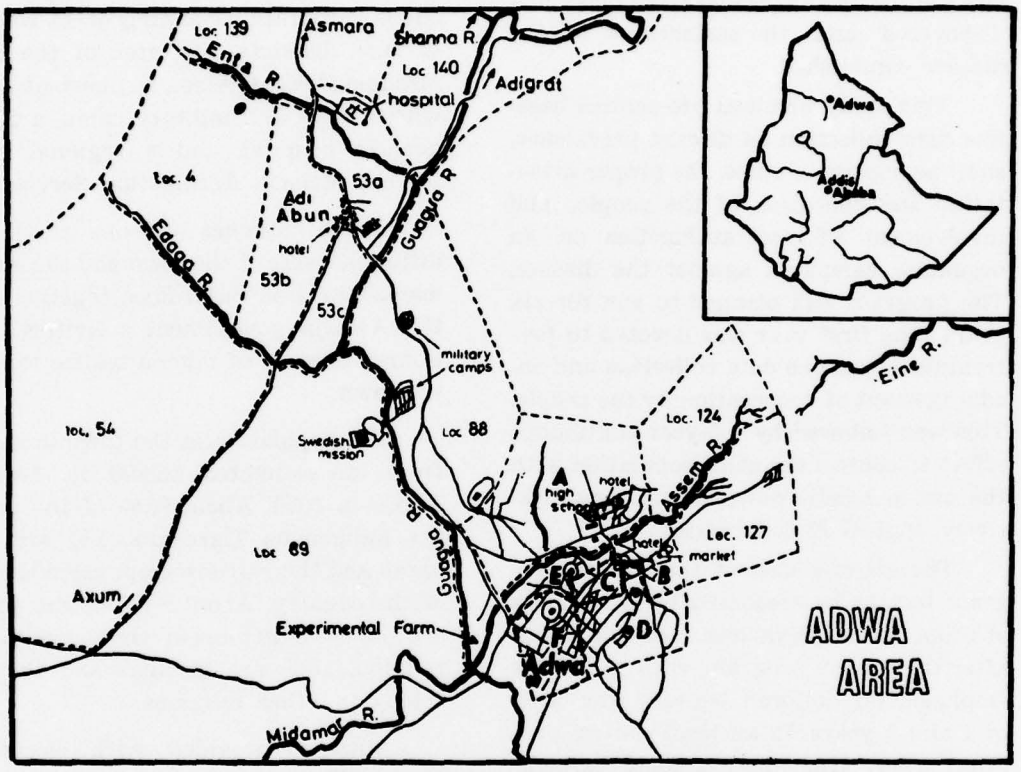


Fig. 1. — Map of Adwa region, Ethiopia

In view of its position, Mai Assem is the most intensively used of the streams in Adwa. Mai Guagua is also frequently used in limited stretches, particularly above and below the Adi Abun bridge. These streams, all of which may become nearly dry in the dry season, provide the fundamental basis of existence for the relatively large populations of Adwa and Adi Abun. They provide water for drinking, cooking, washing, swimming (children), bathing, small-scale irrigation, local beer (talla) brewing and other domestic uses.

All the streams in Adwa support large populations of *Biomphalaria pfeifferi* throughout the year, and are a hazard for *S. mansoni* infection. The extent of hazard in each case depends upon the frequency of contamination and contact by the people, which differ for different rivers and even different parts of the same river. The pH of the water varies between 7.3 to 7.9 and the temperature between 18° to 26°C, throughout the year.

Snail Survey Techniques

For quantitating the snail population, various methods were tested comparatively. The best was found to be simple visual searching and hand picking by two people for a fixed period of time (usually 2 min.) every 100 m on both sides of the river.

Counts were averaged and expressed in terms of number of snails per sample. The sizes (maximum diameter) of collected snails were measured, and percent infectivity determined by exposure of individual snail to light for cercarial shedding and/or by crushing the snails.

The predominant snails in the rivers in Adwa were *Biomphalaria pfeifferi,* the intermediate host of *Schistosoma mansoni ; Bulinus truncatus,* intermediate host of *S. bovis ;* and *Lymnaea natalensis,* intermediate host of *Fasciola gigantica* and *F. hepatica.*

Population and Statistical Sampling

The population estimates used in the study were obtained from the Central Statistical Office in Addis Ababa and from the Malaria Eradication Service branch office in Adwa.

With aerial photographs obtained from the Mapping and Geography Institute and ground maps made by the Malaria Eradication Service, each family dwelling in the greater Adwa area was numbered serially and the area was divided into zones. Numbers were written on a standard metal sheeting and nailed on each front door. The total number of people living in each zone was determined and the area delineated by specific roads and other landmarks. Ten percent of the population in each zone was then randomly selected for interview and stool examination.

Random samples of the population were selected by lot. The randomly selected numbers corresponded to specific houses. Each person in the selected house was asked to give a stool sample for examination. Personal data on each individual (area of origin, profession, age, sex, etc.) were recorded. By this method, 10% of the population from different parts of the town were sampled. The age distribution of the population sampled is given in Fig. 2.

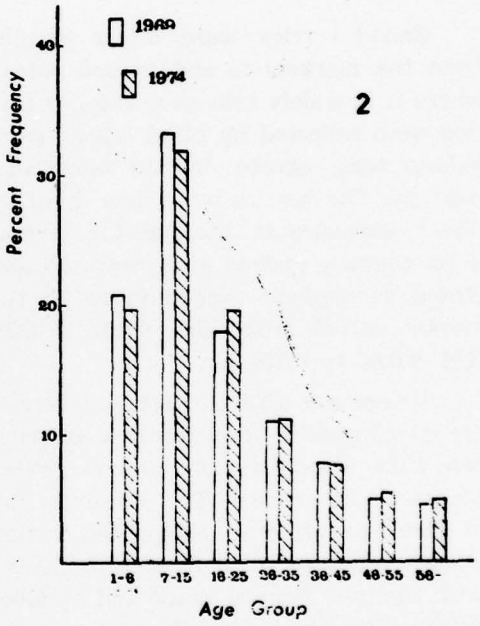


Fig. 2. — Age distribution of population samples in Adwa.

Stool examination techniques

All stool samples taken throughout the control program were examined by one experienced technician (Bahta Mazengia), thus minimizing variation due to individual differences.

Stool examinations were first performed by the direct smear technique, using about 2 mg of fresh stool comminuted with a drop of water on a slide.

Two or 3 such preparations were made from each specimen. All samples negative for *S. mansoni* were further subjected to Ritchie's formol-ether concentration technique (1948), found by Duncan et al. (1970) to be superior to other available methods under our laboratory conditions. Various intestinal parasites other than *S. mansoni*, were also looked for and recorded at the same time.

Endod preparation and application techniques

Endod berries were either bought from the markets in and around Adwa, where it is widely sold as a soap, or berries were collected by hired labour from wild-growing shrubs in the mountains near by. The berries were then dried by direct exposure to the sun for several days, coarsely ground at a local mill and stored as needed. Endod prices in the market varied from Eth. $ 0.15 to 0.30 (US. $ 0.07 to 0.15)/kg.

Before use 0.5 kg of crushed berries are mixed with 10 l of water in a watering can. This mixture is poured into the water bodies to be treated. The concentration of Endod at different spots of the treated stream was monitored by two methods : with the use of caged snails and by laboratory determination of the molluscicidal potency of the treated water sample. In the case of caged snails, 10 snails were loosely tied in a piece of cloth attached by a long string and submerged with one end of the thread tied to an easily identifiable object on the shore. After 6 hr of such exposure the snails were washed, fed and allowed to recover in clean water for 24 hr, after which time mortality was recorded. By the other method, samples of water from different parts of the treated river were taken and serially diluted in clean water. The molluscicidal potencies of the different dilutions were then determined by exposing an appropriate number of snails, usually 10, in each dilution for 24 hr, followed by a proper wash and recovery in clean water for another 24 hr. On the basis of mortality in such dilution, lethal concentrations of Endod in the river water were determined.

Since the primary objective of this project was to determine the usefulness of Endod in the control of schistosomiasis, and as most persons in Adwa become infected in Mai Assem, Endod was systematically used in the treatment of this river.

Other molluscicides used

To compare the effectiveness of Endod with other well-known commercially available molluscicides, Frescon and Bayluscide were also used in some of the streams in Adwa.

Frescon (N-Tritylmorpholine)

The synthetic molluscicide Frescon® (N-tritylmorpholine) was initially tested in Mai Guagua and its three tributaries : Mai Edaga, mai Enta, and mai Shanna. Since the Guagua river originates some 40 km from Adwa, treatment could not be started from the source. Treatment therefore was started about 6 km upstream before it joins Mai Assem at the end of the town. This covered the area where frequent human contact and schistosome transmission was probable. Application was also made above the junction of the three tributaries to Guagua. These tributaries, all only 2-3 km long, were also treated with Frescon. All of these treatments started from their sources until they joined Mai Guagua.

® Registered Trade Mark of a Shell Chemicals Product.

In all cases Frescon was applied at a concentration of 0.025 ppm (a 16.5% concentration of the active ingredient) for 7 days from a special automatic dispenser, as recommended by Crossland (1967).

Bayluscide® (Niclosamide)

As will be discussed later, Bayluscide was substituted when Frescon was found to be unsatisfactory for treatment of the 3 tributaries and Mai Guagua. Bayluscide was sprayed from a Hudson Spray tank in a mixture of 100 g of the 70% emulsifiable powder in 8 litres of water. The final concentration in the streams was calculated to be about 1 ppm for 8 hr.

Results

Precontrol investigations

Although earlier studies indicated that Adwa was suitable for control of the transmission of schistosomiasis, it was essential that a baseline study be first conducted for later comparison. Prevalence of human infections, and abundance, seasonal fluctuation, and distribution of the intermediate snail host in the area were determined.

To be certain that interruption of transmission could be correctly ascribed to the measures taken, prevalence surveys of *S. mansoni* were also periodically conducted in an untreated control or comparison village, Inticho, about 40 km east of Adwa.

In addition to determination of *S. mansoni* infection rates, data were collected on sex, age, and religion of the sampled population to determine the pattern of infection within the community. Observations were also made on human and animal water-contact activities that might suggest activities likely to increase risk of infection. Such behavioural studies were conducted before and after the control programme to ascertain whether, during the 5 years of the programme, there were any gross behavioural changes that might have contributed to the reduction of *S. mansoni* infection observed in the controlled area. In spite of efforts to educate the residents about the disease, the water contact behaviour studies conducted before and after the control programme revealed no difference. Details of the behavioural studies will be published separately (Lemma, et al., in prep.).

Stool surveys in Adwa

Results of parasitological surveys by stool examination are given in Tables 1 to 6. Table 1 lists the parasites recorded in the 1969 pre-control survey and the 1974 post-control final survey. Information on the prevalence of *S. mansoni* infection during 1969 and 1974 according to age and sex is given in Table 2.

In 1969, *S. mansoni* was the most abundant parasite in the Adwa area, with 63.1% of the population infected. *Ascaris* (42.2%), *Entamoeba* (16.1%), and Trichuris (9.5%) were also found frequently. In general, there were no significant differences in the infection of *S. mansoni* in males and females, suggesting that there were no obvious occupational trends. The figures for males and females in each age group show the usual age distribution of *Schistosoma* infection in a population. There is a rising incidence in the younger members with a peak in the 7-15 year age group, followed by a slight drop in the 16-25 group and a further falling off with increasing age (Fig. 3). The increase in prevalence in the 1-15 age group is associated with increasing exposure, the reduction of infection rate in the older group may be due to reduced exposure or to some sort of acquired immunity.

® Registered Trade Mark of a Bayer Chemicals Product.

TABLE 1. — Stool examinations among randomly selected individuals in Adwa before and after the control programme.

Parasites found	Pre-control 1969 survey		Post-control 1974 survey	
	Number positive	% infection	Number positive	% infected
Schistosoma	1080	63.5	544	33.0
Ascaris	715	42.2	401	24.3
Entamoeba	272	16.1	148	9.0
Trichuris	162	9.5	195	11.8
Giardia	39	2.3	25	1.5
Strongyloides	26	1.5	17	1.0
Hymenolepis	70	4.1	26	1.6
Enterobius	10	0.1	2	0.1
Ancylostoma	24	1.4	14	0.9
Fasciola	3	0.2	1	0.1
Taenia	4	0.2	7	0.4
Negative	223	13.4	597	36.2
Total examined	1659	86.6	1651	63.8

TABLE 2. — *S. mansoni* infection according to age and sex in Adwa before and after the control programme.

1969 Survey

Age Groups	Number Examined		Number Positive		% infected		Total
	M	F	M	F	M	F	
1—6	229	172	118	84	51.5	48.8	50.2
7—15	380	249	321	206	84.5	82.7	83.6
16—25	150	102	115	69	76.7	67.6	72.2
26—35	60	113	28	51	46.7	45.1	45.9
36—45	52	53	16	20	30.8	37.7	34.3
36—55	50	22	19	13	38.0	59.1	48.6
56	28	35	6	14	21.4	40.0	30.7
Total	949	746	623	457	65.6	61.3	63.5

1974 Survey

Age Groups	Number Examined		Number Positive		% Infected		Total
	M	F	M	F	M	F	
1—6	145	178	7	17	4.8	9.6	7.2
7—15	224	285	136	140	62.5	49.1	55.8
16—25	122	188	68	59	55.8	31.4	43.6
26—35	59	120	19	31	32.2	25.8	29.0
36—45	42	78	12	13	28.6	16.7	22.7
46—55	37	47	7	6	18.9	12.8	15.9
56	40	39	9	5	22.5	12.8	17.7
Total	669	935	258	271	38.6	29.0	33.8

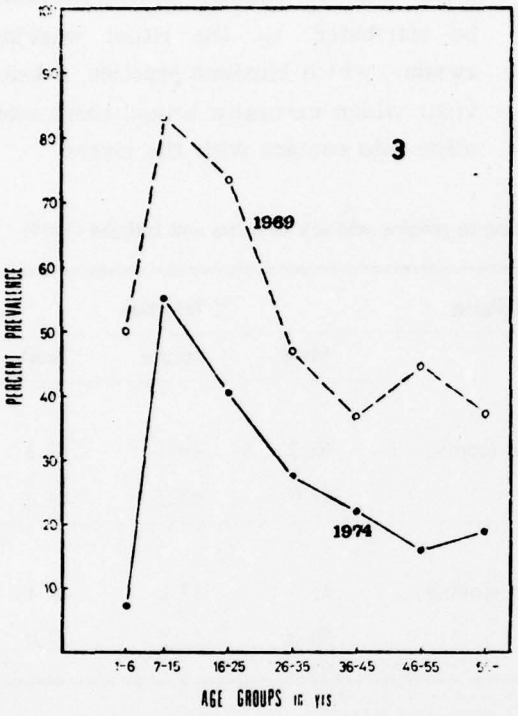


Fig. 3. — Prevalence rates of *Schistosoma mansoni* in Adwa before and after control measures by application of Endod.

Table 3 shows a more detailed analysis of *S. mansoni* infection in the different zones and locations of Adwa. In general, prevalence rates were high throughout and there was no area free of infection. In some areas, i.e. location 124, prevalence was considerably higher than in others, such as location 140 (80% and 43% respectively). This appears to be due to the proximity of a high prevalence location to active transmission sites on the rivers. Location 124 is near an active transmission site on Mai Assem, while location 140 is on the extreme end of Adi Abun near an area known to have very little transmission of the disease on Mai Guagua.

The Adwa Elementary school survey rate in Table 3 corresponds well with the 7-15 age group infection rate shown in Table 2. The low prevalence figure for the Swedish Mission (41%) may be due to the education these students get and also to their being relatively far from active transmission sites.

TABLE 3. — *S. mansoni* infection in different locations in Adwa, 1969 and 1974.

Zone or location	1969 survey		1974 survey	
	No.+/ No. exam.	% infected	No.+/ No. exam.	% infected
Zone A	112/169	67.0	81/217	37.3
Zone B	174/265	65.7	87/248	35.1
Zone C	167/264	63.3	72/206	35.0
Zone D	78/120	65.0	75/159	47.1
Zone E	104/147	70.8	26/98	26.5
Zone F	47/82	57.3	23/73	31.5
Adwa Elementary School	78/93	83.9	—	—
Swedish Mission	20/49	41.0	—	—
Military Camp	61/112	55.0	20/99	20.2
Adi Abun A	63/98	64.3	30/143	21.0
Adi Abun B	54/103	52.4	29/126	23.0
Loc. 4	50/73	68.4	39/95	41.1
Loc. 140	13/30	43.3	—	—
Loc. 54	27/40	60.0	21/43	48.8
Loc. 139	17/33	74.9	—	—
Loc. 127	6/12	50.0	6/16	37.5
Loc. 124	12/15	80.0	—	—
Total	1080/1695	63.7	544/1651	33.0

It was of interest to know whether religion could be a factor influencing rates of infection. Data on the denomination of infected people were recorded both in Adwa and Intitcho. Details are given in Table 4. It seems fairly apparent from this that Muslim men and women show higher infection rates than do their Coptic peers, and that this can almost certainly be attributed to the ritual washing, «wadu», which Muslims practice, a behaviour which naturally brings them more often into contact with the rivers.

TABLE 4. — *S. mansoni* infection according to religion and sex in Adwa and Intitcho (1969)

Area	Religion	% Infection		
		Male	Female	Total
Adwa (Zones A-F)	Christian (coptic)	62.2	60.5	61.3
	Muslim	69.9	68.3	69.6
Intitcho	Christian (coptic)	23.6	17.6	19.4
	Muslim	30.0	23.5	27.0

Stool surveys in the comparison (untreated) village of Intitcho

Intitcho, a somewhat smaller town than Adwa, located about 40 km east of it, was selected as a reference area to show the progress of the disease where no control measures were being applied. A 10% statistically random sample of the population was followed and stools examined in much the same way as in Adwa.

Table 5 lists all parasites recorded by stool examination in Intitcho during 1969 and 1974. In contrast to Adwa, *Ascaris* (29.2% in 1969, and 22.2% in 1974) remained the most prevalent parasite, with *S. mansoni* (22.6% in 1969, and 16.7% in 1974), and *Entamoeba* (7.5% in 1969, and 16.6% in 1974) following.

Trichuris was only 2.8% in 1969 but rose to 9% in 1974. *Giardia* also rose from 0.9% in 1969 to 6.3% in 1974, while *Strongyloides* dropped from 2.8% in 1969 to only 0.7% in 1974. Similar drops in the infection rates of *Hymenolepis, Enterobius* and *Ancylostoma* were also observed. As in Adwa, the male population was more heavily infected with schistosomes (Table 6). This is somewhat surprising as the reverse might be expected, since women serve as water carriers and laundresses and presumably have more frequent contact with infected water.

It is interesting to note that because of the Intitcho river being situated in a deep gorge in a relatively difficult position, young children who could not get free access to it were spared from infection with schistosomiasis.

Biodynamics of the snail population

In order to achieve maximum efficiency of control, especially of the snail host, knowledge of seasonal population

TABLE 5. — Stool examinations in randomly selected inhabitants of Intitcho during 1969 and 1974

Parasites Found	1969 Survey No. + out of 106 Examined	% Infection	1974 Survey No. + out of 144 Examined	% Infection
Schistosoma	24	22.6	24	16.7
Ascaris	31	29.2	32	22.2
Entamoeba	8	7.5	21	16.6
Trichuris	3	2.8	13	9.0
Giardia	1	0.9	9	6.3
Strongyloides	3	2.8	1	0.7
Hymenolepis	5	4.7	3	2.1
Oxyuris	4	3.8	1	0.7
Ancylostoma	2	1.9	2	1.4
Negatives	48	45.3	57	39.6

TABLE 6. — *S. mansoni* infection in different age groups and sexes in Intitcho

1969 Survey

Age Years	No. exam. M	No. exam. F	No. positive M	No. positive F	% Infection M	% Infection F	% Infection Total
1—6	19	8	0	0	0.0	0.0	0.0
7—15	18	16	6	6	33.3	37.3	35.3
16—25	6	9	2	1	33.3	11.1	20.0
26—35	8	7	6	0	75.0	0.0	40.0
36—45	2	6	1	2	50.0	33.3	37.0
46—55	2	3	0	0	0.0	0.0	0.0
56	3	2	0	0	0.0	0.0	0.0
Total	58	51	15	9	25.8	17.7	22.6

1974 Survey

Age Years	No. exam. M	No. exam. F	No. positive M	No. positive F	% Infection M	% Infection F	% Infection Total
1—6	5	5	0	0	0	0	0.0
7—15	74	22	16	1	21.6	4.6	17.7
16—25	17	7	2	3	11.8	42.9	20.8
26—35	4	5	2	0	50.0	0	22.2
36	3	2	0	0	0	0	0.0
Total	103	41	20	4	19.4	9.8	16.7

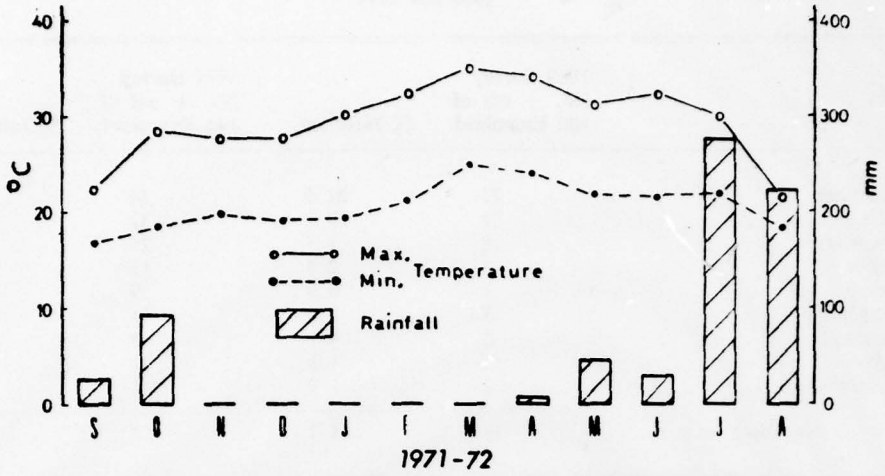


Fig. 4. — Mean temperatures and rainfall, Adwa, Ethiopia.

fluctuation is required. Concomitant infection with *S. mansoni* also requires study, as mollusc populations are subject to rapid and substantial fluctuations, chiefly by temperature and rainfall. In Adwa, it is unlikely that temperature has much effect as it is fairly uniform throughout the year (Fig. 4). Rainfall, however, is restricted to two periods : April-May (short rains) and June-September (long rains). During these times, rain falls chiefly in the form of heavy thunderstorms, small streams become raging torrents for several hours and what might have been a dense snail population of *B. pfeifferi* and other species are virtually eliminated.

Repopulation depends upon the frequency of such rainstorms, but in general small populations survive until the rains ease off. Repopulation is from individuals that survive in sheltered parts of the habitat. Therefore, in order to trace the fluctuations of snail colonies in the various streams, weekly surveys were carried out to determine the abundance and distribution of snail populations and delineate appropriate intervals for molluscicide application.

Various sampling methods were tested but the only one that could be standardized and was thought to give an adequate population estimate was simple hand-searching. All the streams are extremely rocky, rarely showing much mud or silt and usually consisting of interconnected pools. In the dry season, considerable stretches of the rivers dry up completely. Consequently, fixed sampling sites could not be selected, and a more randomized programme was adopted.

Snail surveys during different times of the year along the entire length of Mai Assem, Mai Edaga, Mai Shanna, Mai Enta, and the first 7 km of Mai Guagua, showed abundant populations of *Biomphalaria pfeifferi, Bulinus truncatus* and *Lymnaea natalensis. B. pfeifferi* was far more widely and abundantly distributed in all the rivers. This observation conforms to an earlier study (1966-67) by a

student of our Institute, Mr. Fesseha, who found that throughout the dry season all streams in the Adwa area supported fairly dense population of *B. pfeifferi*.

The sampling programme adopted in the current study made it possible to follow snail population fluctuations and also to monitor the success of molluscicide applications by a direct measure of population reduction following such treatment. The timing of treatments in turn was determined by the appearance of *B. pfeifferi* infected with *S. mansoni*. Infection of the intermediate host was assayed by making collections of this species and examining them in the laboratory by either crushing the snails or isolating them individually for cercarial shedding. By either method percentage infection rates could be calculated. Molluscicide applications were begun when the snail population becomes abundant, whether or not any infected snails were found.

Launching of the control programme

Once the preliminary snail surveys and the parasite prevalence in the human population were assessed, control molluscicide operations were begun ; other measures were also taken. These measures, though subordinate to the molluscicide programme, were undertaken to improve its efficiency, stimulate interest in it, and gain acceptance for the programme by the community of Adwa.

The latter aspect was very ably promoted by the Governor General of Tigre, who showed interest in the work from the start and undertook to address an assembly of the community elders, the priesthood, and local government officials. This followed an inaugural lecture and demonstration given by members of the Institute of Pathobiology on the nature and importance of the disease and the need for controlling transmission. Such talks were given in the schools for the benefit of students and teachers alike in order to foster awareness of the disease and of the project among this more receptive segment of the community.

Health education was augmented by the physical involvement of nearly 2000 people, including students, members of the Army, the police force, the farming community, government officials, and various elderly people of the town, who came as volunteers to participate in various preparations for the project. These activities were designed to instill a lively interest in and eventual responsibility for the programme, as it was to be maintained on a routine basis for some years after the initial 5-year programme. It was hoped too that a build-up of awareness of the public health importance of schistosomiasis would also provoke interest in other problems in public health, which exist both in urban and rural communities lacking a safe domestic water supply and sewage disposal. It was hoped that improvement in general standards of personal hygiene and modification of habits practiced at the site of water contact would develop and contribute to curtailment of water borne communicable diseases.

It was apparent early that application of Endod in whatever fashion would be greatly facilitated by cleaning up and improving the flow of the river. The first task of the volunteers therefore was removal of impeding boulders, straightening stream flow routes wherever possible, and removal of vegetation. A number of Boy Scouts and boys from the junior high school cut and gathered sufficient wood and thornbush for the construction of fences along certain sections of the stream thought to be most frequented, and likely to be important transmission foci. It was

hoped that such fencing would help in determining or preventing access to the stream and the banks, and thus reduce casual activity such as stream crossing, swimming, and defaecation. The Municipality was also directly involved by the provision of zabanyas (guards) for patrolling the streams to prevent contamination in the most densely populated areas. Unfortunately, both the attempts to fence the crucial areas as well as the guarding system, using small fines, failed. The fences soon were broken up and the wood used for fire. Some of the guards who tried to arrest people found defaecating near the stream, were attacked or chased away, chiefly by gangs of youngsters.

As mentioned earlier, the first application of Endod was made in the presence of a large number of town people. Each subsequent application involved the employment of a number of casual labourers, who also acted as a reminder of the importance of the project and its continuance. Other facets of the programme, such as the annual stool surveys and the frequent visits by Institute staff and visitors, also had this incidental effect of increasing awareness of the control efforts.

A further adjunct to enhanced effectiveness of control measures was a programme for construction of pit-latrines in as many households as possible. A survey made in November 1969 showed that there were 518 latrines in Adwa and Adi Abun, i.e. only one out of 10 houses had such a facility. Of these, perhaps only between 50-100 conformed to the standard 1 m^2 × 3 m deep hole overlaid by a wooden platform with a suitable opening which is kept covered when not in use to prevent ingress of flies. In a comparable survey in 1974, about 50% of house-holds questioned during the stool survey claimed to have latrines in their compounds, but only about 19% claimed using the latrine and keeping it in good order. This point will be discussed subsequently.

Molluscicide applications

Endod

As mentioned under Methods and Materials, a mash of Endod berries in water was poured into the river along the shores, with particular attention to the grass growing on the edges, seepage areas, and any ponds at the sides. This method was found to be far more effective than one previously tried that involved dripping concentrated Endod from a locally designed and constructed controlled-flow barrel. The latter was stationed at the head of the river, and the solution was allowed to mix into the river and flow downstream. With this method Endod was not evenly distributed. The centre of the river had high concentrations of molluscicide while the shores did not have enough. Therefore, application of Endod with watering cans were preferred for routine use. With this later method, Endod was applied in sufficient quantitities to make up and maintain a final concentration of 80-100 ppm for 6-8 hr which was sufficient to kill snails along the entire Assem river.

By trial and error, it was found that the whole treatment required two teams, each consisting of a supervisor, two sprayers, one man to carry and supply the Endod to the sprayers. One team started at the junction of the Assem with the Guagua and worked upstream, while the other started at the bridge by the Assem Hotel and also moved upstream. In this way, the river was covered in 1 ½ to 2 hr. Three hours after starting, the teams returned to their starting point and applied the molluscicide once again.

TABLE 7. — Effect of Endod against different snail species using Mai Assem river water (6 hr exposure and 24 hr recovery, average of 3 separate tests in Adwa, April 17-19, 1975)

Snail Species	% mortality at various concentrations of Endod ppm.				
	(100)	(80)	(60)	(40)	(0)
Biomphalaria pfeifferi	100	100	56	22	0
Bulinus trancatus	100	100	100	34	0
Lymnaea natalensis	100	100	100	80	0

From bioassays made on water taken from 6 places equally spaced along the length of the river, it was ascertained that a lethal concentration was maintained throughout during the 6 hr required and indeed for many hours later (Table 7). Even on the following day, large quantities of foam could be seen passing under the Assem bridge. By these procedures, the amount of Endod needed for complete treatment of Mai Assem was 400 to 800 kg, depending upon the water volume, which depended on the time of the year.

With such a treatment of Mai Assem, the following observations were recorded:

1. Small fish, tadpoles and leeches were killed by the concentration aimed at killing snails. But algae, crustaceans and other organisms in the stream did not appear to be affected, and the fish, tadpole and leech populations were quickly replenished. Although no quantitative study was made, no permanent change in the rich flora and fauna of the treated river could be detected over the 5 years of continuous Endod application other than the temporary effects noted. There were no large fish in these streams.
2. Although attempts were made to prevent the use of treated river water by the people and animals for a few days immediately after treatment, people have often taken home such treated water while it was still foaming and used it for various domestic purposes. No complaints about ill-effects of such water have been received. Both large and small animals have been seen freely drinking freshly treated water from the river, again without apparent ill-effect.
3. Over 95% of the snail population dies within 24 hr of each Endod application. Two to 3 weeks later, very young snails, presumably those hatched from egg-masses not affected by the treatment, were seen. No infected snail appeared in the stream until about 7-8 weeks after treatment, approximately the time required for unaffected snail eggs to hatch, the young snails to be infected, and for miracidia to develop to fully mature cercaria.
4. During the first few days after treatment, the water becomes very clean and clear, which the people appear to enjoy. Furthermore, all miracidia and cercaria are killed and the stream becomes safe for the following 7-8 weeks until newly infected snails begin to shed again. Treatment of the stream also appears to have an adverse effect on other parasites such as *Entamoeba* (which are killed within 5 min. at 100 ppm in the laboratory).

5. Double treatment was employed in an attempt to compensate for the non-ovicidal nature of Endod. The first treatment was followed by a second treatment 2 weeks later to kill young snails that hatched from the unaffected eggs. However, this method proved unsuccessful as some snails survived the second treatment and perpetuated themselves. Further, not all egg masses laid just before treatment hatched within the first two weeks. Some would take 3-4 weeks after the treatment, while others hatch immediately after treatment. This method, which also required more molluscicide, was replaced by routine treatment every 7-8 weeks, in order to control by reduction but not by the elimination of every snail. This method appeared to destroy selectively snails with an infection with *S. mansoni*.

6. One important result of the snail control program was elimination of the *Lymnaea* population in Mai Assem during the first 3 years of Endod treatment, presumably due to the fact that Endod is ovicidal for *Lymnaea* eggs while adult snails are highly susceptible to its lethal action.

Frescon

Frescon was initially tried in Mai Guagua, starting about 6 km upstream before it joins Mai Assem.

The automatic dispenser, constructed with the help of the local representative of Shell Chemical Company, worked well and required a minimum of labour and attention. Lethal concentration of the molluscicide was detected by use of caged snails and chemical analysis. Concentration of the chemical further down stream was, however, so low that it was undetectable by either method. Since the pH of the river water was about 7.0, hydrolysis of the active principle was not thought to be responsible for this loss in activity. Dilution in some of the large pools at the lower end of the Guagua, and possible loss by absorption of the active ingredient by non-specific materials may be partially responsible.

In an attempt to get an even distribution of Frescon, application from the dispenser was backed up by spraying the stream margins from a Hudson malaria spray tank. In one instance a trial on a 100 m stretch of river showed that on the day following application the number of *B. pfeifferi* fell from 460 to 110, and a few days later only 12 snails could be found in the same portion of river.

In applying Frescon from dispensers into the Edaga, Enta and Shanna tributaries of the Guagua, some of the effects were seen in subsequent snail surveys. However, since these streams flowed so slowly, the chemicals took 4-6 days to cover most of them. Both Edaga and Enta rivers have swampy distal ends and the chemical never reached a lethal concentration there. This was further complicated by the fact that the Guagua and all of the tributaries, particularly the Shanna, dried along portions of the course of the river. This prevented distribution of molluscicide by a single application. Furthermore, because of the non-ovicidal nature of Frescon, as with Endod, frequent applications were required.

For these reasons, the use of Frescon was discontinued in the Adwa streams after the first year and the chemical was replaced by Bayluscide.

Bayluscide

Niclosamide was applied in Mai Guagua and its 3 tributaries from a Hudson spray tank as stated in Materials and Methods. It was sprayed in the

stream to make a final concentration of about 1 ppm for 6-8 hr. Such treatment gave a very good kill and, because of the ovicidal property of the molluscicide, repopulation, particularly in the tributaries that start from springs, was relatively slow. General application was required only about twice a year with some supplementary spot treatments. In Mai Guagua, however, repopulation was more frequent, and general treatment was required every 10-14 weeks. Presumably adult snails were carried down from untreated parts of the river.

As with Endod, Bayluscide also kills small fish and tadpoles, but, again, these organisms quickly repopulate the streams. Although no quantitative ecological study was done, no ill effect was apparent after continuous application of Bayluscide in the Adwa streams for 4 years.

Evaluation of the control programme (1974)

Evaluation of the molluscicide programme depended upon stool examinations performed in 1974, after 5 years of control operations. If the measures taken had succeeded in interrupting transmission, reduction in *S. mansoni* prevalence in the community at large would be evident, but the interruption would be especially evident in children aged 1 to 6, as they were born during the

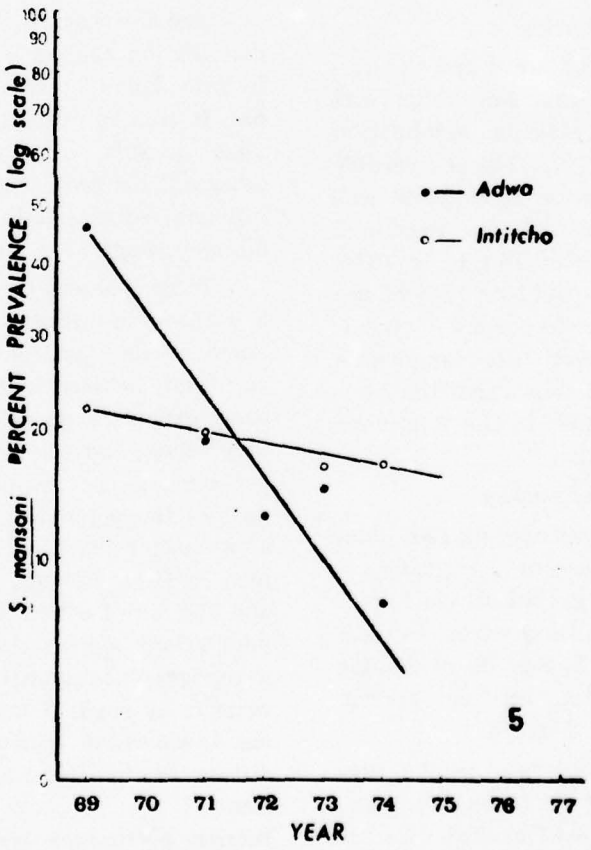


Fig. 5.— Prevalence of *Schistosoma mansoni* in children 1-6 years old in Adwa and in the control area of Intitcho.

control programme. Comparison with the control (untreated) village of Intitcho would be another significant indicator (Fig. 3 and 5).

The 1974 stool survey was conducted in much the same way as was the original 1969 survey, utilizing updated maps of the various locations and most recent population statistics provided by the Malaria Eradication Service.

During the parasitological survey conducted in 1974, socio-economic information on the sampled population was also collected for determination of possible correlation between such factors and infection with *S. mansoni*. Observations were also made on water contact activities, as in 1969.

1974 population statistics

The population of Adwa and the surrounding area, including Adi Abun, was estimated by the Malaria Eradication Service at 17,286 in 1973. The comparable figure for the same area in 1969 was 16,369. In 1969 the sample size examined was 1965 individuals or 10.4%. In 1974, the sample taken was 1651 or 9.5% of the population. The age frequency distribution of the 1969 and 1974 samples is plotted in Fig. 1 and shows that the composition of the samples in the 2 surveys were nearly identical.

Results of 1974 stool survey

S. mansoni prevalence in 1969 and 1974 is listed by age group in Tables 2 and 3 and presented graphically in Fig. 2. *S. mansoni* and intestinal parasites data are shown in Table 1. Results of similar surveys for Intitcho, the comparison village, are given in Table 6.

A significant reduction in the prevalence of *S. mansoni* throughout the Adwa community is evident (Table 2) ; it had dropped from 63.5% in 1969 to 33.8% in 1974, while in Intitcho, it was 22.6% in 1969 and 16.7% in 1974 (Table 5). Maximum age-frequency prevalence of *S. mansoni* between 1969 and 1974 showed a significant reduction in the 1-6 year age group and some in the 7-15 year age group as well, though peak infection rates in both periods remained in the 7-15 year old group (Fig. 2). Disease prevalence in males in 1969 was similar to that of females (65.6% for males, and 61.3% for females). But after the 5-year control programme more females lost their infections than males (prevalence having been reduced to 38.6% for males and 29% for females). Differences in male and female infection rates may have been due to their respective water-contact activities.

The key feature of the age prevalence distribution change is the dramatic drop in prevalence in the children 1-6 years old. It was equivalent to a reduction of about an 85% in the transmission of the disease. This drop was supported by significant reductions in prevalence among all age groups (Fig. 3).

Table 3 shows the prevalence rate of *S. mansoni* in different locations of Adwa. Some of the locations sampled in 1969 were not included in the 1974 survey as some groups, such as the Adwa elementary school and the Swedish Mission, did not need to be sampled twice, once as part of the population at large and once as a special group. Other areas not sampled in 1974 included locations 140, 139 and 124, all of which had relatively small populations spread over a wide area in a topographically difficult area not accessible by road. It was felt that excluding these small groups would make no difference in the final control assessment.

Interim prevalence surveys by stool examination

Between 1969 and 1974 several addi-

tional surveys were conducted, primarily in the 1-6 year age class to measure prevalence changes in the treated area and the comparison village. Results are shown in Fig. 5. The fall in prevalence in Adwa is fairly steep throughout the 5-year control period, whereas in Intitcho there was no significant change over the 5-year period, the infection remaining about 20%.

Assessment of other factors

In order that reduction in schistosomiasis prevalence can be reasonably ascribed to the snail control efforts, other factors should have remained more or less the same. Several of these factors were dealt with in a questionnaire used in the 1974 survey. Although in many instances answers were ambiguous or conflicting, some useful information was obtained.

Of those questioned 66% claimed to have lived in the same house for more than 5 years, indicating that there was no radical change in the habitat of 2/3 of the Adwa population during the control period. With respect to living standards, 49% claimed it was higher in 1969 than in 1974, and only 10% said that the reverse was true. This was due largely to massive inflation in the price of nearly all foodstuffs on which the bulk of the average Ethiopian income must be spent, with no general compensatory increase in income. No factories, companies, or any other substantial industries opened business in Adwa during the 5-year control period.

As a result of the general drought in the northern part of Ethiopia, particularly during the latter part of the control period, there has been an increased shortage of water in the area. In Adwa, there was more dependence and contact with the rivers which were observed to be continuously reduced in size and volume.

Although no dramatic change occurred in stability and income level of the people, the town of Adwa did undergo some improvements. The road from Adi Abun to Adwa was paved, the main road and piazza laid out with trees and flowerbeds, a branch of the Commercial Bank of Ethiopia and a new comprehensive High School were opened. These changes, however, had little impact on the patterns of life or activities of Adwa residents. Although strict comparison with the situation prevailing in 1969 is not possible, 38% of the people said in 1974 that they used stream water even for drinking, in spite of the often available piped water supply. Stream bathing was admitted by 44%, and 67% said they washed their clothes in the streams. Other activities, such as children playing in the water, remained more or less the same. Of those laundering at the streams, 59% also bathe, or at least wash their hands and feet. Of those who go to the streams to collect household water, 50% also indulge in other water-contact activities.

According to figures provided by the Municipality of Adwa, 6969 houses and 3,265 latrines existed in Adwa and Adi Abun in 1974, corresponding to 47% of the houses, which is close to the ratio obtained from the questionaires used during our second stool survey. These figures, possibly exaggerated, show a substantial improvement over 1969. The important factor however, is the use of the latrines. The answers to our questions indicate that latrines were not a favoured place for urination or defaecation. Only 4% of the people admitted to using latrines, 49% preferred the fields and 17% the riverside.

All these relatively high frequencies of water-contact activities, probably all

Cost of Endod		1970-71	1971-72	1972-73
1. Total weight used:		2793 kg	2300 kg	1752 kg
2. Total cost of purchase: . . . . . (including transport, storage, coolies, and incidentals)	Eth. $	562.20	529.60	438.00
3. Average buying price:	„	0.20/kg	0.23/kg	0.25/kg
4. Grinding and associated charges, and labour for application: . . .	„	304.00	257.05	166.60
5. Total cost of application per year	„	866.20	786.65	604.60
6. Average cost for 3 years per year	Eth. $	752.5		

Cost of Bayluscide		1970-71	1971-72	1972-73
1. Total weight used:		16.4 kg	19.1 kg	18.7 kg
2. Price paid for in 1970:	Eth. $	16.00/kg	16.00/kg	16.00/kg
3. Total cost of Bayluscide:	„	262.40	305.6	299.2
4. Transport and labour for application	„	94.50	88.75	121.65
5. Total cost of application . . .	„	356.90	394.35	420.85
6. Average cost for 3 years per year:	Eth. $	390.7		

understated, make it probable that no significant behavioural changes have accurred, inspite of the health education efforts attempted at the beginning of the programme.

Chemotherapy, by home treatment or by a doctor was claimed by about 4% of the people. Since the cure rate among treated people is relatively low, and the total number involved was not high, we feel it unlikely that it made a significant impression on the overall transmission rate.

Costs of Endod and Bayluscide used in the Adwa Schistosomiasis control programme

The average yearly direct costs of purchase and application of Endod and Bayluscide used in all the treated streams in Adwa during the first 3 years of the project, 1970-1973, is given above.

Comparison of costs of Endod and Bayluscide

The figures given are the actual costs incurred during the period of 1970-73.

On the basis of cost of treatment of a unit length of river (i.e. km), Bayluscide appears to be considerably cheaper than Endod. However, cost estimation on the basis of per kilometer treatment of river is misleading. It does not take into account the volume of water to be treated and the benefits (in terms of reduction of risk of infection) to the population. For example the Endod treated Assem river has a larger volume of water than any of the Bayluscide treated rivers. Over 80% of the infection with the disease takes place at Assem, thus the benefits gotten from the Endod treatment are much more

significant than those obtained from Bayluscide.

The cost of purchase of Bayluscide has been increasing considerably in recent years. In 1970 a large quantity of Bayluscide was bought at the cost of about Eth. $ 16/kg and this was what was used throughout the 3-year treatment period. Since then the cost of Bayluscide has been rising at about 10% per annum. More recently the increase has been as high as 30%. Furthermore, Bayluscide had to be bought from Germany with hard-currency. On the other hand the cost of Endod used in these calculations were based on material bought from the local market with local currency during the period of the control programme.

The cost of Endod in the local market has also been rising recently to Eth. $ 0.30 per kg, because of the high demand and limited supply. The cost of Bayluscide in 1974 was $ 24/kg (a 50% increase over the cost during 1970). When selected strains of the Endod plant will be grown on a large scale basis, the cost of Endod is expected to fall considerably. In the case of Bayluscide, however, the cost is expected to rise further as a result of inflation and a general rise in cost of commodities in West Germany. If Bayluscide is to be used for snail control on a large scale basis over a prolonged period of time, the government would need to allocate quite an amount of hard currency, a resource which is in great demand for other development projects.

Cost of the control programme per head of the protected individual

Perhaps the best way to determine the cost benefit of such a control programme is to show the annual cost of the control per head of protected individual. The total cost of purchase and application of Endod and Bayluscide in the Adwa schistosomiasis control programme was $ 1,143.2 ($ 752.5 for Endod and $ 390.7 for Bayluscide). The approximate population of the area where schistosomiasis transmission appears to have been reduced by 85% due the control effort over a period of 5 years was 20,000. Therefore, *the cost of the control programme was about Eth.* $ 0.06 (6 *cents*) *or U.S.* $ 0.03 (3 *cents/head/year*).

Discussion

The reduction in prevalence of schistosomiasis from 63.5% in 1969 to 33% in 1974 among the general population of Adwa, is highly significant and is strong evidence that this reduction is real and was brought about primarily by the snail control efforts. The people in the community on the basis of answers to the 1974 questionnaire, also feel that there are less people suffering from schistosomiasis in 1974 than in 1969. Furthermore, analysis of hospital records in Adwa show a reduction by about 50% in the total number of schistosomiasis cases appearing at the hospital during this period. Some of the reduction, particularly in the older age group, may have been due to loss of an earlier infection. Yet drop in the infection rate has been gradual, suggesting a cause-and-effect relationship with the control effort.

As would be expected, the greatest reduction in infection was in the 1-6 year age group, with a drop from 50% to 7%, an 85% reduction in transmission ; while in the untreated comparison village of Intitcho, the rate has remained more or less the same.

Comparison of prevalence of intestinal parasites with *Schistosoma mansoni* before and after the control period, shows some reduction in prevalence of *Ascaris*

in Adwa, but not in Intitcho. There was also a reduction in prevalence of *Entamoeba*, while infection with *Trichuris* has substantially increased. The remaining parasites were recorded in such small numbers that fluctuations are of doubtful significance. The highly significant reduction in *S. mansoni* is therefore the more striking. The reduction in prevalence of *Ascaris* and *Entamoeba* in Adwa but not Intitcho, while other parasitic infections remained the same, suggest that molluscicide may also have had a controlling effect on these parasites as well.

The data, in our view, strongly suggest that the primary cause for reduction in transmission of *S. mansoni* in Adwa was the use of Endod to control *B. pfeifferi* in Mai Assem, the centre of activity for the majority of the population of Adwa, and the most highly infected and hazardous river in the area, and not to unrelated events. The control programme has also benefited, though to a lesser extent, from the use of Bayluscide in Mai Guagua and its 3 tributaries.

Continuous surveillance of the streams for snails over the 5-year control period has enabled us to establish the patterns of repopulation following molluscicidal application. This, coupled with snail infection data, allowed us to develop a routine treatment programme for each molluscicide in each stream. On the basis of the last 3 years' application of Endod in Mai Assem, the following schedule is thought adequate for snail and infection control: Endod application every 7-8 weeks through the 9 month dry season, beginning at the end of September after the main rains cease. Using Bayluscide, it was only necessary to treat Mai Guagua every 10-14 weeks, and that primarily because of repopulation of snails from untreated parts of the river. For the other small streams, application frequency was even less, about 3 applications per year in Mai Enta, and 2 each in Mai Edaga and Shanna. This may be due both to the ovicidal properties of Bayluscide and to the fact that much of the length of these tributaries dry out during the dry season.

The health education aspects of the control effort did not have any great impact in disease control. Water contact behaviour and the water supply system remained much the same. Efforts to fence some particularly hazardous sections were unsuccessful. Defaecation and urination habits on the river shores, particularly near Mai Assem, have remained unchanged. Employment of guards to prevent this did not succeed. The Muslim population was more heavily infected with schistosomiasis than were their Christian neighbors, owing to the obligatory daily ritual washing in infected waters.

The changes observed in the infection pattern at the end of the 5-year control programme closely resemble those seen elsewhere (Barnish, 1975). A shift in the peak of the age/frequency distribution to a slightly older group is a good indication of reduction in transmission. These reductions were brought about by similar molluscicide programmes. The important observation from the programme was that a simple application of locally acquired unprocessed Endod berries, systematically applied, did control vector snails and interrupt transmission of schistosomiasis.

Acknowledgements: This project would not have been successfully carried out without the strong support and full participation of very many people in Adwa. We are particularly grateful to the Governor-General of the Tigre Province who provided his unreserved leader-

ship and continuous encouragement for the project, the Governor of Adwa Awraja, the Mayor of Adwa, the Chief of the Adwa Police Force, the Christian and Muslim leaders, the Ministry of Public Health (particularly the Malaria Eradication Service), and various other officials and elders of the Adwa community.

This study was in part supported by grants from the Lutheran World Federation, the Municipality of Adwa, the Haile Sellassie I University, the World Health Organization, and, during the final year, the U.S. Office of Naval Research.

The Ministry of Public Health, in collaboration with the local administration of Adwa, has now taken over the project and the schistosomiasis control programme is continuing on a permanent basis.

LITERATURE CITED

Barnish, G., 1978. Control of **Schistosoma mansoni** transmission in an isolated valley in St. Lucia, West Indies. Paper presented at the Int. Conf. on schistosomiasis in Cairo, Oct. 18-25, 1975.

Buck, A.A., Spruyt, D.J., Wade, M.K., Deresse, Asrat and Feyssa, Egetta, 1965. Schistosomiasis in Adwa. Eth. med. J., **3**: 93-106.

Crossland, N., 1967. Field trials to evaluate the effectiveness of molluscicide N-tritylmorpholine in irrigation systems. Bull. Wld Hlth Org., **37**: 23-42.

Duncan, J., Lemma, A. and Mazengia, B., 1970. A comparison of certain stool examination technique with special reference to their efficacy in surveys for schistosomiasis in the Awash Valley. Eth. med. J., **8**: 17-25.

Lemma, A., 1965. Preliminary report on the molluscicidal property of endod (**Phytolacca dodecandra**). Eth. med. J., **3**: 187-190.

Lemma, A., 1970. Laboratory and field evaluations of the molluscicidal properties of endod (**Phytolacca dodecandra**). Bull. Wld Hlth Org., **4**: 597-612.

Lemma, A., Brody, G., Newell, G.W., Parkhurst, R.M., Skinner, W.A., 1972. Studies on the molluscicidal properties of endod (**Phytolacca dodecandra**): I. Increased potency with butanol extraction. J. Parasit., **58**(1): 104-107.

Ritchie, L.S., 1948. An ether sedimentation technique for routine stool examination. Bull. U.S. Army med. Dept., **8**: 326.

Russell, H.B.L., 1958. Final Report. Pilot Mobile Health Team, Ethiopia. Unpublished document WHO FM/PHA/62 Ethiopia 13.

SPERMICIDAL ACTIONS OF EXTRACTS AND COMPOUNDS FROM PHYTOLACCA DODECANDRA

S. J. Stolzenberg
Endocrinology Laboratory
Biomedical Research Department

R. M. Parkhurst
Pharmaceutical Chemistry Department

Stanford Research Institute
Menlo Park, California 94025

ABSTRACT

The crude saponins from three plants, four purified saponins, and two synthetic derivatives were tested for spermicidal activity by means of an in vitro incubation procedure. The crude saponins and two purified saponins derived from Phytolacca dodecandra were found to have potent spermicidal activity. The most active compound tested against human sperm was lemmatoxin, a trisaccharide of oleanolic acid. Based on ED_{50} values obtained in our test system, the crude saponin preparation from P. dodecandra and lemmatoxin possessed nearly three times the spermicidal activities of a synthetic detergent currently used as a spermicidal agent. Biological activity could not be accounted for on the basis of surface activity.

Accepted for publication May 30, 1974

AUGUST 1974 VOL. 10 NO. 2

INTRODUCTION

The crude saponins of Phytolacca dodecandra have been recognized for their potent molluscicidal (1,2), fungicidal (3), and larvacidal (4) activities and for their ability to kill the cercaria of schistosomiasis (5). Previous work directed toward the structural characterization of the most active molluscicidal components (6-8) showed that biological activity did not closely parallel the surface activity of the compounds tested. King et al. (9) showed oleanolic acid hemisuccinate to be a potent molluscicide, although it had poor detergent activity. The surface activity of saponins from P. americana is comparable to that of saponins from P. dodecandra. However, the P. americana saponins are almost devoid of molluscicidal activity, as are a number of commercial detergents (5).

It was of interest to examine the crude saponins of these two Phytolacca species and the saponins of Calendula officinalis (10), as well as related purified compounds for their spermicidal activity in vitro. All the purified compounds studied, including three isolated from P. dodecandra, contained the oleanolic acid aglycone. Nonoxynol-9*, a detergent and spermicidal agent widely used as the active component of numerous intravaginal contraceptive preparations, was tested for comparison.

MATERIALS AND METHODS

Experiments with human sperm were performed only on samples with a high percentage of motility and forward progression. Original sperm counts ranged from 46 million to 186 million per ml, and dilutions with modified Ringer's solution (11) ranged from nine- to fifteenfold. All specimens were used within 1 to 4 hours postejaculation. Test compounds or crude saponin preparations were serially diluted in the same modified Ringer's solution. In each test, 0.1 ml of test solution appropriately diluted was placed in a 10 × 75 mm test tube. Controls contained the same volume of the modified Ringer's solution. To each tube, 0.9 ml of the diluted sperm solution was added. The mixture was incubated in a Dubnoff shaker at 30 rpm, 37°C, air atmosphere, for 1 hour.

At appropriate intervals, small samples from each tube were removed for microscopic examination between a slide and coverslip. The percentage of motile sperm as well as the type of motility based on vigor and forward progression were graded from 1 to 4. However, lemmatoxin-C was tested against rat epididymal sperm by the method of Gwatkin and Williams (12) before human sperm samples were available for these studies.

* Polyoxyethylenated nonyl phenol.

CONTRACEPTION

RESULTS

Table I summarizes the results of spermicidal activities of the materials tested. Table II presents ED_{50} and ED_{90} values with 95% fiducial limits obtained by probit analysis calculated from the percentage of nonmotile sperm according to the method of Finney (13). ED_{50} and ED_{90} values, where obtainable, are based on a 15-minute incubation period because of the increased accuracy of this time measurement over a "zero" time value and because differences in results between the preparations tested are more obvious at this time.

ED_{50} values for P. dodecandra saponins* (10.7 µg/ml) and lemmatoxin (10.7 µg/ml) clearly indicate that one or more saponins from P. dodecandra are highly potent spermicides, possessing nearly three times the activity of Nonoxynol-9 (27.0 µg/ml). ED_{90} values for the saponins compare favorably with those for the widely used commercial product. Although the results with lemmatoxin-C are not comparable with those for other compounds, the activity of lemmatoxin-C on rat epididymal sperm (ED_{50}, 8.7 µg/ml; ED_{90}, 20.0 µg/ml) suggests that it may be highly active against human sperm as well. On the other hand, oleanoglycotoxin-A, when tested against human sperm, had ED_{50} and ED_{90} values of 134.2 and 276.5, respectively. All three compounds mentioned above are trisaccharides of oleanolic acid isolated from P. dodecandra. Chikusetsusaponin IV, also a trisaccharide of oleanolic acid, obtained from Panacis japonica (14) was relatively inactive (Table I). It is also obvious from Table I that the hemisuccinate (8) and the cellobioside (7) synthetic derivatives of the oleanolic acid displayed low spermicidal activities. The crude saponin preparation from P. americana was surprisingly devoid of activity even at 500 µg/ml, the highest level tested.

DISCUSSION

The data demonstrate the existence of plant saponins--particularly from P. dodecandra and C. officinalis--having a high degree of spermicidal activity. In our test system the spermicidal activity of one or more saponins derived from P. dodecandra was even greater than that of Nonoxynol-9, which is currently in wide use as an intravaginal contraceptive. Differences in spermicidal activities between two related plants, P. dodecandra and P. americana, could not be attributed to differences in surfactant activities. It is known that 50% of the crude saponin extract from P. dodecandra consists of oleanoglycotoxin-A, lemmatoxin,

* The crude P. dodecandra saponins contain oleanoglycotoxin-A, lemmatoxin, and lemmatoxin-C in concentrations of approximately 18%, 16%, and 17%, respectively.

Table I Sperm motility after incubation with various concentrations of test material.

Test	Conc. (µg/ml)	No. of Tests	% Motile Sperm (Grade of Motility) Incubation Time: < 3 min	15 min	30 min	60 min
Control	-	13	71.5 (4.0)	66.8 (4.0)	66.8 (4.0)	58.9 (3.8)
Nonoxynol-9	5	1	60.0 (4.0)	70.0 (4.0)	50.0 (4.0)	40.0 (4.0)
	10	2	55.0 (4.0)	75.0 (4.0)	65.0 (4.0)	60.0 (3.0)
	20	4	55.0 (4.0)	47.5 (3.8)	41.3 (3.5)	30.0 (2.0)
	30	1	70.0 (4.0)	60.0 (4.0)	70.0 (4.0)	30.0 (3.0)
	40	3	5.0 (3.0)	3.3 (3.0)	1.7 (0.0)	0
	80	2	25.0 (4.0)	5.0 (4.0)	0	0
Crude saponins of P. dodecandra	5	4	55.0 (4.0)	50.0 (4.0)	50.0 (4.0)	40.0 (3.8)
	10	3	53.3 (4.0)	28.3 (4.0)	33.3 (3.0)	23.3 (3.0)
	20	6	28.8 (4.0)	20.8 (4.0)	19.4 (3.5)	11.8 (2.8)
	40	3	20.0 (4.0)	6.7 (4.0)	8.3 (4.0)	3.3 (3.0)
	80	3	0	0	0	0
	100	1	10.0 (4.0)	10.0 (4.0)	0	0
	500	1	0	0	0	0
Crude saponins of P. americana	20	1	80.0	60.0	70.0	60.0
	100	1	90.0	70.0	70.0	50.0
	500	1	100.0	60.0	80.0	60.0
Crude saponins of C. officinalis	20	1	60.0 (4.0)	60.0 (4.0)	50.0 (4.0)	50.0 (4.0)
	100	1	30.0 (2.0)	30.0 (2.0)	0	0
	500	1	0	0	0	0

Oleanoglycotoxin-A	10	2	55.0 (4.0)	55.0 (4.0)	50.0 (4.0)	50.0 (4.0)
	20	2	55.0 (4.0)	50.0 (4.0)	55.0 (4.0)	55.0 (4.0)
	40	2	60.0 (4.0)	50.0 (4.0)	50.0 (4.0)	55.0 (4.0)
	80	2	60.0 (4.0)	45.0 (4.0)	40.0 (4.0)	55.0 (4.0)
	100	1	50.0 (4.0)	50.0 (4.0)	30.0 (4.0)	40.0 (4.0)
	500	1	0	0	0	0
Lemmatoxin	5	2	55.0 (4.0)	50.0 (4.0)	50.0 (4.0)	35.0 (4.5)
	20	2	30.0 (4.0)	25.0 (4.0)	30.0 (3.5)	22.5 (3.5)
	80	2	0	0	0	0
Oleanolic acid hemisuccinate	20	1	90.0	70.0	80.0	70.0
	100	1	80.0	70.0	70.0	50.0
Oleanolic acid cellobioside	10	1	70.0 (4.0)	60.0 (4.0)	60.0 (4.0)	40.0 (4.0)
	20	1	80.0 (4.0)	60.0 (4.0)	40.0 (4.0)	50.0 (4.0)
	40	1	70.0 (4.0)	50.0 (4.0)	40.0 (4.0)	30.0 (4.0)
	80	1	60.0 (4.0)	50.0 (4.0)	30.0 (4.0)	40.0 (4.0)
Chikusetsusaponin-IV	20	2	70.0 (4.0)	60.0 (4.0)	65.0 (4.0)	60.0 (4.0)
	40	2	65.0 (4.0)	65.0 (4.0)	65.0 (4.0)	55.0 (4.0)
	80	2	60.0 (4.0)	60.0 (4.0)	60.0 (4.0)	55.0 (4.0)
	100	1	60.0 (4.0)	50.0 (4.0)	70.0 (4.0)	50.0 (4.0)
	500	1	70.0 (4.0)	50.0 (3.0)	50.0 (4.0)	40.0 (4.0)
Lemmatoxin-C*	5	2	55.0 (3.5)	40.0 (2.0)	35.0 (1.5)	40.0 (1.5)
	20	2	2.5 (4.0)	5.0 (4.0)	0	0
	100	1	0	0	0	0
Rat epididymal sperm control	-	2	55.0 (3.5)	50.0 (2.5)	35.0 (1.5)	40.0

* Rat epididymal sperm used.

CONTRACEPTION

Table II

PROBIT ANALYSIS WITH ED_{50} AND ED_{90} LEVELS AT 15 MINUTES

Compound or Extract	ED_{50} (95% Fiducial Limits) [µg/ml]**	ED_{90} (95% Fiducial Limits) [µg/ml]**
Crude saponins of *P. dodecandra*	10.7 (7.5-15.2)	40.5 (25.8-63.7)
Nonoxynol-9	27.0 (21.8-33.5)	52.7 (37.9-73.3)
Oleanoglycotoxin-A	134.2 (75.1-239.7)	276.5 (68.4-1118.1)
Lemmatoxin	10.7 (5.2-21.8)	42.3 (17.7-100.9)
Lemmatoxin-C*	8.7 (4.4-16.9)	20.0 (9.9-40.4)

* Tested against rat epididymal sperm.

** Final concentration of incubation medium.

and lemmatoxin-C in approximately equal ratios (6-8). It is also known that the saponins of P. dodecandra contain oleanolic acid, whereas the principal aglycone from the P. americana saponins is phytolaccagenin (15), an oxidized form of oleanolic acid.

Other differences in activity may be attributable to the types and numbers of sugars and their positions of attachment to the aglycone. Surface activity could not account for the differences in spermicidal activity among purified compounds such as lemmatoxin, oleanoglycotoxin-A, and chikusetsusaponin IV--all of which contain the same aglycone, and all of which are trisaccharides. On the other hand, the low activity of chikusetsusaponin IV may be attributable to the lack of a free C-28 carboxylic acid group.

The total content of saponins in C. officinalis represents a small percentage of the dry matter from the flowers. In contrast, saponins represent one-quarter of the dry weight of the P. dodecandra fruit and are available in considerable quantities from pilot plants producing a molluscicide on the African continent.

It is important to point out that there was some variation in spermicidal activity of a compound or extract in different tests, which was undoubtedly due to differences in seminal fluid content and sperm cell fragilities between donors. If washed spermatozoa had been used instead of whole diluted semen, it might have reduced this variability to some extent. Because of severe limitations in supply, particularly of purified plant saponins and synthetic derivatives, we were restricted in the number of possible trials with some compounds, particularly at the higher concentrations tested.

Conventional tests, such as the methods of Brown and Gamble (16) or Sander and Cramer (17), are designed to compare the contraceptive efficacy of commercial products or purified compounds by determination of the highest dilution that achieves instantaneous immobilization of sperm. However, our primary objective was to demonstrate that saponins derived from plants had potent spermicidal activities and that slight modification in structure could profoundly alter their effectiveness. Further work will be required to determine the potential usefulness of one or more naturally occurring plant saponins or synthetic derivatives.

In recent years, the development of new vaginal contraceptive agents has received limited attention. Recently, a highly effective detergent, polyoxyethylenated p-menthanyl phenol (TS-88), formulated as a vaginal foam tablet, has appeared in Japan as a replacement for mercurial-containing products (18). Field tests with this product indicated a pregnancy rate of 0.7 per 100 woman-years (18). Using an aerosol form of 8% polyoxyethylenated nonyl phenol, Bernstein obtained a pregnancy rate of 3.98 per 100 woman-years (19). With appropriate use, a vaginal preparation has been considered to be one of the simplest, safest, most effective, and most economical contraceptives (18). Side effects are virtually nonexistent, except for occasional localized irritation to the female or male genitalis exposed to the preparation. Our previous experience with the saponins from P. dodecandra indicates that they are not irritating and are relatively free of oral toxicity when administered in large quantities to rats (5).

ACKNOWLEDGEMENTS

The authors are indebted to Dr. E. J. Lamb and Mrs. O. Izdebski of Stanford Medical School for obtaining the human sperm samples and to Professor Isao Kitagawa of Osaka University for the sample of chikusetsusaponin IV. We are also indebted to Dr. R. H. Foote of Cornell University for his valuable suggestions, and to Drs. W. A. Skinner and C. Mitoma for encouraging this work. Dr. G. Pryor contributed substantially to this report by obtaining ED_{50} and ED_{90} values and probit analysis of the data.

REFERENCES

1. Lemma, A. A preliminary report on the molluscicidal property of endod (Phytolacca dodecandra). Ethiopian Med J 3: 187 (1965).

2. Lemma, A., Brody, G., Newell, G.W., Parkhurst, R.M., and Skinner, W.A. Studies on the molluscicidal properties of endod (Phytolacca dodecandra): I. Increased potency with butanol extraction. J Parasitol 58: 104 (1972).

3. Maxwell, W.A. Unpublished observations of studies at SRI.

4. Spielman, A., and Lemma, A. Endod extract, a plant-derived molluscicide: Toxicity for mosquitoes. Am J Tropical Med Hygiene 22: (6) 802 (1973).

5. Lemma, A. Studies on the molluscicidal properties of endod (Phytolacca dodecandra). Final Report, Project LSU-8972, ONR, Contract N00014-71-C-0123. Stanford Research Institute, Menlo Park, California (June 1972).

6. Parkhurst, R.M., Thomas, D.W., Skinner, W.A., and Cary, L.W. Molluscicidal saponins of Phytolacca dodecandra: Oleanoglycotoxin-A. Phytochem 12: 1437 (1973).

7. Parkhurst, R.M., Thomas, D.W., Skinner, W.A., and Cary, L.W. Molluscicidal saponins of Phytolacca dodecandra: Lemmatoxin. Canadian J Chem 52: 702 (1974).

8. Parkhurst, R.M., Thomas, D.W., Skinner, W.A., and Cary, L.W. Molluscicidal saponins of Phytolacca dodecandra: Lemmatoxin-C. Indian J Chem (in press).

9. King, T.A., Jewers, K., Richardson, H., and Falshaw, C.P. Endod saponin, the molluscicide of the fruit of endod (Phytolacca dodecandra). Fifth International Symposium on the Chemistry of Natural Products, London, 1968.

10. Kasprzyk, S., and Wojciechowski, Z. The structure of triterpenic glycosides from the flowers of Calendula officinalis. Phytochem 6: 69 (1967).

11. Mann, T. The biochemistry of semen and of the male reproductive tract. Methuen and Co. Ltd., London, and John Wiley and Sons Inc., (1964), p. 347.

12. Gwatkin, R.B.L., and Williams, P.T. Inhibition of sperm capacitation in vitro by contraceptive steroids. Nature 227: 182 (1970).

13. Finney, D.J. Probit Analysis. 2nd Ed., Cambridge University Press, 1952.

14. Kondo, N., Shoji, J., Nagumo, N., and Komatsu, N. Studies on the constituents of Panacis japonici rhizoma II. The structure of chikusetsusaponin IV and some observations on the structural relationship with araloside-A. Yakugaku Zasshi 89: 957 (1964).

15. Stout, G.H., Malofsky, B.M., and Stout, V.F. Phytolaccagenin: A light atom X-ray structure proof using chemical information. J Am Chem Soc 86: 957 (1964).

16. Brown, R.L., and Gamble, C.J. Method of testing relative spermicidal effectiveness of contraceptives and its application to 10 commercial products. Human Fertil 5: 97 (1940).

17. Sander, F.V., and Cramer, S.D. Practical method for testing action of chemical contraceptives. Human Fertil 6: 134 (1941).

18. Ishihama, A., and Inoue, T. Clinical field tests of a new contraceptive vaginal foam tablet. Contraception 6: 401 (1972).

19. Bernstein, G.S. Clinical effectiveness of an aerosol contraceptive foam. Contraception 3: 37 (1971).

REPRINTED FROM
FEDERATION PROCEEDINGS
VOL. 34, NO. 3, MARCH 1, 1975
PRINTED IN THE U.S.A.

BLASTOCIDAL AND CONTRACEPTIVE ACTIONS OF SAPONINS FROM PHYTOLACCA DODECANDRA L. Sidney J. Stolzenberg, Robert M. Parkhurst,* and Elmer J. Reist.* SRI, Menlo Park, CA 94025

Saponins derived from the triterpene oleanoleic acid occur in the plant Phytolacca dodecandra L., found in Africa. They exhibit molluscicidal, larvicidal, fungicidal, and spermicidal activities. A crude saponin extract and purified derivatives of the extract have shown extremely low toxicity by the oral route in mice and a lack of mutagenicity in tissue culture studies. We now report blastocidal and contraceptive activities by the extract and two purified compounds derived from the extract when administered in utero to rats.

The saponin extract and purified compounds in 0.1 ml of physiological saline were injected at a series of concentrations into one of the two uterine horns of rats on Days 1, 4, or 6 of pregnancy. The contralateral horn served as an untreated or sham-injected control. Saline had no detectable effect on embryo counts when injected on Days 1, 4, or 6. However, treatment with the crude saponin extract or either of two purified compounds has terminated pregnancy in most cases or reduced embryonic counts in the treated horns down to the lowest levels tested to this date. The following figures represent the lowest levels tested to this date and found active: crude saponin extract--500, 500, 100 μg on Days 1, 4, and 6, respectively; lemmatoxin--500 μg on all three days; oleanoglycotoxin-A--100 μg on Day 4, no data available for Days 1 and 6. Microscopic studies of treated uteri are in progress. (Supported by NIH Contract NO1-HD-4-2833)

BLASTOCIDAL AND CONTRACEPTIVE ACTIONS BY AN EXTRACT AND COMPOUNDS FROM ENDOD (*PHYTOLACCA DODECANDRA*)

S. J. Stolzenberg
Endocrinology Laboratory
Biomedical Research Department

R. M. Parkhurst
E. J. Reist
Pharmaceutical Chemistry Department

Life Sciences Division
Stanford Research Institute
Menlo Park, California 94025

ABSTRACT

An extract consisting primarily of the saponins derived from the Endod plant (*Phytolacca dodecandra*), three purified trisaccharides derived from the extract, and a synthetic disaccharide were tested for antifertility activity on Days 1, 4, and 6 of pregnancy. Known quantities of the crude extract or purified compounds were injected into one of the two uterine horns of rats, and the contralateral horn served as a control. Separate control groups received physiological saline in one of the two horns. Vaginal smears were routinely observed each morning, and the presence of vaginal sperm determined Day 1 of pregnancy. Then, intrauterine injections were performed by surgery on Days 1, 4, and 6 of pregnancy. Treatment with proper dose levels of the crude saponin extract, lemmatoxin, and oleanoglycotoxin-A was found to be capable of preventing pregnancy or reducing the embryonic count on all three days. Lemmatoxin-C-C' was also tested on Day 1 and found effective at proper dose levels. On Days 1 and 4, oleanolic acid cellobioside had little or no effect on pregnancy when injected *in utero* at doses up to 1,000 µg. Endod extract caused substantially lower decreases in surface tension of water than Nonoxynol-9, yet showed 5 to 10 times the antifertility activity when administered on Day 1. To test whether a substance acted on the uterine horn to prevent implantation, Endod extract was injected on Day 1 into one of two uterine horns at a high level, sufficient to prevent pregnancy on the treated side. When the oviducts were flushed the third day after mating, 4 out of 5 rats had embryos on the treated side which were in similar stages of development as on the untreated side. Under the conditions of these experiments, there was no obvious damage to the uterine endometrium observed histologically due to treatment with purified compounds. It is suggested that this or related classes of compounds may be useful as abortifacients during early stages of pregnancy.

Accepted for publication May 12, 1976

INTRODUCTION

Saponins derived from the triterpene, oleanolic acid, occur in the plant Endod (*Phytolacca dodecandra*), which is found on the African continent. These saponins have been known previously for their molluscicidal properties (1,2) and, as such, have been used for vector control of schistosomiasis. The saponins are also known for their piscicidal properties (1) and for their ability to destroy mosquito larvae at a particular stage of the larva's development (2); in addition, they exhibit selective activity against dermatophytes but show little or no activity against other fungi or bacteria tested (2). More recently, a butanol extract of saponins from Endod, representing about 25% by weight of the dried berries, and three purified derivatives isolated from the extract demonstrated *in vitro* spermicidal activity (3). The blastocidal, abortifacient, and antifertility activities shown by the crude extract and three purified compounds are reported here.

MATERIALS AND METHODS

The *P. dodecandra* extract used in these experiments is a tan colored powder representing a butanol extract by a method previously described (1). The three purified trisaccharides of oleanolic acid isolated from the berries of *P. dodecandra* are designated as lemmatoxin, oleanoglycotoxin-A, and lemmatoxin-C, and a synthetic disaccharide of oleanolic acid is designated as oleanolic acid cellobioside. Isolation procedures and proof of structural configurations for three natural products have already been published (4,5,6). The C compound used in these experiments was contaminated with an isomer, in which the rhamnose occupied a different position on the chain, and is therefore referred to as lemmatoxin C-C'. The cellobioside was prepared by condensation of oleanolic acid with acetobromocellobiose according to the conditions described by Lemieux et al. (7). This afforded us an opportunity to compare a disaccharide with trisaccharides of the same triterpene. Structures of oleanolic acid and compounds containing the triterpene that were tested in these experiments are shown in Figure 1.

To carry out the study, we used mature, virgin, 60-day old, female Sprague-Dawley rats. The animals were acclimatized for a minimum of 4 days in a room with a controlled 14 hours of lighting per day and temperatures ranging from 73° to 75°F; then they were mated with males of proven fertility. Vaginal smears were checked each morning, and the presence of copulation plugs and vaginal sperm determined Day 1 of pregnancy. When mating had occurred, the females were assigned randomly to a designated treatment group.

Surgery was performed on the rats under aseptic conditions and ether anesthesia. An incision measuring 1½ to 2 cm was made along the linea alba in the lower abdominal region to exteriorize the uterus. Half the animals in each group received an injection of the test substance in

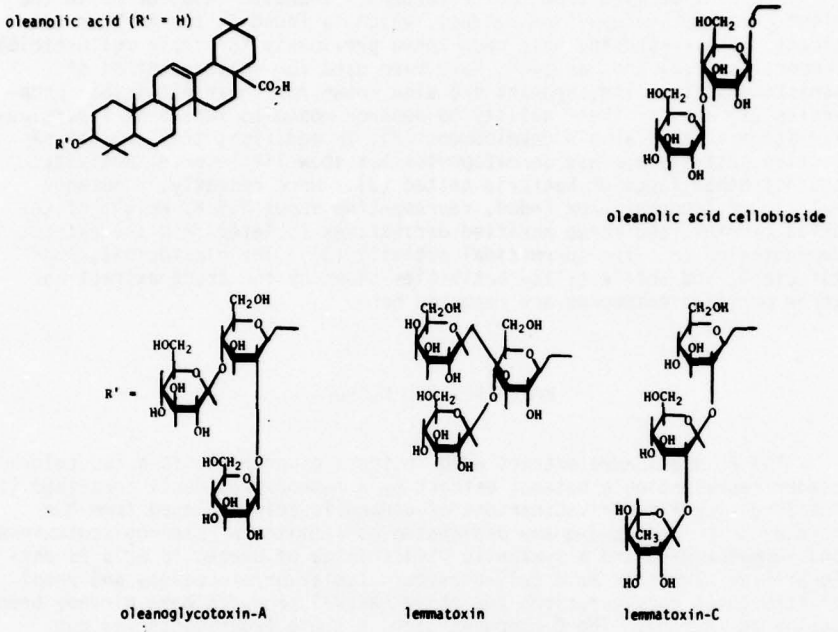


FIGURE 1 STRUCTURES OF PURIFIED COMPOUNDS TESTED

0.1 ml saline into the left or right uterine horn approximately 1 cm above the cervix; the injection was administered on Days 1, 4, or 6 of pregnancy.* The contralateral horn served as a control and received either a sham injection with a 26.5 gauge needle or no treatment. A separate control group received 0.1 ml saline in either the right or left uterine horn.

Following the injection, the abdominal musculature was sutured with 3-0 chromic catgut and the skin incision closed with 9-mm autoclamps. An autopsy was performed between Days 8 and 12 of pregnancy, during which embryo counts of the treated and control horns were made.

---

* Day 1 represents a period that is less than 24 hours after coitus, Day 4 represents a day of uterine sensitivity when implantation is occurring or about to occur, and Day 6 represents a time after implantation has occurred.

Repeated analyses of measures of variance were done, comparing the two horns for each rat and the effect of drug treatment. This was followed by "t" test comparisons between control and treated means within each horn. The P values listed in the tables represent the statistical significance of the "t" test comparisons.

Six-micron tissue sections were stained with hematoxylin-eosin for examination by light microscopy. These tissue sections included uterine horns prepared from animals in which pregnancy had been terminated by means of intrauterine injection of purified compounds. Also, uterine tissue sections were taken between conceptuses of control (saline-treated and non-treated) horns and from nonpregnant rats with normal 4- to 5-day estrous cycles on Day 2 of diestrus (2 days after a vaginal cornified smear).

RESULTS

The data in Table I indicate that on Day 4 of pregnancy, intrauterine injection of the crude saponin extract (at levels ranging from 2,000 µg down to 500 µg), lemmatoxin (500 µg), and oleanoglycotoxin-A (at levels of 250 µg and 100 µg) prevented implantation in almost every case. Embryo counts made from the drug-treated horns were significantly lower than those made from the saline-treated control horns ($P < 0.01$). Embryo counts from the nontreated contralateral horns were significantly lower ($P < 0.05$) in the groups that received 1000µg of the crude saponin extract and 500 µg of lemmatoxin. This suggests that a substance injected into one horn may be reaching the contralateral horn at a low concentration but in an amount that is sufficient to influence the embryo count of the untreated horn.

In every case, the lowest level of natural trisaccharide products tested on Day 4 of pregnancy was active. Further experiments are required to determine the lowest effective dose levels. On the other hand, the cellobioside caused a 50% decrease in the number of animals with pregnancy in the injected horn only at the highest level tested, 1,000 µg. Nevertheless, no statistically significant differences occurred in mean embryo count between the cellobioside-treated and control horns.

The data in Table II indicate that the crude extract of *P. dodecandra*, lemmatoxin, and oleanoglycotoxin-A terminated pregnancy on Day 6 when administered *in utero* at all levels tested ($P < 0.01$). The lowest level tested for each substance was 100 µg. In the untreated contralateral horns, only the group treated with 100 µg lemmatoxin showed an increase in viable embryo counts ($P < 0.05$).

Large numbers of nonviable decomposing embryos were found in all drug-treated uterine horns ($P < 0.01$); however, few nonviable embryos were present in the contralateral untreated horns. These large numbers of nonviable embryos occurred only in animals treated on Day 6, indicating that pregnancy was terminated after the occurrence of implantation.

Table I

PREVENTION OF IMPLANTATION BY A CRUDE EXTRACT FROM *P. DODECANDRA*, TWO PURIFIED TRISACCHARIDES, AND A SYNTHETIC DISACCHARIDE
(Day 4)

Exp.	Test Substance	Injected (μg)	Number Pregnant / Number Treated: Treated Horn	Number Pregnant / Number Treated: Control Horn	Mean Number Viable Embryos at Autopsy ± S.E.: Treated Horn	Mean Number Viable Embryos at Autopsy ± S.E.: Control Horn
1	Control	--	6/6	6/6	7.2 ± 0.8	7.8 ± 0.8
	P. dodecandra extract	2000	0/6	6/6	0.0 ± 0.0†	5.8 ± 1.2
	"	1000	0/6	6/6	0.0 ± 0.0†	5.5 ± 0.8*
	"	500	2/6	6/6	0.5 ± 0.3†	6.2 ± 0.4
	Lemmatoxin	500	0/6	6/6	0.0 ± 0.0†	5.3 ± 0.4*
2	Control	--	6/6	6/6	5.8 ± 0.4	6.8 ± 0.8
	Oleanoglycotoxin A	250	1/6	6/6	0.2 ± 0.2†	6.2 ± 0.5
	"	100	0/6	6/6	0.0 ± 0.0†	5.3 ± 0.5
3	Control	--	4/5	5/5	3.8 ± 1.3	7.0 ± 0.6
	Oleanolic acid cellobioside	250	4/4	4/4	3.8 ± 0.5	6.0 ± 0.3
	"	500	5/6	5/6	2.8 ± 0.8	4.3 ± 1.0
	"	1000	3/6	6/6	1.7 ± 0.8	4.7 ± 1.0

* $P < 0.05$

† $P < 0.01$

Table II

EMBRYOCIDAL ACTION BY A CRUDE EXTRACT OF *P. DODECANDRA* AND TWO PURIFIED EXTRACTS
(Day 6)

Exp.	Test Substance	Injected (µg)	Number Pregnant / Number Treated: Treated Horn	Number Pregnant / Number Treated: Control Horn	Mean Number Viable Embryos at Autopsy ± S.E.: Treated Horn	Mean Number Viable Embryos at Autopsy ± S.E.: Control Horn	Mean Number Nonviable or Decomposing Embryos: Treated Horn	Mean Number Nonviable or Decomposing Embryos: Control Horn
4	Control	--	6/6	5/6	6.7 ± 0.7	5.5 ± 1.2	0.2 ± 0.2	0.0 ± 0.0
	P. dodecandra extract	500	0/6	6/6	0.0 ± 0.0†	7.0 ± 0.6	6.2 ± 0.7†	0.7 ± 0.3
	"	250	0/6	6/6	0.0 ± 0.0†	6.7 ± 1.1	6.7 ± 1.2†	0.2 ± 0.2
	"	100	0/6	6/6	0.0 ± 0.0†	7.3 ± 1.1	5.8 ± 0.5†	0.0 ± 0.0
	Lemmatoxin	500	2/6	5/6	0.3 ± 0.2†	5.6 ± 1.2	5.2 ± 0.8†	0.0 ± 0.0
	"	100	0/3	3/3	0.0 ± 0.0†	8.3 ± 0.9*	4.7 ± 0.9†	0.0 ± 0.0
	Oleanoglyco-toxin-A	100	2/6	5/6	1.0 ± 0.8†	5.5 ± 1.1	4.2 ± 1.2†	1.5 ± 1.5

* $P < 0.05$

† $P < 0.01$

In the remaining experiments, all test substances were administered on Day 1 of pregnancy. The objectives were to determine (a) if pregnancy occurred when the substance was administered at the earliest possible time following coitus and (b) if there were structure-activity relationships between the compounds tested.

Table III (Experiment 5) indicates that oleanoglycotoxin-A, administered on Day 1 of pregnancy, caused no significant decrease in embryo count in the treated horns at the 250-µg level. Therefore, we raised the level of oleanoglycotoxin-A to 500 µg (Experiment 6). At that level, the difference in embryo counts between the treated and the control group was statistically significant ($P < 0.05$). In the same experiment, the crude extract was tested from levels of 250 µg down to 25 µg. At the 250- and 100-µg levels, there were no viable embryos in the treated horns. At the 50- and 25-µg levels, the differences in mean number of embryo counts were not statistically significant. Lemmatoxin was tested at lower levels ranging from 250 µg down to 25 µg (Experiment 7). A decrease in the mean number of embryo counts occurred at the 250 µg ($P < 0.05$), 100-µg ($P < 0.05$), and 50-µg ($P < 0.01$) levels. Nevertheless, pregnancy was not completely terminated in the treated horns since four of six animals in the three groups still had viable embryos, although at a reduced number compared with the control group. At the 25-µg level, lemmatoxin was not active. The mean embryo count decreased slightly in the contralateral untreated horn in the 50-µg lemmatoxin treated group ($P < 0.05$). Experiment 7 also indicates that lemmatoxin C-C′ caused a significant decrease in embryo count at the 500-µg ($P < 0.01$) and the 250-µg ($P < 0.01$) levels, but the difference was not statistically significant at the 100-µg level.

In contrast to the crude extract and the three trisaccharides isolated from the crude extract, the synthetic disaccharide, oleanolic acid cellobioside, did not prevent pregnancy in the injected horns of six rats per group at the 500- or 1,000-µg levels. The decrease in embryo count in the treated horn at the 1,000-µg level was not statistically significant although the difference was significant ($P < 0.05$) in the contralateral control horn.

An experiment was performed to determine if a substance that prevented pregnancy when injected *in utero* on Day 1 of pregnancy had an effect either on the uterine endometrium to prevent implantation or in the oviduct where it could influence embryonic development. Endod extract was injected at the level of 500 µg into one of the two uterine horns of five rats on Day 1 of pregnancy. This substance and dosage were selected because in previous experiments no embryos were found in the treated horns when inspected 8 to 10 days later at the level of 100 µg or greater. When the oviducts were flushed with physiological saline by means of a 23-gauge blunt ended needle attached to a 3-cc syringe, the embryo counts in both the treated and the untreated horns were similar (Table IV).

Table III

PREGNANCY PREVENTION CAPABILITY OF *P. DODECANDRA* EXTRACT AND FOUR STRUCTURALLY RELATED COMPOUNDS
(Day 1)

Exp.	Substance	Injected (μg)	Number Pregnant / Number Treated: Treated Horn	Number Pregnant / Number Treated: Control Horn	Mean Number Viable Embryos at Autopsy ± S.E.: Treated Horn	Mean Number Viable Embryos at Autopsy ± S.E.: Control Horn
5	Control	--	6/6	6/6	6.3 ± 1.1	5.5 ± 1.0
	Oleanoglycotoxin-A	250	5/6	5/6	3.5 ± 0.8	4.8 ± 1.1
6	Control	--	4/6	5/6	4.1 ± 1.5	4.0 ± 1.0
	Oleanoglycotoxin-A	500	1/6	5/6	1.0 ± 1.0*	4.8 ± 1.1
	P. dodecandra extract	250	0/6	4/6	0.0 ± 0.0†	4.5 ± 1.6
	"	100	0/6	5/6	0.0 ± 0.6	4.6 ± 0.5
	"	50	2/6	6/6	2.5 ± 1.6	5.0 ± 0.7
	"	25	5/6	6/6	5.5 ± 1.3	6.5 ± 0.8
7	Control	--	7/7	7/7	5.6 ± 0.7	5.7 ± 0.6
	Lemmatoxin	250	4/6	5/6	3.0 ± 1.0*	4.5 ± 1.4
	"	100	4/6	6/6	2.7 ± 1.0*	6.7 ± 0.6
	"	50	4/6	5/6	2.2 ± 0.9†	3.2 ± 0.9*
	"	25	3/4	3/4	4.0 ± 1.6	3.0 ± 1.1
	Lemmatoxin-C-C'	500	2/6	5/6	0.6 ± 0.4†	6.2 ± 1.3
	"	250	1/6	6/6	0.3 ± 0.3†	6.3 ± 0.8
	"	100	3/6	6/6	3.2 ± 1.4	5.2 ± 0.4
8	Control	--	6/6	6/6	6.2 ± 0.8	6.8 ± 0.3
	Oleanolic acid cellobioside	500	6/6	6/6	8.2 ± 0.8	4.5 ± 1.2
	"	1000	6/6	6/6	3.3 ± 1.1	4.3 ± 0.8*

* $P < 0.05$
† $P < 0.01$

Table IV

EFFECT OF ENDOD EXTRACT ADMINISTERED ON DAY 1 IN UTERINE HORN OF EMBRYOS IN THE OVIDUCT ON DAY 3

Number Pregnant / Number Nonpregnant		Mean Number Viable Embryos at Autopsy ± S.E.	
Treated Side	Control Side	Treated Side	Control Side
4/5	5/5	3.00 ± 1.05	4.40 ± 0.87

Further, the embryos in both oviducts of the same rat were at similar stages of cleavage, usually 4 cells, although some at 2 and others at later stages of cell division.

To test the hypothesis that the antifertility activity of saponins from *P. dodecandra* was not due solely to their detergent activities, we compared surface tension lowering activity and antifertility activity of the *P. dodecandra* with those of Nonoxynol-9.* The latter compound is a potent detergent, presently in widespread use as a spermicidal agent with intravaginal contraceptives. Unfortunately, comparisons with purified compounds containing the triterpene, oleanolic acid, were not possible because supplies were depleted.

A Fischer Surface Tensiometer was used for measuring the decrease in surface tension obtained by adding known quantities of Nonoxynol-9 or *P. dodecandra* extract to distilled water. A correction factor, which accounted for temperature of the water and properties of the tensiometer ring, was ignored because it had no effect on the final results. Each point on the plots in Figure 2 is representative of the mean of at least four determinations with the tensiometer at each concentration. The surface tension obtained for our distilled water (68.3 dynes/cm^2) deviated from the theoretical value of 73 dynes/cm^2, possibly because of minute impurities. However, it is clear from the plots in Figure 2 that the surface tension lowering capability for the *P. dodecandra* extract is considerably lower than that of Nonoxynol-9. Yet, the antifertility activity of the *P. dodecandra* extract on a weight basis was 5 to 10 times greater than that of Nonoxynol-9 when administered *in utero* on Day 1 of pregnancy (Table V). It is difficult to make these comparisons on a molar basis because of the mixture in the saponin extract. However, Nonoxynol-9 has an average molecular weight of 516, compared with 916 for compounds such as oleanoglycotoxin-A or lemmatoxin. The average number of hexoses for

*Poly(ethylene glycol)p-nonylphenyl ether. Kindly supplied by Union Carbide Company.

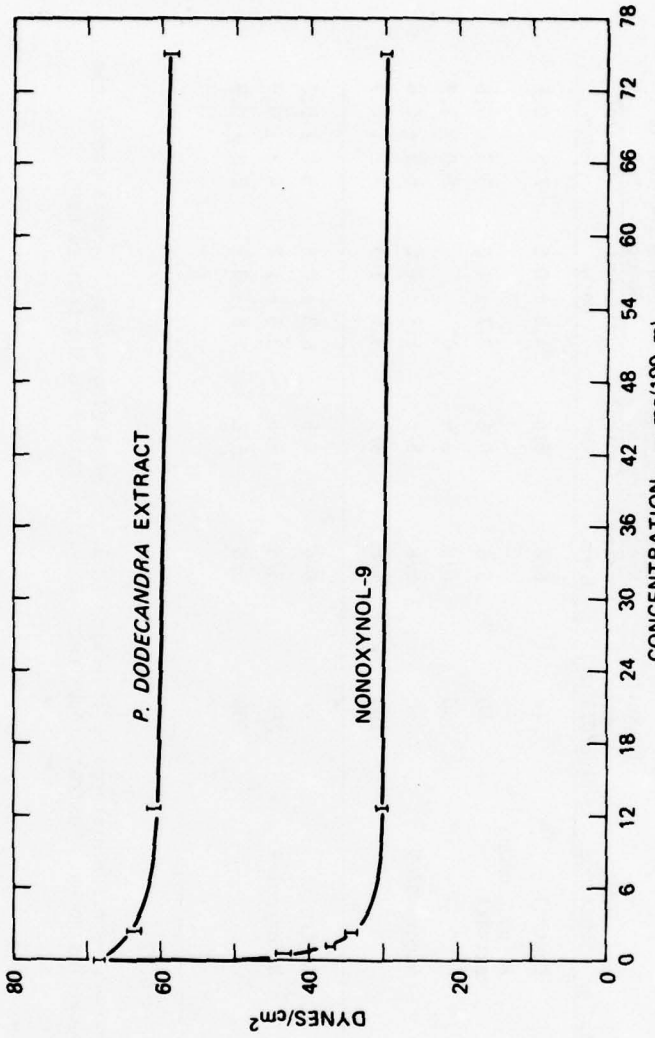


FIGURE 2 SURFACE TENSIONS AT A SERIES OF CONCENTRATIONS BY CRUDE EXTRACT OF *PHYTOLACCA DODECANDRA* AND NONOXYNOL-9

Table V

COMPARISON OF ANTIFERTILITY ACTIVITIES OF *P. DODECANDRA* AND NONOXYNOL-9

Exp.	Test Substance	Quantity Injected (μg/0.1 ml)	Number Pregnant / Number Treated: Treated Horn	Number Pregnant / Number Treated: Control Horn	Mean No. of Viable Embryos at Autopsy ± S.E.: Treated Horn	Mean No. of Viable Embryos at Autopsy ± S.E.: Control Horn
1	Control	--	6/6	6/6	4.8 ± 0.5	7.3 ± 0.6
	P. dodecandra extract	50	3/6	6/6	2.7 ± 1.2	5.7 ± 0.6
	"	100	0/6	5/6	0*	5.0 ± 1.2
	Nonoxynol-9	50	6/6	6/6	5.2 ± 0.8	6.5 ± 0.6
	"	100	4/6	6/6	3.5 ± 1.3	6.2 ± 0.9
2	Control	--	6/6	6/6	6.8 ± 0.8	5.0 ± 0.7
	Nonoxynol-9	250	5/6	6/6	3.5 ± 1.2	4.8 ± 0.9
	"	500	3/6	5/6	1.8 ± 0.9*	2.8 ± 0.9

* $P < 0.01$

Compounds were administered by injection in 0.1 ml physiological saline into one of the two uterine horns. Control groups received 0.1 ml saline in the treated horn.

each triterpenoid molecule in the extract has been estimated as 3.8 (8). Therefore, on a molar basis, it is possible that the *P. dodecandra* extract may be 10 to 20 times more active than Nonoxynol-9.

Histological Observations. Uterine horns were obtained from rats treated on Days 1 and 4 with up to 500 µg of lemmatoxin, on Day 4 with 250 µg of oleanoglycotoxin-A, and on Day 1 with 250 and 500 µg of lemmatoxin C-C′. All rats treated on Day 4 were sacrificed on Day 8, whereas those treated on Day 1 were sacrificed on Day 10. Under these conditions, none of the uteri appeared to have damage to the mucosal or connective tissue cells of the endometrial tissue surrounding the lumen or the endometrial glands.

Body Weight Gains. Although no statistical analyses were performed, body weight gains of treated animals were similar in most cases to those of saline-treated controls. There were no mortalities or signs of toxicity owing to intrauterine injections of any of the substances.

DISCUSSION

The present studies suggest that compounds such as those isolated from *P. dodecandra* may be useful as antifertility and possibly abortifacient agents. The crude extract was the most active substance tested, which may indicate the existence of still more active compounds that remain to be isolated from the extract. From the data in Table III, it is difficult to determine any clear-cut differences in antifertility activities between the three purified compounds tested on Day 1 of pregnancy. The trisaccharides were more active than the one disaccharide tested. Of the trisaccharides, oleanoglycotoxin-A appeared to be the least active of the trisaccharides tested, suggesting a structure-activity relationship due to differences in hexose moieties, to differences in the arrangement of the hexoses on the aglycone, or to both. In previous experiments, lemmatoxin was ten times more active than oleanoglycotoxin-A in its spermicidal activity (3).

The mechanisms of action for contraceptive activity by these compounds or the extract have not been studied in depth. Changes in surface tension within the uterine lumen would be expected to cause changes in permeabilities of the cell membranes in the endometrial tissue and possibly in the blastocysts. Nonoxynol-9 has a much greater capacity for lowering the surface tension than the extract of Endod but a substantially lower contraceptive activity. This suggests that other mechanisms of action may contribute to the effectiveness of the substances tested. In five of six cases, embryos at similar stages of development were found in both oviducts on Day 3 of pregnancy after injection of Endod extract on Day 1 at a concentration sufficient to prevent embryonic development in the injected horn. This may suggest an effect on the uterine endometrium to prevent implantation. The use of other substances for interruption of pregnancy by intrauterine application of solutions has been reviewed (9).

The crude extract and purified compounds obtained from *P. dodecandra* caused no changes in pH of distilled water or physiological saline. There were no signs of toxicity due to intrauterine injection of any of the substances tested, and body weight gains of treated groups were similar to those of the saline-injected controls. The crude extract has previously been shown to be devoid of mutagenic activity (2).

ACKNOWLEDGMENTS

This research was supported by a contract from the National Institute of Child Health and Human Development. The authors are grateful to Dr. Gordon Pryor for statistical analysis, to Dr. Kurt Metzner for his help with the chemical synthesis, and for the technical assistance of Diane Shoaf and Shirley Madan.

REFERENCES

1. A. Lemma, G. Brody, G. W. Newell, R. M. Parkhurst, and W. A. Skinner. Studies on the molluscicidal properties of endod (*Phytolacca dodecandra*): 1. Increased potency with butanol extraction. *J. Parasitol.* 58: 104 (1972).

2. A. Lemma. Studies on the molluscicidal properties of endod (*Phytolacca dodecandra*). SRI Final Report, prepared for the Office of Naval Research, June 1972.

3. S. J. Stolzenberg and R. M. Parkhurst. Spermicidal actions of extracts and compounds from *Phytolacca dodecandra*. *Contraception* 10: 135 (1974).

4. R. M. Parkhurst, D. W. Thomas, W. A. Skinner, and L. W. Cary. Molluscicidal saponins of *Phytolacca dodecandra:* Oleanoglycotoxin-A. *Phytochemistry* 12: 1437 (1973).

5. R. M. Parkhurst, D. W. Thomas, and W. A. Skinner. Molluscicidal saponins of *Phytolacca dodecandra:* Lemmatoxin-C. *Indian J. Chem.* 11: 1192 (1973).

6. R. M. Parkhurst, D. W. Thomas, and W. A. Skinner. Molluscicidal saponins of *Phytolacca dodecandra:* Lemmatoxin. *Can. J. Chem.* 52: 702 (1974).

7. R. V. Lemieux, K. B. Hendricks, R. V. Stick, and K. James. Halide ion catalyzed glycosidation reactions. Synthesis of α-linked disaccharides. *J. Amer. Chem. Soc.* 97: 4056 (1975).

8. R. M. Parkhurst. The Chemotaxonomy of *Phytolacca* species. *Indian J. Chem.* 13: 757 (1975).

9. Y. Manabe. Interruption of pregnancy at midterm by intrauterine application of solutions. *Obstet. Gynecol. Surv.* 27: 701 (1972).

Lemma, A., Demisse, M., and Mezengia B. (1968) *Eth. med. J. 6.* 61

PARASITOLOGICAL SURVEY OF ADDIS ABABA AND DEBRE ZEIT SCHOOL CHILDREN, WITH SPECIAL EMPHASIS ON BILHARZIASIS

Aklilu Lemma, B.A.(Wisc.) D. Sc. (Johns Hopkins)
Mesfin Demisse and Bahta Mezengia

From the Pathobiology Research Unit,
Faculty of Science, Haile Sellassie I University.

INTRODUCTION

Until recently, it was believed that bilharzia infection was absent from several major towns and cities of Africa. But planned surveys have now revealed that this disease is endemic in Cairo, Ibadan, Nairobi, Dar-es-Salaam, Salisbury, Johannesburg, and other major towns of Africa (W.H.O. *Epidemiological and Vital Statistics Report*, 1960). It was of interest, therefore, to know whether or not Addis Ababa should be included in this group.

Intestinal bilharziasis is widespread in Ethiopia, whereas urinary bilharziasis is localized only in some low and warm places in the Awash and the Webi Shebele Valleys. Possible bilharzia intermediate host snails (both *Biomphalaria* and *Bulinus* spp.) are present in the highlands (over 9000 ft. above sea level in Begemdir as well as in the lowlands all over the Empire (Ayad 1956, Brown 1964, Lemma 1965). The improvement of highways and rapid shifts of population in search of work and better opportunities, particularly towards the newly developing areas of the Awash Valley and some of the major towns such as Addis Ababa, Asmara, Dire Dawa and Nazareth will inevitably increase the spread of the disease. Even if the disease is absent from some of these areas now, it may be just a matter of time before it appears. For all these reasons, there is now an increasing awareness of bilharziasis and its possible public health and economic importance in Ethiopia.

A project for the examination of stool, and in some cases urine, specimens from school children in Addis Ababa and Debre Zeit was carried out during the months of July — August 1967. Debre Zeit was included in this study because the Pathobiology Research Unit obtains its large supply of *Bulinus* snails from Lake Hora. The presence of these possible urinary bilharziasis intermediate hosts, coupled with the fact that this area is extensively used for recreation (swimming, boating and fishing) by Imperial Ethiopian Air Force personnel and tourists, necessitated the need for a thorough search for the occurrence of the disease in that area as well.

Even though the main interest was bilharziasis, other parasites found during the stool examination are also reported and discussed herein.

MATERIALS AND METHODS

I. *Sampling Technique*

a) *Addis Ababa:*

As far as possible, the stool samples were collected from school children born

and raised in Addis Ababa and who had had very little chance of travelling outside the city and therefore of becoming infected elsewhere. The age of these children ranged from 5 — 15 years. They were from nine different tribes, and all were from the low socio-economic group.

In order to get a reasonable sample to detect the presence or absence of bilharzia in Addis Ababa, stool specimens were collected from children living in different parts of the city. The areas used for the survey were selected primarily on the basis of their geographical location in the city, and also on the presence of a river or a stream appropriate for possible transmission of the disease.

The city was divided into five different zones, each having a river or a stream running through it. *Zone A* was the Municipality — Tekle Haimanot Church area, *Zone B* was the Princess Tsehai Hospital — Building College area, *Zone C* was Africa Hall — Princess Zenebework School area, *Zone D* was Merkato— St. Paul Hospital area, and *Zone E* was the English School — British Embassy area. (See map for their location.)

Since the main government schools were closed for the summer vacation, small private schools had to be used. Ninety to a hundred stool samples were collected from children living in each of the above mentioned five zones. A total of 468 stools were collected and examined.

b) *Debre Zeit:*

In Debre Zeit, 90 male students (ages 8 - 17) out of about 300 Atse Libnedingle School students were selected on the basis of their ability to swim and their having been born, raised in and never having left Debre Zeit. Both stool and urine specimens were taken from these students for examination.

II. *Stool and Urine Examination*

The stool specimens were collected in paper cups and a small amount of each was immediately transferred into plastic vials containing 5-10 cc. of 5% formalin. These were transported to the laboratory, thoroughly mixed by shaking, and filtered through three layers of cheese cloth. The filtrate was then centrifuged at about 3000 RPM for 10 minutes and the sediment examined for ova and parasites.

III. *Malacological Study*

Six major rivers and streams of different sizes in Addis Ababa were surveyed for snails. The first two of these rivers are perennial, while the other four are seasonal. They are: 1, Kebena; 2, Little Akaki; 3, Kurtume; 4, Kechenie; 5, Buche, and 6, Ginfile. (See map for their location.)

In Debre Zeit, only Lake Hora was surveyed.

The following points were considered in the malacological study.

1. The presence or absence of appropriate snails
2. The suitability of the topography and general vegetation of the area for snail breeding
3. Contamination of the river with human waste
4. Human contact with the river
5. Examination of the snails for cercariae, and
6. Determination of possible host snails to transmit the disease in the laboratory

II. *Epidemiological and Malacological Studies*

a) *Addis Ababa:*

In Addis Ababa all six rivers surveyed were found contaminated with faeces and urine. It was observed that at several sites in the different streams, people wash themselves and their clothes with contaminated water. It was also observed that workers stand in the middle of Kurtume and Ginfile Rivers while collecting riverbed sand used for building purposes. People also use the apparently clean water of the river for drinking and washing household utensils.

In spite of a thorough search for snails in these rivers during the rainy season, none were found probably because of the rather large amount of water and its rapid flow. A similar search carried out during the dry season (December) has revealed the presence of numerous *Biomphalaria, Bulinus, Lymnaea,* and other unidentified species of snails in most of the above mentioned rivers However, none of the 100 *Biomphalaria pfeifferi* collected from Ginfile River shed any cercariae.

b) *Debre Zeit:*

Human contact with the lake water for recreational purposes (boating, fishing and swimming) is extensive throughout the year. Villagers in the neighborhood of the lake wash their bodies and clothes in the water. Large herds of animals also use the water for drinking.

Lake Hora is an excellent environment for the breeding of *Bulinus (Bulinus) truncatus sericinus* (Jick.), which is the only species of snails found there. During the rainy season, these snails are found in abundance almost everywhere on the shores of the lake. Subsequent surveys in the early parts of the dry season (December) have shown only a slight decline in population.

Over 90% of the snails found in this lake are free of cercariae, the remaining 10% harbour unidentified straight-tailed cercariae.

c) *Susceptibility Tests:*

B. pfeifferi collected from Addis Ababa was maintained in the laboratory and subjected to infection by *Schistosoma mansoni* miracidia. Fresh stool specimens from an infected boy from Adwa, in the northern part of Ethiopia, were obtained and the ova hatched in tap water. Previously examined clean *B. pfeifferi* snails were then exposed to the miracidia so obtained. The exposed snails were then kept in an air-conditioned laboratory at about 24°C. Frequent examinations of these snails showed that abundant cercariae were being shed about 6 weeks after infection. These cercariae were indistinguishable from the cercariae of *S. mansoni.* Mice were infected with these cercariaej at autopsy the mice had fully mature *S. mansoni* parasites and their ova showed in the portal veins and among the liver cells.

A similar experiment was carried out to determine the susceptibility of *B. (B.) t. sericinus* to *S. haematobium* ova. A patient with urinary bilharziasis was specially brought from Gewani, in the middle Awash Valley, for the purpose of giving fresh miracidia with which to infect the snails. The exposed snails were then put in an air-conditioned room at an average temperature of 24°C. Weekly examination of these snails for cercarial shedding over a period of 7 months showed no cercariae of any kind being shed. It was evident that these snails are not capable of transmitting urinary bilharziasis.

DISCUSSION

From the results of the present experiment, in which 468 Addis Ababa and 90 Debre Zeit students were examined, it appears that bilharziasis has not yet been introduced to Addis Ababa or Debre Zeit. In this respect, our results agree with those obtained by Ayad (1956) who found none of the 48 Addis Ababa and 12 Debre Zeit school children he examined to be positive for either intestinal or

urinary bilharziasis. Pavars (1956) also had not found bilharziasis among 7,500 school children of Addis Ababa. Furthermore, according to Ayad "negative reports were given for Addis Ababa by Nagelsbach (1934), with regard to *S. haematobium*, and by Mariani (1938), with regard to *S. mansoni*." However, the absence of the disease at this time does not mean that it is unlikely to be introduced in the near future. The migration of infected people to Addis Ababa and the presence of *B. pfeifferi*, which has now been proved in the laboratory to be susceptible to *S. mansoni*, is certainly evidence for the possible introduction of the disease into Addis Ababa any time. Undoubtedly the habitual tendency of the people to bathe, wash and work in the streams and rivers will contribute a great deal to the rapid spread of the disease once it is introduced.

Famigliari (1938) did not find either form of bilharziasis in Debre Zeit, but in 1939 L'Abbate drew attention to one patient suspected of having acquired a haematobium infection in "Lake Bishoftu" (Lake Hora); this case has also been cited by Scarpa (1940) and others. Ayad, however, after a series of investigations during his visit in Ethiopia, says "it appeared that the one case of haematobia infection reported from Lake Bishoftu was now generally considered to have contracted the disease while fishing in one of the affluents of the lake, because the lake itself is much frequented by Europeans, none of whom is reported to have acquired the disease."

Although the examination carried out on 90 students in this study is not sufficient to draw a firm conclusion on the absence of the disease from Debre Zeit, the fact that none of the numerous school children, the Air Force personnel, and other residents in the town had been shown to have either form of bilharziasis, and the failure of *B.(B.)t.sericinus*, the only species of snail found in the lake, to transmit *S. haematobium*, suggests that bilharziasis may not be easily introduced and is unlikely to become a major public health problem in this town in the near future.

The parasitological analysis of the survey shows ascariasis to be the most common infection both in Addis Ababa and Debre Zeit school children. Although eating of raw meat is a common practice in Ethiopia, taeniasis seems to have a surprisingly low incidence (2-7%). strangely, and paradoxically, this infection was detected in a predominantly Arab school in Zone A. Muslims are generally believed not to eat raw meat, but according to these results this may not be so. In the predominantly Christian (Amhara) school in Zone B, not a single case of tapeworm was found, possibly because children of the low socio-economic class have little access to raw meat. Out of 12 Gurage children whose normal diet consists of raw ground meat (Kitfo), only one had *Taenia*. This may be due to possible damage done to the cyst during the grinding of the meat.

Hymenolepis diminuta was found in only two out of the *468* stools examined. *H. nana* on the other hand was found to be more common (about 9%). This is probably due to its direct hand-to-mouth transmission, while an intermediate host is needed in the case of *H. diminuta*.

Amoebiasis also appears to be low (0.5%). Considering that the standard of sanitation is very poor, it was expected that the incidence of amoebiasis in Addis Ababa would be much higher.

Seventy-two per cent of all the Addis Ababa school children examined were positive for one or more parasites. The sex and age distribution of these cases show some differences. In the lower age group (5-10), males have more parasites than

females. But this is reversed in the 11 - 15 age group. This may be correlated with the behaviour and activities of male and female children at different ages.

The differences in the incidence of parasites in the children studied can however, only be accepted with some reservation. No statistical tests have been performed to determine the significance of these differences. Also, it must be noted that the sensitivity of the method used in the stool and urine examination may not be good enough to detect the presence or absence of all different species of parasites.

The results obtained in this experiment are very similar to those obtained by Wang (1963) in a survey of school children carried out in Begemdir Province. The rates of single and multiple infection correspond very closely, and ascariasis was also the most common infection in school children in Begemdir.

The rainy season is not a good time for malacological studies in the field. No snails were found in Addis Ababa during the months of July, August and September; but the *B. pfeifferi* collected from the same streams during the dry season has been proved to be capable of transmitting the disease in the laboratory. *B. pfeifferi* is also known to act as immediate host for the disease in other parts of the world (Mandahl-Barth 1960, Fisseha H. Meskal 1967).

Although *B.(B.)t.sericinus* is known to be the intermediate host of *S. haematobium* in Aden, we have failed to achieve transmission of the disease in our laboratory, even under optimal conditions of temperature and care. In a similar previous trial, Wright (1963) failed to transmit the Aden strain of *S. haematobium* through *B.(B.)t.sericinus* from Ethiopia. He attributed his failure to a possible difference of parasite-strain rather than the inability of this snail to transmit the disease. In our case, however, the infective parasite was obtained from an area only about 150 km. away; we realized the possible role of temperature in the development of *S. haematobium* parasites, and we were therefore careful to keep the miracidia-exposed snails in aquariums at an average temperature of 24°C. Although these snails were kept under these conditions for over 7 months, laboratory conditions are not identical to those in the field, and so this may not necessarily mean that this species of *S. haematobium* does not transit urinary bilarziasis in a natural environment. It is possible that the parasite will adapt itself, eventually, to transmission by this species, and it might therefore be valuable to study the differences in the chromosome numbers between *B.(B.)t. sericinus* from Aden (which is a known intermediate host of *S. haematobium)* and those from Debre Zeit or any other part of Ethiopia where it is abundantly found (Burch 1967).

SUMMARY

Four hundred and sixty-eight stool specimens from different parts of Addis Ababa and 90 stool and urine specimens from Debre Zeit school children were examined for *Schistosoma* and other parasites. The results of the examination are analysed. It is concluded that *S. mansoni* and *S. haematobium* have not yet been introduced into these two towns. *B. pfeifferi* collected from Addis Ababa streams was found to be able to transmit *S. mansoni* in the laboratory, whereas *B.(B.)t.sericinus* from Debre Zeit was unable to transmit *S. haematobium* recovered from a patient who was specially brought in from Gewani (middle Awash Valley).

RESUME

Quatre cent soisante huit échantillons de selles prélevés à Addis Abeba et 90 échantillons de selles et d'urine prélevés chez les enfants de l'Ecole de Debre Zeit furent soumis à un examen de laboratoire en vue de détecter la présence de *Schistosoma* et autres parasites. Les résultats des examens indiquent d'une façon concluante que le *S. mansoni* et le *S. haematobium* n'infectent pas encore les endroits où habitent ces enfants. On a la preuve que les *S. pfeifferi* récoltés dans les ruisseaux d'Addis Abeba peuvent tranmettre le *S. mansoni* in vitro tandis que le *B.(B.)t.sericinus* en provenance de Debre Zeit ne put pas transmettre le *S. haematobium* de Gawani.

Acknowledgements

The authors wish to acknowledge with gratitude the cordial assistance given by Shell Ethiopia, who provided the necessary petrol, and Shell Chemical Company of Eastern Africa, who donated a car for this and other work on bilharziasis in Ethiopia. They are also indebted to Dr. J. Duncan, Ato Fisseha H. Meskal and Dr. W.A. Foster for having read the manuscript and making some suggestions for improvement.

References

Ayad, N. (1956). Bilharziasis surveys in British Somaliland, Eritrea, Ethiopia, Somalia, the Sudan, and Yemen. *Bull. Wld. Hlth. Org. 14*, 1-117.

Bilharziasis in Various Countries, 1947-1959. Epidem. Vital Statist. Rep., *Wld. Hlth. Org.* Vol. 13, No. 6-7/1960.

Brown, D.S. (1964). The distribution of intermediate hosts of schistosoma in Ethiopia. *Eth. med. J. 2*, 250-9.

Burch, J.B. (1967). Some species of the genus Bulinus in Ethiopia possible intermediate hosts of schistosomiasis haematobia. *Eth. med. J. 5*, 245-56.

Famigliari, P. (1938). G. ital. Clin. trop. 2: 189.

L'Abbate, G. (1939). Rass Sanit. A.O.I. 1: 43.

Lemma, A. (1965). Schistosomiasis in Adwa: a report on an ecological pilot study. *Eth. med. J. 3*, 84-92.

Lo Wang. (1965). Helminthiasis in Begemdir and Semien Province, Ethiopia. *Eth. med. J. 4*, 19-26.

Haile Meskal F. (1967). A preliminary identification key to fresh water gastropod molluscs of Ethiopia with some comments on the medical significance of a few species. *Eth. med. J. 5*, 227-34.

Mandahl-Barth, G. (1960). Intermediate hosts of schistosoma in Africa. Some reccent information. *Bull. Wld. Hlth. Org., 22*, 565-73.

Pavars, J. (1957). (Personal Communication with Ayad).

Scarpa. (1940). Progr. Terap., Milano, No. 1 & 2.

Wright. (1963). (As quoted by Dr. Browning in personal communication).

Lemma, A. (1969 *Eth. med. J.* 7, 147.

BILHARZIASIS IN THE AWASH VALLEY: I. AN EPIDEMIOLOGICAL STUDY WITH SPECIAL EMPHASIS ON ITS POSSIBLE FUTURE ECONOMIC & PUBLIC HEALTH IMPORTANCE

Akliku Lemma, Sc. D.

From the Institute of Pathobiology, Faculty of Science, Haile Sellassie I University, P. O. Box 1176, Addis Ababa, Ethiopia.

INTRODUCTION

Ethiopia's new opportunities for large-scale agricultural development

The Imperial Ethiopian Government, with the assistance of the UN/FAO Special Fund, has started a project to develop over 150,000 hectares (1,500 sq. km.) of irrigated land in the Awash Valley. According to the FAO/SF Report (1965), this agricultural development project is expected to increase the national income by Eth. $181 (U.S.$72.4) million a year. In addition, it is envisaged that by building dams and hydro-electric power supplies in the Tendaho and Kassem areas on the Awash River, a total of 237 million kwhr of electricity worth Eth. $16 (U.S. $6.4) million a year can be generated; also, the water controlled by these dams would increase the irrigable area in the Awash Valley to a total of over 500,000 hectares, possibly enabling a total national earning of about Eth. $800 (U.S. $320) million a year.*

The Awash Valley Authority has been established and entrusted to direct, promote and co-ordinate the entire development plan for achieving the above mentioned objectives. Thousands of people from all over the country, especially from the more heavily populated highland areas, have already started moving into the newly developing areas of the Awash Valley in search of work and better opportunities. This mass migration is expected to increase as development proceeds. To facilitate transportation and to open up the area under development, a new highway and railway system are being planned to link the town of Nazareth, about 100 km. south-east of Addis Ababa, with the seaport of Assab, about 900 km. north-east of Addis Ababa.

Now that malaria, the disease which for centuries had made this and other potentially rich valleys inaccessible, is being rapidly controlled, the country is emerging into a new phase of unlimited opportunities for development. In addition to the Awash Valley, other potentially rich valleys, such as that of the Wabi Shebelle, Blue Nile, Tekese, Baro, and Omo, are opening up for agricultural and hydro-electric development. Various experts from all over the world are presently engaged in planning, promoting, and co-ordinating these development projects.

---

* Calculated from figures given in FAO/SF Report 1965.

The public health and economic importance of bilharziasis in agricultural development projects.

Experience around the world, especially in the tropical parts of Africa, Asia and South and Central America, shows that any extensive agricultural development scheme, such as the Awash Valley project, is almost always crippled by the problem of bilharziasis. Irrigation canals and water reservoirs provide excellent new breeding sites for the intermediate host snails of the parasite. Large numbers of infected and uninfected people working in these places and utilizing the water for washing, bathing, and drinking, enable the parasite to spread rapidly and create ideal transmission sites for repeated infections.

Pesigan *et al.* (1958) noted that a single female *Schistosoma* can lay up to 3,500 eggs per day, averaging 1,200. The eggs are normally deposited in the liver mesenteric tissues and urinary bladder where they cause considerable pathological damage and combat the various body defence mechanisms. When one realizes that up to 20 to 40 worms can live in an infected person for as long as 20 to 30 years, it is indeed cause for alarm. From the public health point of view it is seen that the presence of the bilharzia parasite, especially heavy infections can lead to very serious consequences for the individual concerned. Even in those persons who are not incapacitated, their capacity for hard work may be lowered considerably. This will, of course, reflect on the economic potential of a country especially one which relies to a large extent on agricultural or more specifically irrigated agricultural pursuits for its main source of income.

With chronic schistosomiasis, it is particularly difficult to make quantitative estimations of its economic importance. Furthermore, surveys for the incidence of such diseases may be completely meaningless if they bear no relation to the intensity or morbidity of the infection. In schistosomiasis, where most cases show no obvious acute episode, it is easy enough to dismiss the disease on the whole as unimportant. However, Forsyth and Bradley (1966) reported that in Tanzania *S. haematobium* gave rise to hydronephrosis, ureteric lesions or non-functioning kidneys in more than 20% of the children, whereas in adults such lesions occurred in only 10% of the cases examined. These figures tend to indicate that a considerable mortality may exist in children.

Certain other socio-economic consequences of the lack of disease control should be pointed out. Those countries, which are dependent on the tourist trade could easily be crippled because visitors will avoid them on account of their reputation. Possibly even more serious for the potential long-term growth of a country are the effects which parasitism may have on absenteeism in schools children. Moreover, it has been shown in Mexico (Biagi, 1963) that generalized heavy infections of *Schistosoma* parasites can affect mental growth and learning ability. A number of studies have also been made on the learning ability, activity and growth of rats and mice infected with schistosomiasis (Kershaw *et al.* 1955; Stretch *et al.* 1960; Hearnshaw *et al.* 1963). One of the conclusions from these works was that an acute infection with low-grade schistosomiasis reduces the motivation to solve a problem rather than reducing learning ability; i.e. produced a state of lethargy in the experimental mice. Maze-habit was also less readily acquired in infected as compared with non-infected albino rats.

Development projects which are initially full of good intentions may later have unfortunate corollaries as far as the spread of disease is concerned. Two examples will illustrate this in the case of bilharziasis. In the Aswan province

Egypt, it has been shown that *S. haematobium* increased from four to forty per cent within three years of the introduction of perennial irrigation (Blair, 1964). Secondly, when the incidence of the disease began to reach alarming proportions, the Umshandige Irrigation Scheme in Rhodesia had to be abandoned in 1949 after ten years of construction at a total cost of three million pounds sterling.

The extent of economic loss due to schistosomiasis in Egypt, Iraq, Japan and the Philippines was given by Farooq (1964). In Egypt, the loss is estimated to be about 80 million Egyptian pounds a year (Khalil, 1949). In Iraq, with one million infected individuals, Watson (1958) estimated a loss of U.S.$24 per infected individual. In Japan, the estimated loss is U.S.$18 million. In the Philippines, Farooq (1964) estimated the loss as 13 million pesos annually, and stated that "by comparison with a recent estimate of the economic loss due to malaria in the Philippines, it appears that a heavier economic burden is probably imposed by *S. japonicum* infection than by malaria."

It is estimated that about 200 million people in the world are presently suffering from bilharziasis and this number is increasing rapidly (WHO Bilharziasis Exp. Rep. 1965). In many of the developing tropical and subtropical countries, where problems are many and resources limited, bilharziasis is spreading more rapidly than it is being controlled. In areas where large agricultural development plans are being implemented, it is imperative that the settlers and labourers be healthy to realize the full economic potential of the area. The debilitating effects of bilharzia may involve such a heavy loss in manpower (hence in economy) that any large-scale agricultural development plan, made without due consideration of the importance of this disease, may be unrealistic. This holds true for the Awash Valley development project.

Bilharziasis in Ethiopia

Both intestinal and urinary bilharziasis are known to occur in undetermined degrees of prevalence in various localities in Ethiopia. The first few case reports on the occurrence of the disease in the country were reported by Italian physicians during World War II. Ayad (1956) made the first schistosomiasis survey, giving a comprehensive review of the literature up to 1952. He reported that intestinal bilharziasis due to *Schistosoma mansoni* was endemic in Lake Tana, Akaki, Bahr-Dar, Harar and Eritrea. This has since been repeatedly confirmed by the Ethiopian Nutrition Survey Team (1958), Chang (1961), Kubasta (1964), and others. Buck *et al.* (1965) and Lemma (1964) showed that intestinal bilharziasis is also highly endemic in Tigre province, particularly in the town of Adwa. On the other hand, Lemma, in a later survey, did not find any schistosomiasis in Addis Ababa or Debre Zeit. Also examination of 80 urine and 80 stool specimens collected from the indigenous inhabitants on the Baro River, particularly in Gambela and Pokwo villages, were all negative for schistosomiasis (Lemma, 1964 unpublished study).

In general, it appears that intestinal bilharziasis is predominantly found in the highlands, whereas urinary bilharziasis is restricted to the warm and arid lowlands. Russell (1958) had found *S. haematobium* to be common amongst the Danakils at Gewani, in the middle Awash Valley. Military physicians working in different parts of the Wabi Shebelle Valley have verbally reported seeing "bloody urine" assumed to be caused by *S. haematobium*. This, however, needs confirmation.

Possible intermediate host snails of *Schistosoma* are very widely distributed in the country. Brown (1964) studied the distribution of freshwater gastropods in Ethiopia, showing that *Bulinus* and *Biomphalaria* species, important intermediate hosts, are very widely and abundantly distributed in areas as high as 9,600 ft. (2,926 m.) above sea level (Begemdir). Snails of these genera are present in some of the lakes used as resort areas, e.g., Lakes Hora, Abijata, Zuwai, Awasa, and others, as well as in the irrigation canals of some of the agriculturally developing areas in the Awash Valley, such as the Wonji-Shoa, Metehara, Abadir, Melka Werer, and Tendaho plantations.

The problem of schistosomiasis in Ethiopia is relatively new, but the abundance and wide distribution of the intermediate host snails, rapid movement of possibly infected people, agricultural and industrial developments, improvements of highways, and exploitation of resort areas in the lake regions, are making it of increasing public health significance.

Specific aims in the present study

The present study was initiated by the author in 1965, shortly after the series of extensive reports on "Survey of the Awash River Basin", prepared by the joint efforts of the Imperial Ethiopian Government and the United Nations Special Fund project (through FAO), were released, indicating that the Awash Valley development scheme was to operate on a very large scale. These reports, written by known experts in the fields of agriculture and economics, did not consider the public health and economic importance of bilharziasis which was known to occur in undetermined degrees of prevalence in different parts of the Awash Valley. It was, therefore, deemed necessary to gather more facts about this disease and to recommend and urge the incorporation of adequate control measures in the development plan, if economic benefits from the project were to be realized.

The present report is the first of a series, and is designed to give the necessary background on the Awash Valley, to determine the prevalence of bilharziasis in different parts of the Valley, to project the possible future public health and economic importance of the disease, and to make recommendations for future action. The epidemiological information presented was obtained from 1965-68. Although the emphasis is on bilharziasis, the presence of other intestinal parasites found during the stool examinations is also reported. A detailed malacological study, involving transmission and factors affecting the spread of the disease, will be covered in a subsequent report.

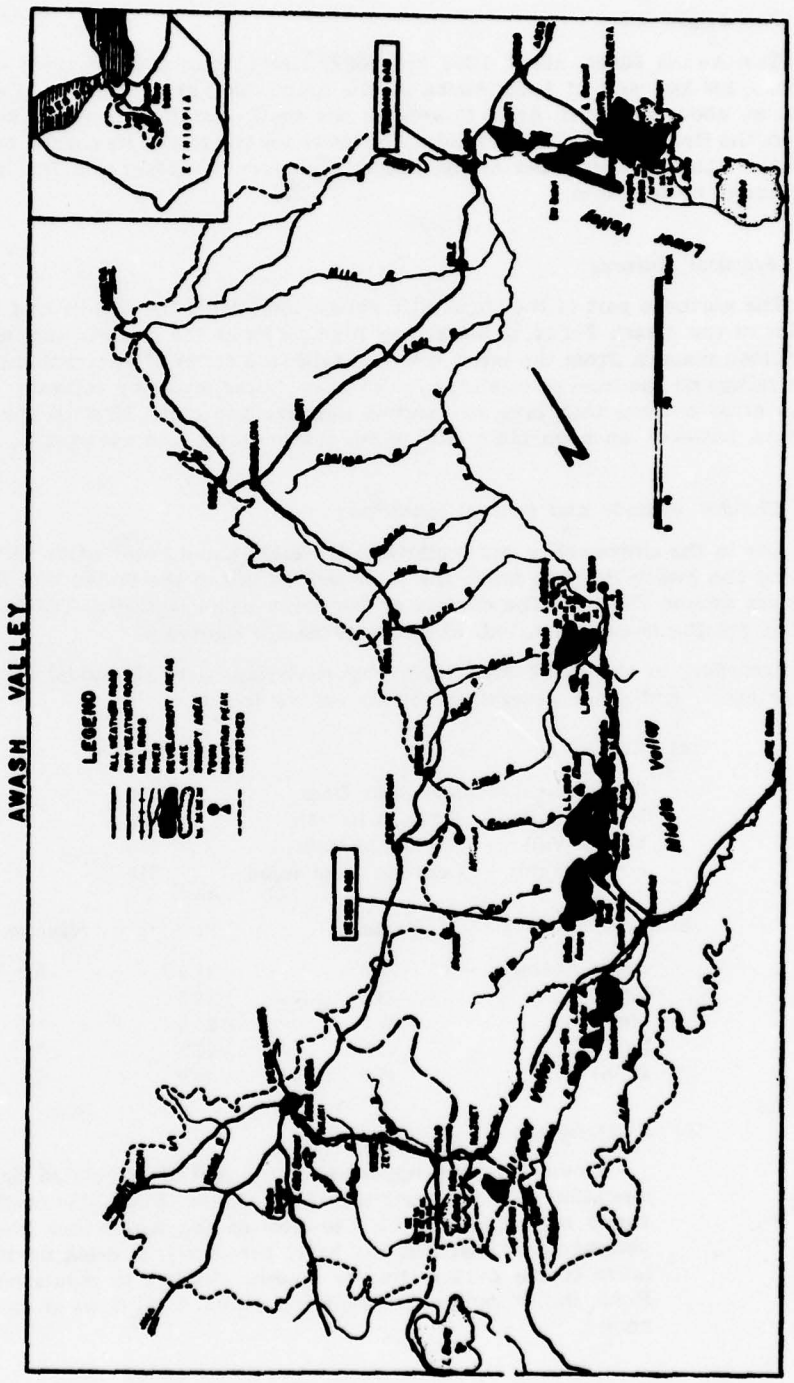
GEOGRAPHICAL AND DEMOGRAPHIC DATA

The Awash Valley

I. *Location and extent*

The Awash Valley occupies the eastern part of central Ethiopia from latitude 8° to 12°N., and longitude 38° to 42°E. It covers a total area of 70,000 sq.km. (6% of Ethiopia's land area), almost all of it lying geologically within the Great Rift Valley (see map).

151



152

II. *The Awash River*

The Awash River, about 1,200 km. long, starts near a small town called Ginche, 150 km. west of Addis Ababa on the Ambo road, at an altitude of about 2,100 m. above sea level. After flowing to the south-east for about 250 km., it enters the Great Rift Valley. It follows the latter for the rest of its course, ending in Lake Abbe on the border of the French Territory of Affars and Isas at an altitude of about 250 m.

III. *Physical features*

The northern part of the Great Rift Valley, comprising the middle and lower plains of the Awash Valley, is marked by high peaks of the eastern edge of the Ethiopian plateau. From the latter the land falls in a series of alternating peaks and valleys on the lines of extensive faults. It is characterized by volcanic cones, large areas covered with lava, hot spring, pumices and tuffs. Most of the vast area is, however, an open plain with some acacia forest and savanna.

IV. *Climate, altitude and general conditions*

Life in the entire valley, particularly in the middle and lower parts, revolves around the Awash River in much the same way as life in the Sudan and Egypt revolves around the Nile. The climate of the River Basin is varied. The land is fertile, grazing is extensive, but water resources are limited.

According to the UNSF/FAO (1965) report, typical normal annual rainfall, temperature, and other general conditions are as follows:

(a) *Rainfall*

	Millimetres
Upper Basin — above Koka Dam	1000
Middle Valley — Koka to Hertale	850
Middle Valley — Hertale to Dubti	610
Lower plains — Dubti to Lake Aobe	215

(b) *Temperatures*

	Maxima	*Ave.*	*Minima* (°C)
Addis Ababa	28.5	13.5	6
Koka	34.5	19.5	7
Metehara	39.5	23.5	7
Gewani	41.8	26.2	8.1
Dubti	45.6	29.0	6

(c) *River flow*

Flows from the upper valley are already regulated by the operation of Koka Reservoir (Lake Galila). Since this reservoir began operation in 1961, the flow of the Awash has become permanent throughout the year; previously, it dried up in its lower course during the dry seasons. Subject to regulation at Koka, the following are estimated annual total flows at various points:

	hm³*	hm³
Inflow to Lake Galila	1,895	
Less losses from reservoir (evaporation and leakage)	— 695	
Outflow from Lake Galila		1,200
Flow at Awash Station		2,460
Flow at Hertale		2,840
Flow at Dubti		3,490

V. *Land in use*

Until 1952, only the upper reaches of the valley in the highlands produced cash crops for the nearby markets. The middle and lower valleys were completely inaccessible because of malaria and lack of roads.

In 1952 the HVA-Ethiopia Share Company was established. It obtained a concession for 5,000 hectares of land, which later was increased to 6,600 hectares, for sugar plantations in Wonji and Shoa. The area has since developed very well, presently with a total population of about 30,000 and an annual production of 60,000 tons of refined sugar.

In 1958, the Mitchell Cotts Company obtained a concession for growing cotton on 18,000 hectares of land along the lower part of the Awash River. This area, officially known as the Tendaho Plantations Share Corporation, is also developing very well, with a peak population employment of about 30,000 and an estimated annual production of 30,000 metric tons of seed cotton (FAO/SF Report 1965).

In 1966, the HVA Company obtained another concession for about 16,000 hectares of land for a similar sugar plantation in the Metehara area. This is now under rapid development.

There are a few other smaller concessions given for growing cotton and other crops in different parts of both the middle and lower Awash Valley.

VI. *Human and animal population*

Except for the part above Nazareth, the Awash Valley is sparsely populated. Nomads seek grazing lands and water for their herds. It is estimated that there are 240,000 cattle and 60,000 sheep, goats and camels between Awash Station and Lake Abbe (FAO/SF Report 1965). Apart from domestic animals, the Awash Valley has a very large variety of wild game. In fact, a great part of the area from Metehara to Gewani is now being used as a national park and wild game reserve. The large number of baboons and different species of monkeys are of special interest, being potential bilharzia reservoir hosts.

VII. *The people*

The highland area from the origin of the Awash River to the town of Nazareth is predominantly occupied by Galla and Amhara people. South-east of Addis Ababa, the rural population is mainly of Galla origin, while south-west it is composed of Gurage, Wollamo, and Soddo tribesmen. The Amhara people are settled mostly in the cities and towns.

---

* $hm^3 = 1{,}000{,}000\ m^3$ (cubic hectometre).

Further down in the valley, the Arusi Galla (pagans) constitute a large part of both cultivators and shepherds. East of Nazareth, the sedentary Galla cultivators are replaced by Galla pastoralists of the Kereyu tribe. These are the first of the nomads to be encountered in the middle Awash Valley. Beyond them are the Danakils, and further down the valley are the Issas.

The highlands of the northwestern part of the valley, as far north as Debre Sina, are populated by the Amhara (Shoa) people. Further north-east, the highlands and the mountainous valleys are occupied mostly by Galla tribes, and the region of Dessie by the Wolloyé and Argoba people.

The Danakils, or Adals in the Amhara language, call themselves Afars. They occupy the largest part of the present bilharziasis survey area. These people, of semitic origin, are unique amongst the Ethiopian people. Because of their nomadic nature, houses are of a temporary nature, namely, dome-shaped huts made of matting spread over sticks. Their household utensils are primitive; pottery is unknown to them. Goat skins serve for churning butter and carrying water. Their main diet is milk and corn. All the work at home and with the animals is done by women, while the men, formerly only fighters, spend their time scouting for water and pasture. The Danakil man is known to be very individualistic, proud and independent.

Intermingled with these various tribes is a very important and discrete foreign population, the Arabs. Arab merchants have long been travelling through the Danakil Desert with their merchandise. They come with their goods from the southern part of the Arabian peninsula via the Red Sea. Some smuggled merchandise, being transported through the unguarded sea shores by camels, is sold to the Danakils and Issas as well as to the other inhabitants of the area.

Apart from the Arabs, there are also very limited numbers of foreign technicians from different parts of the world employed in the large agricultural enterprises in the Wonji-Shoa, Metehara, Melka Werer, and Tendaho plantations.

EXAMINATION TECHNIQUES

I. *Sampling technique*

An attempt was made to collect representative stool and urine specimens from both sick and healthy as well as rich and poor people. Usually in surveys of this kind, more sick than healthy people come for examination in hope of receiving medical treatment. Therefore, the prevalence rate may be biased towards a higher percentage of positive cases.

II. Examination of stool and urine

When possible, both urine and stool specimens were collected from each survey area. The specimens were preserved in 5% formalin and brought to the laboratory for examination. The stool specimens were filtered through three layers of cheese-cloth and centrifuged at 3000 rpm for 10 minutes. The urine specimens were centrifuged without filtering. The sediments were then examined for ova of parasites.

RESULTS

As a convenience in presenting adequately the results of the present and subsequent investigations in the Awash Valley, the area is divided into three parts: upper, middle and lower Awash Valley. The upper part extends from the origin of the river to the village of Awash Station, the middle from Awash Station to the Tendaho area, and the lower from the Tendaho area to Lake Abbe, where the river ends.

The fact that urinary bilharziasis was found to exist among the Danakils in Gewani (Russell, 1958), and that the Mitchell Cotts Company was rapidly developing the Tendaho Plantation (only about 200 km. downstream from Gewani), raised the serious possibility of the 30,000 employees in Tendaho being in danger of getting the disease. This was of further concern when it was realised that almost all the employees on the plantation were Galla, Amhara and Tigre peoples who came from the highlands where they have had no previous exposure to urinary bilharziasis, thereby being more susceptible to the harmful effects of this disease.

The lower Awash Valley was, therefore, chosen as the starting point for the present study and a team of eight people, including the Provincial Medical Officer of Wollo and a representative of the Awash Valley Authority, surveyed the Tendaho and Assayta areas from February 4th - 21st, 1965. The following information and materials were collected:

(1) Stool and urine specimens from the various sectors of population (school children, farmers, wives, and the nomadic Danakils):

(2) Epidemiological data on the movements of people, sources of water, habitats, and occupations; and

(3) Snails from the irrigation canals in use, swamps, and Awash River.

I. *Results of studies in the lower Awash Valley.*

The villages surveyed were Dubti (commonly known as Tendaho), Assayta, Hadeleguera, and Barga.

The Amhara, Galla and Tigre populations in these villages were co-operative in giving stool and urine samples. It was difficult getting samples from the Danakils as they considered the handling of urine, and especially stools, disgraceful. However, with the special assistance of the Sultan of the Aussa, a very enlightened and helpful spiritual leader of the region, it was possible to get some specimens from them.

Along with stool and urine collections, finger-prick blood smears were made from 150 people for the diagnosis of blood parasites and for differential white blood cell counts.

The results of the urine, stool and blood examinations are given in Tables 1, 2 and 3, respectively.

The prevalence of intestinal and urinary bilharziasis

The results as presented in Table 1 show that none of the 104 urine samples from Dubti had any *S. haematobium*, but 2 of 94 stool samples from the same

people had *S. mansoni*. Of the 144 urine samples from Assayta, 5 had *S. haematobium* ova, and of 116 stools examined, 6 were positive for *S. mansoni*. In Hadeleguera 4 of 10 urine samples were positive for *S. haematobium*. In Barga, one of 112 stool samples was positive for *S. mansoni;* none of the 99 urine samples had *S. haematobium* ova. The age, sex and area of origin of all the positive cases are given below.

TABLE 1

Summary of urine and stool examinations showing the prevalence rates of *S. haematobium* and *S. mansoni* in the lower Awash Valley

Village	Total No. Examined		Total No. Positive		Per cent of Total Positive	
	Urine	Stool	Urine	Stool	S. haematobium	S. mansoni
Dubti	104	95	0	2	0	2.1
Assayta	144	116	5	6	3.5	5.2
Hadeleguera	10	0	4	0	40	0
Barga	112	99	0	1	0	1

The prevalence of different intestinal parasites

Table 2 shows that *Ascaris, Trichuris, Entamoeba histolytica* and *Strongyloides* are commonly encountered, whereas *Hymenolepis nana, Schistosoma mansoni, Taenia, Oxyuris,* and *Giardia* are relatively rare intestinal parasites in the lower Awash Valley.

TABLE 2

The relative endemicity of some intestinal parasites in Dubti, Assayta, and Barga in the lower Awash Valley

Parasite	Dubti: % Positive of 94 stools examined	Assayta: % Positive of 116 stools examined	Barga: % Positive of 100 stools examined
Entamoeba histolytica	18	7	10
Giardia	0	0.8	0
Schistosoma mansoni	2	5	2
Hymenolepis nana	3	10	1
Taenia	2	3	0
Ancylostoma	11	4	3
Ascaris	26	25	45
Oxyuris	0	0	3
Strongyloides	13	3	5
Trichuris	24	21	14

Results of blood smear examinations for parasites and differential white blood cell counts.

Examination of a total of 150 blood samples collected from Dubti, Assayta, and Barga showed no blood parasites, although the area is known to have malaria, and on the basis of some clinical manifestations, filarial infections were very strongly suspected. However, none of these was detectable in the blood smears examined.

The differential white blood cell counts show no significant differences from the normal. Due to the rather high rate of parasitic infections, and suspected filarial cases, at least the eosinophil counts were expected to be much higher than they were.

General observations on the surveyed areas and comments on the schistosomiasis cases found in the villages.

Dubti: This village is located about 600 km. north-east of Addis Ababa on the road to Assab. It is a new settlement area which was just being developed at the time of the suvey in 1965, and is the headquarters of the Tendaho Plantations Share Corporation. This rapidly developing cotton plantation covers an area of over 6,600 hectares.

The great majority of the people working on the plantation in Dubti were Galla tribesmen from the Bati (Wollo) area. The rest were Amhara and Tigre people from further up in the highlands. These people were employed on a temporary basis, so they were not settling and building houses. Except in a few cases, most of the people did not have their families with them. They work for a few months during the cotton picking season, save some money, go back to their families in the highlands, and stay there until the next cotton picking season.

In 1965 the living quarters of the labourers were inadequate. In most cases, they consisted of shacks made of corrugated iron and pieces of wood and old clothes. There were no provisions for clean water supply and latrines. The people used canal water for drinking and washing. It was not unusual to see the labourers coming from the burning sun and soaking themselves in the canal or ditch water. They also defaecated and urinated in the immediate neighbourhood, making an ideal environment for the rapid spread of bilharzia as well as other diseases.

The Mitchell Cotts Company was building rather attractive villas to house the plantation managers and officers. The completed administration buildings were all in use. There was no hospital in Dubti at the time of the survey, but a nurse was available to take care of emergency cases. However, since 1966 the Mitchell Cotts Company has employed a full-time doctor to look after the health of the people.

The results of the Schistosoma findings from 94 stool and 104 urine specimens from this village are presented in Table 1. Two persons (male) from Tigre Province, one from Adiquiha and the other from Akaleguzai, 27 and 17 years of age respectively, were positive for *S. mansoni*. One had been in Dubti for 2 months and the other for about a year. In view of the relatively short period of time of residence here, and the fact that intestinal bilharziasis is common

in Tigre Province (Lemma, 1965a; Buck *et al.* 1965), it was concluded that these *S. mansoni* cases were imported. In view of the fact that none of the long time residents had intestinal bilharziasis and none of the more than 100 *Biomphalaria* and *Bulinus* snails from the irrigation canals had human *Schistosoma* cercariae, it was concluded that this disease was not established in Dubti at that time.

Since none of the 104 urine specimens examined was positive for *S. haematobium*, it was also concluded that urinary bilharziasis was not established. This may be due to the fact that Dubti was a new plantation and settlement area with virtually no Danakils permanently residing there up to the time of the team's visit.

The search for snails in the swamps on the sides of the river and in the irrigation canals in use, revealed that *Bulinus* and *Biomphalaria* spp., possible intermediate hosts of *S. haematobium* and *S. mansoni* respectively, were present in various numbers. There were also numerous *Melanoides, Cleopatra*, and *Lymnaea* spp. present in the surveyed areas. No snails were recovered from the rather fast-flowing Awash River itself, but they were abundantly found in ditches and swamps filled with the river water during the rainy season when the Awash overflows. A detailed account of this and other studies on snails will be given in a separate report.

"Dubti Disease": During the bilharziasis survey, it was noted that people working in the plantations were suffering from an unknown disease which causes a localised swelling near the knee. Verbal information obtained from the people in the area has revealed that a large number of them get this disease every year and those who suffered from it were unable to bend their legs; they suffered a great deal of pain and were unable to work for a period of two or three weeks after which time they were normal again. The disease was seasonal, appearing mostly in October and November, after the long rainy season and when the insect population is high.

The aetiologic agent of the disease is unknown. From the symptoms, it appeared to be a filarial infection resembling Calabar swelling due to *Loa loa* or *Acanthochellonema perstans*. But neither of these parasites was detected in the numerous blood samples taken both at night and during the day from people suffering from this disease. *Dracunculus* is believed to have been introduced to this area by Arab merchants from Yemen, but this was not encountered during the present survey.

There are many wild and domestic animals in the neighbourhood of the Tendaho plantations. It is possible that some aberrant filarial or viral agent from these animals may be causing the temporarily disabling disease we have called "Dubti Disease". An extensive finger-prick blood examination from 150 people in Dubti, Assayta, and Barga showed no evidence of filarial infection. Differential white cell counts made on the same people showed no gross abnormality, especially with respect to the eosinophil count (Table 3).

The significant loss of man-power due to "Dubti Disease" warrants a detailed further study of this ailment.

Assayta, Barga, Hadeleguera: Assayta is the ancient headquarters of the Danakil people. Descendants of the Sultan of Aussa, the spiritual leader of the region, reside in this town. It is still primarily a Danakil town where masses

TABLE 3

Differential white blood cell counts of blood samples collected from Dubti, Assayta and Barga

White Blood Cell Type	Dubti: Average %. for 68 people	Assayta: Average %. for 25 people	Barga: Average % for 57 people	Normal % count according to Guyton (1961)
Neutrophils	60.0	57.5	57.0	63.0
Lymphocytes	31.0	31.4	32.3	30.0
Monocytes	5.2	5.8	5.7	5.0
Eosinophils	2.9	4.5	4.2	1.6
Basophils	0.9	0.8	0.8	0.4

of nomads come from all over the neighbouring desert once a week for marketing. The monuments and ruins found in the area suggest a very long established Danakil settlement.

Barga and Hadelguera are suburbs of Assayta. Barga is a big farm just across the Awash River. There is no bridge so one crosses from Barga to Assayta only by boat or swimming. About 2,000 people are settled on its well-developed cotton and cereal plantations. At the time of the survey, the farm management was being taken over by Mitchell Cotts Company; it was expected that the farm would expand.

Hadeleguera is the Danakil section of Assayta and is only a few kilometres from the main town. At the time of the survey the population was about 100. Although there were no permanent buildings or other structures, the Danakil seem to have been settled here for a relatively long time. They were running several small scale farms, and had large herds of cattle, camels, sheep, and goats. When the bilharzia team visited them, the chief of the village and his colleagues were extremely hospitable and interested in having their children treated for various diseases. But when they were asked for stool and urine samples, they were not willing to give any. After many hours of persuasion and pressure, only 10 urine samples were collected and 4 were found positive for *S.haematobium*. The Danakils in this village complain about the disease they get from the swamp which causes them to urinate blood. From conversations with them it appears that the disease is very common among the people here.

The population of Assayta is about 3,000, and of these 70% are Moslems (Danakils, some Gallas, Arabs) and the rest Christians (Amharas, some Gallas, Tigres).

There are some shops in town owned mostly by Arabs, and some middle and low class hotels and restaurants owned by others. Apart from the Danakils, there are many Somali and Arab merchants. A few of the Amhara, Galla, and Tigre highlanders are civil servants, but most are farmers engaged in the various agricultural enterprises. There is an elementary school with about 150 students.

The frequent movements of the Arab traders who travel with their merchandise all through the lower and middle Awash Valley, and occasionally across the

Red Sea to the lower parts of Arabia, may contribute to the introduction and spread of various diseases including bilharzia to these areas. This point will be discussed further in another section of this paper.

In the present survey, a total of 266 people, comprising school children, farmers, merchants, civil servants, and other residents of Assayta, Barga and Hadeleguera, were examined. Of these, seven were found to be infected with *S. mansoni* and nine with *S.haematobium*. The history of the *S.mansoni* infected cases were as follows:

2 males, ages 11 and 30, both Amhara from Bati, have been in Assayta for 3 and 4 years respectively.
1 male, age 18, Danakil, claimed to have lived all his life in Assayta.
1 male, age 15, Arab, claimed to have lived all his life in Assayta.
1 male, age 13, Tigre boy from Maitchew, has been in Assayta for 2 years.
1 female, age 19, Tigre girl from Dessie, has been in Assayta for 3 years.
1 female, age 30, Galla woman from Bati, has been in Assayta for 1 year.

The history of the *S. haematobium* cases were as follows:

3 males, ages 12, 14, and 22, all Danakils, have lived all their lives in Assayta
1 male, age 15, Amhara from Dessie, has been in Assayta for 3 years.
1 male, age 20, Arab, has been in Assayta all his life.
4 males, ages 14, 16, 20, 30, all Danakils permanently residing in Hadeleguera.

It is important to note that two boys who claimed never to have gone out of Assayta had *S.mansoni* infections. This suggests that *S.mansoni* is being introduced to the lower Awash Valley by labourers who come from the highlands where it is prevalent. It is also important to note that a girl from Dessie, where *S. haematobium* is unknown, had contracted *S. haematobium* infection in Assayta. It is apparent that the highlanders, while introducing intestinal bilharziasis to the Awash Valley, may acquire urinary bilharziasis here and transport it to their homelands where it is presently unknown.

It was noted that the Awash River and some of the main irrigation canals in Assayta, Hadeleguera, and Barga did not have any snails, whereas the numerous swamps and water-filled ditches formed by the overflow of the main river showed abundant *Lymnaea*, *Biomphalaria*, *Bulinus*, and *Cleopatra* spp. Abundant fossil shells of *Melanoides* were also found in various reclaimed (originally riverbed) areas. The swamps and ditches were observed to be most frequently used by human and animal populations because the relatively clean water, after the mud of the Awash River had settled, was preferred for drinking.

The Awash River is very muddy and is not suitable for washing white clothes such as those the Danakils wear. Therefore, the people go to the swamps and ditches in search of the clean water. Unfortunately, these are the areas where the snails also breed and live in large numbers. The people's preference for such areas for bathing, washing, drinking, and watering of animals, the presence of appropriate intermediate host snails, and the very inadequate sanitary conditions, make such areas ideal for bilharzia and other disease transmission. The author believes that the simple drainage of such swamps and ditches and the proper application of molluscicides in the irrigation canals could dramatically reduce the incidence of the disease and result in its eventual control in this part of the Awash Valley.

II. *Results of studies in the middle Awash Valley*

The area designated as the middle Awash Valley extends from Awash Station to the Tendaho area. Most of the land within this area is uninhabited. Life is concentrated in small aggregates of temporary houses along the rivers. The Imperial Ethiopian Government Institute of Agricultural Research, in collaboration with the Awash Valley Authority, has established an agricultural experimental station in Melka Werer. Also, the Awash Valley Authority, in collaboration with the Ministry of Community Development, has established a new settlement area called Amibara located a few kilometres from Melka Werer. The nomadic Danakils are being persuaded and helped to settle in these areas, and are given proper training in farming technology.

Stool and urine samples, snails, and epidemiological data were collected from Awash Station, Melka Werer, Angelele, Cortume, Hertale, and Gewani. The Danakils in this area, like those in the lower Awash Valley, were in general very unco-operative in giving urine and especially stool samples. They consider the handling of stools so disgraceful that even the children could not be bribed

TABLE 4

Prevalence rates of *S. haematobium* in different villages and towns in the middle Awash Valley

Village	Number of urine specimens	Positive cases:	
		Number	Per cent
Awash Station	188	0	0
Melka Werer	47	10	21.3
Angelele	36	21	58.3
Cortume	38	26	67.9
Hertale	51	21	41.2
Gewani	272	165	60.7

TABLE 5

Prevalence rate of various intestinal parasites in Awash Station

Parasite	Number Positive of 120 stool specimens	Per cent Positive
Entamoeba coli	41	34.2
Entamoeba histolytica	8	6.7
Giardia	15	12.5
Schistosoma mansoni	3	2.5
Hymenolepis nana	13	10.8
Taenia spp.	4	3.3
Ancylostoma	6	5.0
Ascaris	23	17.5
Oxyuris	1	0.8
Trichuris	4	3.3

TABLE 6

Age and sex distribution of 243 *S. haemgtobium* positive cases in the middle Awash Valley

Age Group	Male:		Female:		Total:	
	Number	%	Number	%	Number	%
0 — 10	61	49.6	34	28.3	95	39.3
11 — 20	41	33.3	34	28.3	75	30.8
21 — 30	12	9.8	30	25.0	42	17.3
31 — 40	8	6.5	25	12.5	23	9.4
41 — 50	0	0	4	3.4	4	1.6
51 — 60	0	0	0	0	0	0
61 — 70	0	0	2	1.7	2	.8
71 — 80	1	.8	0	0	1	.4
Over 80	0	0	1	.8	1	.4
Total	123	100	120	100	243	100

to do so. However, by repeated persuasion a sufficient number of urine specimens were collected from each of the above-mentioned villages. The results of the urine examination are given in Tables 4, 5, and 6.

Awash Station: Awash Station is a railway station about half-way from Addis Ababa to Dire Dawa. It is 225 km. from Addis Ababa at an altitude of about 1,025 metres. Until recently all trains from Addis Ababa to Dire Dawa, and vice versa, stopped overnight at this station, providing the main source of income for the villagers. There is very little farming in the area. The Danakils from all around the area come to the village once a week (Mondays) for marketing. There is a government school with about 250 students. The population of the village is about 5,000, the great majority being Moslem (mainly Somalis and Arabs).

The Awash River is the only source of water for the town. The town is located on a small plateau, and although the river is only a few kilometres from the town, there is a difference of about 500 metres in altitude between them. Water is pumped by motor from the river to the village and distributed by tap. People wash their clothes, swim, and water their herds in the river. The beach is sandy, and the water slow-flowing. Snails are absent here.

A total of 188 urine and 120 stool specimens were collected from the primary school children and adults in the market place in Awash Station. Examination revealed that none had *S. haematobium*, but three had *S. mansoni*. Of these, two were 27 and 25 year old teachers originally from Tigre Province, the third a young boy from Wonji. It was apparent that all were imported cases.

The other intestinal parasites found are listed in Table 5. In general, none of these parasites is found in high prevalence rates; and they are, therefore, no cause for alarm.

Melka Werer: Melka Werer is a relatively new settlement area about 50 km. south-east of Awash Station. The Institute of Agricultural Research and the

Awash Valley Authority have some experimental agricultural projects going on in the area. There are some very interesting agricultural, agronomical, and animal experiments being conducted by FAO experts and their Ethiopian counterparts at the Melka Werer Experimental Station. Similar studies are expected to be conducted in other parts of the Awash Valley. The Institute has invested 1.5 million dollars in developing the station. There are adequate modern houses for both senior and junior employees on the station, and the area is developing very rapidly.

Most of the people in Melka Werer have come from the highlands to work on the new projects. The nomadic Danakils in the region are also being encouraged to settle down and do some farming. To date (1968), there are about 250 Danakils employed by the Institute.

At the Melka Werer Agricultural Experimental Station, some irrigation canals are already in use and many more will be built. Search for snails in the canals in use has revealed the presence of abundant *Bulinus*, *Biomphalaria*, and other species of snails. Of 47 urine specimens collected from Melka Werer, 10 were positive for *S.haematobium* ova. All of the positive cases were Danakil people, some of whom were working in the Experimental Station. The presence of abundant numbers of the possible intermediate host snails in these canals along with infected Danakils may result in a very rapid increase in the incidence of urinary bilharziasis in this area.

Angelele: Angelele (or Adengelele) is a typical Danakil village about 40 km. from Melka Werer. There are about 200 Danakils living here, and they have very large herds of cattle, sheep and camels. There is no structure of any sort to indicate the permanency of the village. In fact, the Danakils are known to abandon this area during the rainy season when the insect population in the nearby swamp gets too high and troublesome.

Of 36 urine specimens collected from this village, 21 (58%) were positive for urinary bilharziasis. Stool specimens were not obtainable from the Danakils here nor in Cortume, Hertale, and Gewani. Therefore, the question of whether there is any intestinal bilharziasis in these areas is yet to be answered.

Cortume: Cortume is another typical Danakil village very similar to Angelele. It is about 20 km. south of Angelele and near a very big swamp. It has a population of about 100 people. There is no farming or any business in the village. As in Angelele, the nomadic Danakil abandon this village during the rainy season.

Of 38 urine specimens collected from this village, 26 (68%) had *S.haematobium* ova. The age and sex distribution of the positive cases are given in Table 6.

Hertale: this is another Danakil village very similar to the others. It is about 150 km. from Awash Station. The Isas are great enemies of the Danakils, and it is not uncommon for the former to start tribal wars in the vicinity of Hertale, involving shooting and slaughtering. Hertale and Gewani are in the heart of the Danakil Desert. There is no farming or business in Hertale. The nomadic people are known to abandon the village occasionlly. Hertale village is about 5 km. from Lake Hertale. The lake has salty water which seems to discourage snail habitation. Apart from the Awash River and Lake Hertale, there is nothing else but savanna and the burning sun. Occasionally, one might see wild animals such as zebra, giraffe, ostrich, lion, cheetah, and others by the roadside.

Of 51 urine specimens collected here, 21 (41%) had S.haematobium ova. The age and sex distribution of the positive cases is given in Table 6.

Gewani: is the largest of all the Danakil villages in the middle Awash Valley. It is located about 198 km. from Awash Station and at an altitude of about 700 metres. The village has a population of about 20,000 living very primitively. The nomadic tribesmen used to abandon this village when the Awash River dried up or flooded the area. With the construction of the Koka Dams, the water flow is now regulated and the people have begun to settle here more permanently. The Ministry of Community Development has built a school and is helping in settling and educating the people. There are signs of some farming and business development. There are also some shops and government buildings.

During the course of conversation with the Danakil concerning bilharziasis, it was learnt that they believe it is a relatively new disease which came after the Italian occupation of Ethiopia (1935-1941). They also know of the severity of the disease, calling it "Daho Geta" (loosening of urine) and "Asa Abala" (red urine). Whenever this disease is mentioned to them, they point to the lower part of their back (kidneys) and say they feel pain there. They believe they get the disease from Ba'adu, the big swamp in Gewani, where they go to collect the root of a special water plant they like very much because of its sweet taste. The latter is very similar to the sweet potato. The Danakils are known to come great distances (Melka Werer, Angelele, Cortume, etc.) in search of this plant.

The Gewani swamp has numerous snails, and it is an ideal site for the transmission of bilharziasis. The swamp is relatively big, covering several hundred square kilometres. Before the Koka Dams were built, it dried up during the dry season, but now always contains water. The Danakil people use the water at the edges of the swamp for washing, drinking, bathing, and watering their animals. People are frequently seen wading through the swamp in search of the root of the plant mentioned above.

Of 272 people examined in Gewani, 165 (61%) were positive for *S. haematobium*. The age and sex distribution of the positive cases are given in Table 6.

Age and sex distribution of S. haematobium positive cases in the middle Awash Valley.

Table 6 shows the age and sex distribution of *S. haematobium* positive cases in Melka Werer, Angelele, Cortume, Hertale, and Gewani. As is evident from the table, about equal numbers of men and women suffer from the disease. The age distribution of the positive cases shows that 40% are children between 0-10, and 30% are children between 11-20. Although this is in accordance with that reported from other parts of the world, it may be that the statistics from the present study are biased as more children under 10 were sampled.

One interesting aspect of this age and sex distribution is that significantly more boys than girls under 20 are infected; however, between 20-40 more women than men are affected. This may be partly due to women traditionally doing most of the work, including collecting water, washing and searching for the plant with the sweet root.

III. *Results of studies in the upper Awash Valley*

The upper Awash Valley studies covered the area from Melka Kontoure (about 50 km. south-west of Addis Ababa) to Awash Station (about 225 km. south-east of Addis Ababa). About two-thirds of this area is either on the high plateau or on the slope leading to the Rift Valley. As explained earlier, the temperature, rainfall, altitude and other climatic and ecological conditions in this part of the survey area are very different from the other parts of the Awash Valley.

TABLE 7

Parasitological survey in Melka Kontoure
Results of 47 stool specimens examined

Parasite	No. Positive	% Positive
Entamoeba coli	25	53.2
Entamoeba histolytica	1	2.2
Hymenolepis nana	2	4.4
Taenia sp.	1	2.2
Trichuris	4	8.7
Ascaris	3	6.5
Ancylostoma	1	2.2

The villages and areas surveyed were: Melka Kontoure, Koka Village, Wonji and Shoa HVA sugar estates, Sodere area, the HVA sugar estate in Metehara, and the Abadir farms.

Melka Kontoure: lying at 2,000 m. elevation, is a relatively new settlement area with no more than a few hundred people. It was probably established during the building of the Wollamo-Sodo (Buta-Jira) road, and more particularly during the building of the bridge across the Awash River. Because of the tectonic movements and the volcanic eruptions and consequent lava flow, the Awash here has sometimes been turned into a lake with waters overflowing its banks.

The village has recently been in the news because of archeological discoveries in its vicinity. Archeologists have found what is believed to be the oldest Palaeolithic site with cultures going back over one million years, superimposed by all African cultures neatly shown in stratification. Remains of pre-historic man and his tools have been excavated. With proper exploration of this site, it is expected that this area will become a very good tourist attraction. At present, there is very little business going on in the village as there are only a few shops and bars; most of the people in the area are farmers.

Of 46 stool and 47 urine specimens collected, none had *Schistosoma* ova. The other parasitological findings are given in Table 7.

A thorough search for snails on the shores of the slow-moving Awash River in this area has revealed the existence of abundant *Biomphalaria* and *Bulinus* spp. However, none of over 50 snails collected had cercariae.

166

Koka Village (near Koka Dam I): Koka Village, about 2,000 m. above sea level with a warm and very agreeable climate, is also a relatively new settlement area at the site of Ethiopia's largest hydro-electric power source. Almost all of the approximately 500 people are those who came in search of work during the hydro-electric power installations; about 90% are of the Shoa-Galla tribe. The modern Galila Palace Hotel was built in 1958 as a summer-house for His Imperial Majesty. About 10 km. west of the Galila Palace Hotel is the "hippo-farm", a very favourite tourist attraction. The construction of the dam across the river has resulted in the formation of an artificial lake, Lake Galila, the most dominant feature in the area.

In view of the possibility that tourists may encounter bilharziasis in this area, and also because of the desire to know whether the people in Koka suffer from it or not, a parasitological survey was carried out. Out of 30 stool and 30 urine specimens collected from school children, one case of *S. mansoni* and one of *S. haematobium* were found. The *S. mansoni* case was a 16 year-old Galla boy who lived in Wonji prior to coming to Koka a few months earlier. The circumstantial evidence suggests that he had imported the disease from there. The urinary schistosomiasis case was a Gurage boy of 15 who claims to have lived only in Dire Dawa prior to the one year he had been in Koka. It appears that he too may have imported the disease from that area. This was of particular interest since to date no *S. haematobium* case has been reported from Dire Dawa. The other intestinal parasites found are given in Table 8

TABLE 8

Examination results of 30 stool and 30 urine samples collected from Koka Village

Parasite	Number	Per cent
E. coli	15	50
Iodamoeba	3	10
Giardia	3	10
E. histolytica	1	3.3
S. mansoni	1	3.3
S. haematobium	1	3.3
H. nana	1	3.3
Ascaris	15	50
Ancylostoma	5	16.6
Trichuris	5	16.6
Strongyloides	1	3.3

Various species of aquatic snails, including *Biomphalaria* spp. and *Bulinus* spp., were collected from the lake and the river-bed at the bottom of the fall but were not examined for bilharzia cercariae. The people have a clean water supply from the lake by pipeline, and they have very little direct contact with the river. Very fortunately, the Koka Dam guards forbid people from using the lake for swimming, bathing or any other purpose. This has protected the lake from being a bilharzia transmission site. However, with the introduction of

infected people from other parts of Ethiopia, Koka Village becomes a potential area of infection with both types of bilharzia parasites.

Wonji and Shoa HVA Sugar Estates: The 6,000 hectare well-cultivated Wonji and Shoa sugar plantation is located about 110 km. south of Addis Ababa, near the town of Nazareth, at an altitude of about 1,500 m. It is partially owned and operated by a Dutch company known as HVA (Handels Vereniging Amsterdam). The company produces 67,700 metric tons of sugar per annum (for 1967-68, according to information obtained from the Company), and is one of the major agricultural enterprises in the nation and particularly in the Awash Valley.

Both estates jointly have 285 km. of irrigation canals and 210 km. of open drains. There is a total of about 30,000 people working there. Although the company provides housing for the labourers, this accommodation, especially the latrines, is very inadequate. The toilets in small shacks walled with corrugated iron hang above the canals. The open drains carrying the sewage frequently mix with the irrigation water which the labourers work in and use for washing and drinking.

The results of the stool and urine examinations performed on different groups of people during 1966 and 1968 are given in Table 9.

TABLE 9

Summary of results of stool and urine examinations for *Schistosoma* ova in Wonji and Shoa HVA Estates performed October 1966 and April 1968

Number of people examined	Survey Period	Number and Percent Positive:	
		S. mansoni	S. haematobium
220 labourers randomly picked from 2,000 new employees	October 1966	3/220 (1.4%)	0/220 (0%)
100 randomly picked 10-15 year-old school children in Wonji School	"	3/100 (3%)	0/100 (0%)
78 randomly picked individuals of "0" camp dwellers in Shoa	April 1968	19/78 (24.4%)	— —
47 adult male labourers selected from "0" camp dwellers for their close association with irrigation canals and irrigation water	"	28/47 (60%)	— —
81 randomly picked individuals from "K" camp dwellers in Shoa	"	2/81 (2.5%)	— —

Until recently, it was believed that no bilharzia existed in the Wonji and Shoa estates, the few cases found being attributed to importation. However, results of the present study reveal that the disease is not only being introduced by new employees into the estates at the rate of 1 to 2% per year, but also that it is well established and rapidly spreading. In 1966, only 3% of the school children in Wonji had *S. mansoni*. In 1968, examination of Camp 0 dwellers of both sexes and all ages showed 24.4% infection rate. Examination of selected adult male labourers residing in the same camp showed 60% infection rate, suggesting a definite occupational association of the disease.

The fact that *Biomphalaria pfeifferi* is the vector of *S. mansoni* in Wonji and Shoa was experimentally proved by infecting these snails with miracidia hatched from ova obtained from a *S. mansoni* case. The cercariae obtained from these snails 6 weeks after infection were used to infect laboratory bred mice. When the mice were sacrificed 8 weeks later, abundant adult *S. mansoni* were recovered. Since *B. pfeifferi* is the only *Biomphalaria* species present in the whole area, it appears that it is the only vector of *S. mansoni* in the Wonji and Shoa estates.

The fact that no *S. haematobium* was encountered may be due either to the local *Bulinus* spp. not being appropriate intermediate hosts or to the possibility that the disease has not yet been introduced to the area.

Sodere area: this area extends from the border of the Wonji and Shoa sugar estates down to Metehara. It includes the relatively new and very popular recreation place at Sodere Hot Springs, Melkasa Village (Koka Dams II & III), and various Kereyu Galla-inhabited small villages such as Dodota, Bobe, Waga, Gorgo, and Semon.

TABLE 10

Results of bilharziasis survey in the Sodere area

Village	Total Number of Urine Samples	Total Number of Stool Samples	Total Number Positive	
			Urine	Stool
Melkasa	33	32	0	0
Dodota	66	59	0	2
Bobe	81	67	0	3
Waga	20	20	0	0
Gorgo	30	29	0	0
Sodere	24	18	0	0
Total	254	225	0	5

The results of stool and urine examinations in the above villages are given in Table 10. It appears that *S. haematobium* does not seem to be established in this part of the Awash River. In Dodota and Bobe, there were 3.4 and 4.5%, respectively, of *S. mansoni* cases. The infected people were 23 and 18 year old

Kereyu Gallas respectively, both of whom claim to have lived all their lives in this area. It appears that the disease is endemic here to a low degree.

The survey was made shortly after Koka II & III Dams were constructed and the Awash River was blocked with the formation of a relatively big body of water at the junction where the road to Arusi crosses the Awash River. Abundant *Biomphalaria* and *Bulinus* spp. were found along the shores of this artificial lake and also in the slow-moving parts of the river.

Melkasa is a rapidly growing village with an approximate population of 1,000. There are many children in the village, and they are frequently seen freely swimming in the unprotected lake. The villagers also get their water supply and do their washing there. The presence of *Biomphalaria* snails, coupled with the presence of infected people and the rapid introduction of large numbers of people from uninfected areas, may in the long run result in a serious situation with regard to this disease.

One interesting epidemiological note is that bilharziasis was unknown to the people in this area until about 30 years ago when some 60 Sudanese settled here during the Italian occupation of Ethiopia (1935-41). They were all employed by Mr. Gorgo, a Greek farmer and business man who had a large citrus fruit farm in Melkasa. They lived here for a long time, and later some moved to Metehara in search of better opportunities. It is inferred that these Sudanese may have introduced *S. mansoni* to the Sodere and the Metehara areas.

Metehara area: is characterized by abundant past volcanic activity. There is a very large area of burnt land which is virtually of no agricultural use. However, there are also wide plains suitable for irrigation by the sides of the Awash River. The National Wildlife Reserve, a game park, includes part of this area; one sees wild animals grazing in the long and open tropical savanna as well as pink and white cranes in the salty lake of Metehara. The area is very sparsely populated, being primarily inhabited by nomadic Kereyu Gallas.

In 1966, the Imperial Ethiopian Government gave a special concession to the HVA-International to cultivate by irrigation over 12,000 hectares of land for sugar plantations. This area is now being very rapidly developed, and various irrigation canals and water reservoirs are being built. One very important improvement the HVA Company has made on its Wonji and Shoa estates is that it has avoided the use of open sewage drains and instead has installed septic tanks for sewage collection. This will no doubt have a very important impact on the spread of bilharziasis and other similarly transmitted diseases.

Only a few kilometres north of the HVA estate (generally known as Merti), is Metehara Village, a railway station with about 500 people. Also about five kilometres west of Merti, there has been some agricultural development involving irrigation for a long time. Since early 1940, some Greek farmers grew citrus fruits and bananas for the Addis Ababa market. The recent large-scale sugar plantation scheme has now taken over almost all the previous agricultural enterprises. The Abadir area involves over 8,000 hectares of land and is being developed as an independent national agricultural project.

As in the case of Wonji and Shoa estates, the HVA Company is rapidly developing the Metehara estate. It is estimated that the latter will soon have an area equivalent to twice the Wonji and Shoa estates together. It is also expected

(70)

that thousands of people from the highlands, mostly from the Shoa, Gemu Gofa, and Sidamo plateaus, will be brought here to work. As of May 1968, there were 2,500 working on the Metehara HVA estates and about 7,000 on the Abadir government farm. These numbers vary from season to season, but in general it has been increasing rapidly from year to year.

A few kilometres downstream from Merti is the area known as Fowa Fowati (waterfall), inhabited for a long time by Kereyu Gallas. This area was also surveyed.

The results of urine and stool examinations from Merti, Abadir, Metehara Village, and Fowa Fowati are given in Table 11.

The two infected people from Merti who claim to have lived here all their lives were an 8 year old Sudanese boy and a 9 year old Amhara boy.

The one infected from Metehara Village was an 8 year old Amhara boy who had lived in Wonji for a number of years before coming to Metehara, presumably having imported the disease from there.

TABLE 11

Results of bilharziasis survey in the Metehara area

Village	Total Number of Samples examined:		Bilharzia Positive:	
	Urine	Stool	Urine	Stool
Merti (Nomads)	25	24	0	0
Merti (HVA Employees' residence)	95	74	0	2
Fowa Fowati	31	28		
Metehara	102	58	0	1
Abadir Farm	92	55	1	2
Total	245	238	1	5

In Abadir three *S. mansoni* cases were found. On the basis of the history of these cases, all seem to have contracted the disease here. One *S. haematobium* case was found which, according to the history of the infected person, could only have been contracted locally. He was an 18 year old boy from Kembata, Wollamo, where so far no urinary bilharziasis is known to exist. He has been in Abadir for over 3 years.

Search for snails in the long and stagnant irrigation canals (the only sources of water for the labourers) in Merti and Abadir revealed the abundant presence of *Biomphalaria*, *Bulinus*, and *Lymnaea* spp. The situation appears to be ideal for the transmission of both types of bilharziasis as well as liver fluke of cattle. Although there are tens of thousands of people working on the government owned farms in Abadir, there is no provision for housing or medical care for the labourers. Unless the situation is improved, this area may soon become the most endemic bilharziasis zone in Ethiopia.

DISCUSSION

Earlier studies on the occurrence of bilharziasis in the Awash Valley are very limited primarily because of malaria and the lack of roads.

The first reported public health survey of the Awash Valley was by D'Ignazio and Mira (1949) who did not find any bilharziasis among the Danakils at Gewani. Ayad (1956) cited a doctor at Sheikh Osman Hospital in Aden, who had previously worked in Ethiopia, as having reported to him that he had seen two *S. haematobium* cases which were contracted in the Ba'adu (swamp) region situated not very far from Gewani across the Awash River. In the same year, in addition to an extensive review of all work done up to 1952, he studied the occurrence and distribution of intestinal and urinary bilharziasis in Ethiopia. He stated that, "In the plains of the Danakils Desert, bilharziasis has not been reported. The disease does not seem important in the centre (of Ethiopia) and appears to be absent in the south, namely in the desert and semi-desert regions of the south-east lying in the Somali arid zone, as well as in the more lush areas of the south-west."

Russell (1958) made a WHO sponsored health survey in different parts of Ethiopia, including the Awash Valley. He found 48% of 189 Danakils in Gewani suffering from *S. haematobium*. This was the first conclusive evidence of the occurrence of urinary bilharziasis in the Awash Valley and in Ethiopia as a whole.

In 1964 a WHO Bilharziasis Advisory Team visited various places, including the Awash Valley. After an intensive two weeks survey, the team concluded that amongst other places, bilharziasis was not present in Nazareth, the Wonji and Shoa sugar plantations, and the Tendaho cotton plantation.

In the present study, which was done during the period from 1965-1968, *S. haematobium* has been found to be well established in differing degrees of prevalence (60% in Gewani) in the middle and lower but not upper Awash Valley. *S. mansoni*, however, seems to be newly introduced to some places in the lower and upper Awash Valley. Since no stool specimens could be collected from the Danakils in the middle Awash Valley, the occurrence or absence of this disease there could not be ascertained.

It is interesting to note that *S. haematobium* appears to be spreading to Koka and Abadir Villages in the upper Awash Valley. The disease is not yet established in these villages, but the presence of infected people and possible intermediate host snails make them potential areas for its spread.

The available evidence gathered from the present and other studies leads to the conclusion that both *S. mansoni* and *haematobium* have been recently introduced to the Awash Valley. Since *S. mansoni* is known to occur in the highlands of Ethiopia (Ayad, 1956; Chang, 1961; Kubasta, 1964; Lemma, 1965a; Buck *et al.* 1965), it is apparent that people from infected areas such as Adwa, Harar and Kembata, amongst other places, are introducing the disease to the lower and upper Awash Valley as they come to these areas in search of jobs and better opportunities. *S. haematobium*, on the other hand, is so far found only in the lower and middle Awash Valley. There is enough evidence to indicate that before about 1945, this disease did not exist in the Awash Valley. None of the thousands of Italian physicians, troops, planners, and wanderers in this part of Ethiopia had reported seeing any form of bilharziasis. The Danakils themselves

know and say that "Daho Geta" (the Danakil term for bilharziasis) is a disease which has been introduced in their land since the Italian occupation of Ethiopia. It is possible that this disease was introduced by Arab merchants from the Southern Arabian Peninsula (Yemen) as they moved through the Danakil Desert by foot and camel with their merchandise.

Now that the occurrence of both types of bilharziasis in the Awash Valley is well established, there are some very important problems to be considered. The first is to determine the degree of pathogenicity caused by these parasites. This aspect of the investigation would be extremely important in assessing the impact which urinary bilharziasis might have on the large numbers of people brought from the highlands where they have had no previous exposure to it. Similarly, intestinal bilharziasis is new to the Danakils. The question will have to be answered as to whether the Danakils, who are now being exposed to this disease, will suffer more from it than the highlanders who have had previous exposure and perhaps developed some immunological protection against it.

The second consideration, and perhaps a more serious one, is the situation in respect to the intermediate host snails. Both *Biomphalaria* and *Bulinus* spp. are abundantly found in different parts of the Awash Valley. The open canal irrigation system, which is to be utilized extensively to develop at least 150,000 hectares of land in the Awash Valley in the coming few years, is providing excellent habitats for the well-being and rapid population growth of the snails. With the opening of new highways, rapid movement of people, settlement of the nomadic Danakils, and introduction of masses of new people, the spread of the disease will no doubt be enhanced. Unless something is done about it soon, it may seriously hinder the development of the whole Awash Valley.

The third consideration is based mainly on the first two, and it is perhaps more of a speculation than a conclusion on what may happen in the Awash Valley unless some control measures are taken. When the Awash Valley project is in full development, it is expected to have a total population of more than a million. Judging from the rapid pace at which bilharziasis is spreading in the few so far developed areas in the Awash Valley, it is possible that an 80-90% infection rate may easily be attained. This would mean that in the Awash Valley alone there could be hundreds of thousands of people infected with this disease. In addition to the public health and community importance of the disease, one may even estimate the tremendous economic loss it will impose on the country. Based on the simple average of the estimations of Ansari for Egypt, Watson (1958) for Iraq, and Farooq (1964) for the Philippines, one would envisage the annual loss per infected individual in the Awash Valley to be in the neighbourhood of Eth.$60 (U.S.$24). The total annual economic loss due to reduction in power and absenteeism caused by this in the Awash Valley could, therefore, be very many millions of Ethiopian dollars.

Because of this possible future significant public health and economic importance of bilharziasis in the Awash Valley, the Awash Valley Authority, Ministry of Public Health, Ministry of Community Development and Social Welfare are strongly advised to take immediate action to control this disease at this early stage. One should bear in mind that bilharziasis in the Awash Valley is primarily an occupational disease promoted by government projects for economic development. Therefore, these agencies should consider this as their moral obligation and take all necessary actions to safeguard the people.

SUMMARY

Ethiopia's new opportunities for large-scale agricultural development in the Awash Valley, and the general public health and economic importance of bilharziasis in such agricultural development projects are discussed. The physical and climatic features as well as the development areas in the Awash Valley are described and comments on the human and animal population in the area made.

Results of the survey in the lower Awash Valley reveal the following percentages for *S. mansoni* and *haematobium*, respectively: Dubti 2.1 and 0, Assayta 5.2 and 3.5, Hadeleguera 0 and 40, Barga 1 and 0. The other parasites found are also recorded. The differential white blood cell counts of 114 people in this area were normal. The intermediate host snails of both specimens of parasites are abundantly found in the irrigation canals and ditches but not in the main Awash River.

At Awash Station, in the middle Awash Valley, none of 188 people examined had *S. haematobium* ova, but in Melka Werer 21%, in Angele 58%, in Cortume 68%, in Hertale 41% and in Gewani 61% infection rates with this parasite are recorded. The age and sex distribution of the *S. haematobium* cases show that both sexes are equally effected and more children under 20 than adults have the disease.

In Awash Station, 2.5% of the school children have *S. mansoni*. No stool specimens could be obtained from all the other areas in the middle Awash Valley; therefore, the existence or absence of the disease there is not known. Appropriate intermediate hosts of *S. mansoni* and *S. haematobium* are abundantly present in this part of the Awash Valley.

In the upper Awash Valley, no *Schistosoma* infection was encountered at Melka Kontoure where 47 people were examined. In Koka Village, one each of *S. mansoni* and *haematobium* ova were found out of 30 each of stool and urine specimens of school children examined.

In Wonji in 1966, 1.4% of new employees at the HVA estate and 3% of school children at the Wonji School were positive for *S. mansoni*. In 1968 about 25% of the Camp 0 dwellers on the HVA estate had *S. mansoni*. A selective examination of adult male labourers in the same village had 60% infection rate, showing the occupational association of the disease. In Sodere and in Metehara areas also, a few *S. mansoni* positive cases were encountered.

The introduction and establishment of *S. mansoni* in the upper Awash Valley is discussed. The few *S. haematobium* cases found were attributed as imported cases; this parasite does not seem to be established here yet. Appropriate intermediate host snails of both species of parasites are abundant throughout the upper, middle and lower Awash Valley.

Some hypotheses on the introduction of *S. mansoni* and *S. haematobium* into the Awash Valley are given. The possibility that these diseases may be very rapidly spreading and seriously affecting the national development programme in the Awash Valley is discussed.

Quelques hypothèses au sujet de l'introduction de *S. mansoni* et *haematobium* dans la Vallée de l'Awash sont mentionnées. L'eventualité que ces maladies se répandent rapidement et serieusement affectent ainsi le programme de développement national dans la Vallée de l'Awash est discutée.

ACKNOWLEDGEMENT

The author wishes to express his gratitude to Ato Bahta Mazengia, Ato Asrat Woldeyes, Ato Shibru Tedla, P. Neri, and Dr. John Duncan for the valuable technical assistance they have given at different times. Also to Prof. J.H. Fischthal for the editorial comments, and to Mrs. T. Haile for making valuable suggestions on the presentation of the manuscript.

The author also wishes to acknowledge with gratitude assistance given by the Awash Valley Authority and the donation of a vehicle by Shell Chemical of East Africa, Nairobi and gasoline by Shell Ethiopia Ltd., which greatly facilitated many of the field trips.

REFERENCES

Ayad, N. (1956) Bilharziasis survey in British Somaliland, Eritrea, Ethiopia, Somalia, The Sudan, and Yemen *Bull. Wld. Hlth. Org. 14*, 1.

Biagi, F.F. (1963) unpublished WHO/Helminth/42 quoted by Ansari, N. and Junker, J. "The Economic Aspects of Parasitic Diseases" presented at the Global Impacts of Applied Microbiology Conference, Addis Ababa, Nov. 1967.

Blair, D.M. (1964) unpublished BIL/Exp. Com. 3/WP/22 quoted by Ansari, N. and Junkers, J. "The Economic Aspects of Parasitic Diseases" presented at the Global Impacts of Applied Microbiology Conference. Addis Ababa, Nov. 1967.

Brown, D.S. (1964) The distribution of intermediate hosts of Schistosoma in Ethiopia *Eth. med. J, 2*, 250.

Buck, A.A., Spruyt, D.J., Wade, M.K., Deressa, A. and Feyssa, E. (1965) Schistosomiasis in Adwa. A report on an Epidemiological Pilot Study *Eth. med. J. 3*, 93.

Chang, W.P. (1961) Preliminary Report on Demographic Survey in the Gondar Region, Ethiopia *Gondar Health Series, 1*, 5.

D'Ignazio, C. and Mira, M.G. (1949) Epidemiological Research in certain parts of Abyssinia *Trop. Dis. Bull. 47*, No. 2, 1134.

Ethiopian Nutrition Survey (1958) International Committee on Nutrition for National Defence, U.S.A. quoted by Brown, D.S. (1964).

FAO/SF Report on Survey of the Awash River Basin (1965), *1*.

Farooq, M. (1964) New Partnership in Schistosomiasis Control *J. Trop. Med. & Hyg. 67*, 105.

Forsyth, D.M. and Bradley, D.J. (1966) The consequences of bilharziasis, medical and public health aspects *Bull. Wld. Hlth. Org. 34*, 715.

Hearnshaw, G.R., Kershaw, W.E., Brewster, D.J. and Leytham, G.W.H. (1963) The effect of schistosomiasis upon the activity and growth of mice *Ann. Trop. Med. Parasit. 57*, 481.

Kershaw, W.E., Leytham, G.W.H. and Dickerson, G. (1959) The effect of schistosomiasis on animal intelligence *Ann Trop. Med. Parasit. 53*, 504.

Khalil Bey, M. (1949) The national campaign for the treatment and control of bilharziasis from the scientific and economic aspects *J. roy. Egypt. Ass. 32*, 817.

Kubasta, M. (1964) Schistosomiasis mansoni in the Harar Province *Eth. med. J. 2*, 260.

Lemma, A. (1965a) Schistosomiasis in Adwa: A report on an ecological pilot study *Eth. med. J. 3*, 84.

Lemma, A. (1965b) A preliminary report on the molluscicidal property of endod *(phytolacca dodecandra) Eth. med. J. 3*, 187.

Lemma, A. (1964) unpublished; Pathobiology Institute, Haile Sellassie I University, Internal Report.

Pesigan, T.P., Farooq, M., Hairston, N.G., Jauregui, J.J., Garcia, E.G., Santos, A.T., Santos, B.C. and Besa, A.A. (1958) Studies on *Schistosoma Japonicum* infection in the Philippines *Bull. Wld. Hlth. Org. 18*, 345.

Russell, H.B.L. (1958) unpublished WHO EM/PHA/62 Ethiopia, 13.

Stretch, R.G.A., Leytham, G.W.H. and Kershaw, W.E. (1960) The effect of acute schistosomiasis upon learning in rats under different levels of motivation *Ann. Trop. Med. Parasit. 54*, 487.

Watson, J.M. (1958) Proceedings of the Sixth International Congresses on Tropical Medicine and Malaria, *2*, 203.

World Health Organization Expert Committee on Bilharziasis (1965) *Tech. Rept. Ser. Wld. Hlth.Org.* No. 299.

Annals of Tropical Medicine and Parasitology, 1970. *Vol.* 64, *No.* 4

The molluscan fauna of the Awash river, Ethiopia, in relation to the transmission of schistosomiasis

BY D. S. BROWN

(*From the British Medical Research Council, Zoology Department, British Museum (Natural History), London*)

AND

AKLILU LEMMA

(*From the Institute of Pathobiology, Haile Sellassie I University, Addis Ababa, Ethiopia*)

(*Received for publication February 4th*, 1970)

The Awash river rises on the south-eastern slopes of the central Ethiopian plateau, descends to the floor of the Rift Valley and flows north-eastwards towards the Red Sea. The planned development of over 500,000 hectares of irrigated land along the middle and lower courses of the river is described by Aklilu Lemma (*in press*) who reports the occurrence of both urinary and intestinal schistosomiasis in the area. In the present paper we describe the distribution of actual and potential intermediate hosts, consider some possible effects on them of the development of irrigation and assess prospects for snail control.

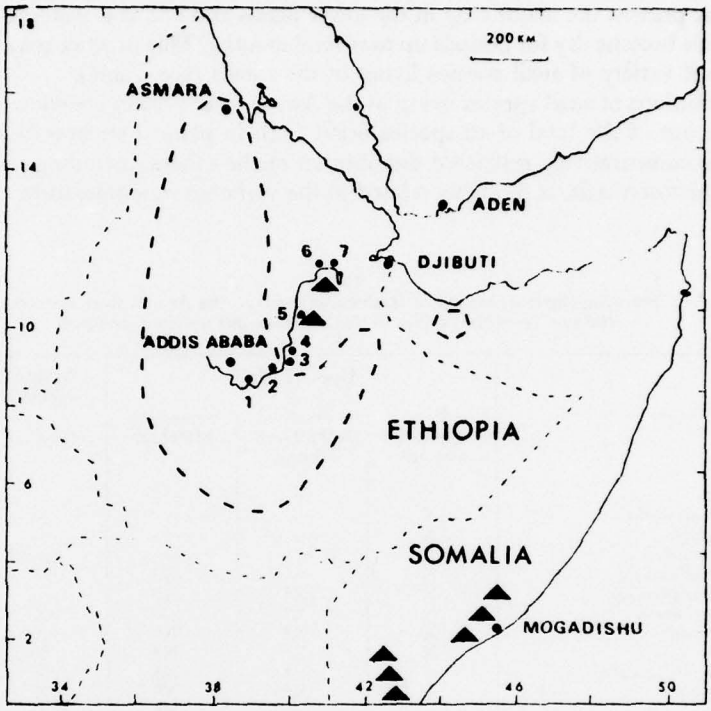
Topography and Climate

The major tributaries of the Awash system, the Awash, Cassam, Borchenna and Mille rivers rise on the cool highland plateau at about 3,000 m. (9,800 ft). The Awash itself rises near the village of Ghingi 70 km. west of Addis Ababa and flows for about 1,200 km. before terminating in Lake Abbe near the border of Somalia (see text-fig.). The course of the river may be subdivided into three parts; the upper basin (above Awash town), the middle valley (Awash town to Dubti), and the lower plains (Dubti to Lake Abbe). Rainfall and temperature vary considerably in relation to altitude. Annual precipitation decreases from 1,000 mm. in the upper basin to 215 mm. in the lower plains, while average annual air temperatures increase from 13·5°C. at Addis Ababa (2,408 m.) to 29°C. at Dubti (300 m.).

Prevalence of Schistosomiasis

Several foci of endemic intestinal schistosomiasis are known on the Ethiopian plateau and Aklilu Lemma (*in press*) reports cases believed to have been acquired locally in irrigation systems at Wonji and Metehara in the upper Awash basin, and at Assaita in the lower plains (for localities see text-fig.). *Schistosoma mansoni* has not been found in the middle Awash valley, perhaps because of the reluctance of the nomadic Danakil people to give stool specimens. Urinary schistosomiasis is comparatively rare, being known only in the Awash valley, at Gewani in the middle region and in the lower plains at Assaita (Russell, 1958; Aklilu Lemma, *in press*). Possibly infection with *S. haematobium* is increasing at Assaita, for in 1965 Aklilu Lemma found an infection rate of 3·5 per cent. in 144 children,

while a subsequent survey (Brown and Santi, *unpublished observations*) showed 12 per cent of 70 children to be infected.



TEXT-FIG. The Horn of Africa, showing the distribution of the snail hosts of schistosomiasis *Biomphalaria pfeifferi* (thick broken line) and *Bulinus abyssinicus* (solid triangles) in relation to the Awash river, Ethiopia. The range of *B. pfeifferi* in Ethiopia conforms broadly to the plateau, the altitude of the lowest recorded locality being about 1,000 m.

Localities in the Awash river valley: 1 Koka Dam and Wonji sugar plantations; 2 Metehara; 3 Awash town; 4 Melka Werrer; 5 Gewani; 6 Dubti; 7 Assaita.

SNAIL FAUNA OF THE AWASH SYSTEM

The present account is based on previous observations (Brown, 1964, 1965, 1967*a*) supplemented by the study of other material collected by members of the Institute for Pathobiology, Addis Ababa and by Brown in 1969 (*unpublished observations*). The Awash river itself has been more thoroughly examined than the Mille, Borchenna and Cassam rivers, which descend from the plateau in deep, inaccessible gorges. However, the snail faunas of the source streams of all these rivers are known to be similar.

Some streams are perennial on the plateau and, having gentle gradients, clear water and abundant aquatic vegetation, provide excellent habitats for snails. At lower altitudes many streams flow temporarily carrying heavy loads of silt and the snail fauna is usually impoverished in such waters. The Awash river in its middle and lower courses is turbid and flows between banks of rock or mud with wide fluctuations in level; these conditions

are unfavourable for snails, though many species live in marshes fed by the river at Gewani, Dubti and Assaita. The presence of freshwater shells in dry alluvium on the lower plains indicates that marshes and perhaps lakes formerly covered a far greater area that at present.

Water for irrigation is taken from the Awash river by pumping and gravity feed. Cotton is at present the major crop in the lower plains and and it is watered intermittently so that canals become dry for periods up to several months. This practice may be responsible for the small variety of snail species living in the canals (see Table).

Distributions of snail species living in the Awash river system are shown in the Table. Only three out of the total of 16 species occur both in plateau streams and on the lower plains. The comparatively restricted distribution of the others, including the intermediate hosts of schistosomiasis, is probably related to the variation in temperature associated with altitude.

TABLE
Showing the distribution of freshwater snails in the Awash river system.
Bulinus 'truncatus' refers to the *B. 'truncatus sericinus'* complex

	Upper basin			Middle valley	Lower plains
	Plateau streams	Koka Dam, Wonji	Metehara	Gewani	Dubti, Assaita
Valvata sp.	X	—	—	—	—
Lymnaea truncatula	X	—	—	—	—
Burnupia caffra	X	—	—	—	—
Ancylus fluviatilis	X	—	—	—	—
Gyraulus costulatus	X	—	X	—	—
Biomphalaria pfeifferi	X	X•	X•	—	—
Lymnaea natalensis	X	X•	—	X	X
Bulinus 'truncatus'	X	X•	X•	X	X•
Anisus spp.	X	X	X•	X	X•
Melanoides tuberculatus	—	X•	X•	X	X•
Bulinus forskali	—	X•	X•	X	X•
Ferrissia sp.	—	—	X•	—	X
Cleopatra bulimoides	—	—	—	X	X
Bulinus abyssinicus	—	—	—	X	X
Gabiella senaariensis	—	—	—	—	X•

• Present in irrigation canals

The genus Biomphalaria

This genus is represented in Ethiopia by the widely distributed *B. pfeifferi* (Krauss) (see text-fig.), and by *B. sudanica* (Martens) which is confined to some lakes in the southern Rift Valley. *B. pfeifferi* is usually found amongst vegetation in streams and it also lives in stony watercourses. It has been obtained only in the upper basin of the Awash river. Large populations are present in irrigation canals at Wonji and Metehara, where *S. mansoni* is transmitted (Aklilu Lemma, *in press*). No living *B. pfeifferi* or shells have been found below Metehara, and shells of this genus obtained at Assaita represent an apparently undescribed and probably extinct species (Brown, *in preparation*). However, two of the cases of *S. mansoni* infection found at Assaita by Aklilu Lemma (*in press*) were apparently autochthonous and it is possible that a species of *Biomphalaria* may be living in the lower

Awash plains, though it is undoubtedly rare as both marshes and irrigation canals have been thoroughly searched without success. It is surprising that *B. sudanica* has not been found in marshes in the Awash middle valley or lower plains, for this snail is common in marshes or lake shores further south in the Rift Valley.

Bulinus africanus *species group* (*subgenus* Physopsis)

This group of snails has a discontinuous distribution in Ethiopia, being represented in the southern Rift Valley in Lake Margherita by *B. ugandae* Mandahl-Barth, in the districts of Jimma and Lake Tana by *B. africanus* (Krauss), and in the middle valley and lower plains of the Awash river by *B. abyssinicus* (Martens) (see text-fig.). *B. abyssinicus* occurs otherwise only in southern Somalia, where it transmits urinary schistosomiasis in the Juba and Webi river valleys (Maffi, 1960). Colonies of this snail live in Ethiopia in the marshes near Gewani and Assaita and it is the presumptive intermediate host of *S. haematobium* in these localities as no other suitable living species of *Bulinus* have been found (Brown, 1967*a*). A few empty shells resembling *B. truncatus* (Audouin) were found in a dried irrigation canal at Dubti, but none were obtained near Assaita where urinary schistosomiasis is endemic. *B. abyssinicus* has not been found in irrigation systems in Ethiopia.

Bulinus forskali (*Ehrenberg*)

B. forskali occurs in Ethiopia in the lowlands and at moderate altitudes, most abundantly in small pools. In the Awash valley this species has been found as high as Koka Dam and large populations live in irrigation canals at Melka Werrer and Dubti. *B. forskali* is not known to serve as an intermediate host of *S. haematobium*, though closely related species do so in the former Western Aden Protectorate and on islands in the Indian Ocean (Wright, 1963 and *in press*).

Bulinus 'truncatus'

Snails showing some resemblances to *B. truncatus* of Egypt and the Mediterranean region have been recorded from many Ethiopian localities *Bulinus* (*Bulinus*) sp., Ayad, 1956; *B. t. sericinus*, Brown, 1964; Mandahl-Barth, 1965). It is now known that these varied populations comprise at least four distinct forms having different chromosome numbers (Brown and Burch, 1967; Burch, 1967) and their taxonomic status has not yet been established. The known chromosome numbers appear to constitute a polyploid series with the following haploid complements; 18 (diploid), 36 (tetraploid), 54 (hexaploid), 72 (octoploid). The tetraploid, having the same number of chromosomes as *B. truncatus* of Egypt and the Mediterranean region, is particularly suspect as a potential intermediate host of *S. haematobium*.

Snails belonging to this complex are abundant in the upper Awash basin, particularly in streams flowing on the plateau and in some pools and irrigation canals, but they are apparently rare in the middle and lower courses of the river, where a few empty shells have been found. Octoploid and hexaploid populations predominate in a high altitude zone (Brown and Burch, 1967), below which only tetraploid and diploid snails have been found. Tetraploid snails are known from a tributary of the Awash river at Moggio (Burch, 1967) and from an irrigation canal at Wonji (Brown and Wright, *unpublished observations*).

DISCUSSION

B. pfeifferi is clearly able to flourish in canals, as large populations have developed in the irrigation systems at Wonji and Metchara in the upper Awash basin and it is surprising that this species is apparently absent from irrigation canals and natural habitats in the middle valley and lower plains. It is possible that irrigation systems will be colonized eventually by dispersal from the upper basin, or from local populations if any exist. However, Sturrock (1966) concluded that high temperatures are a major barrier to the colonization by *B. pfeifferi* of the coastal plain of Tanzania, where waterbodies have temperatures exceeding 28°—30°C. for several months on end, and as the average annual temperature at Dubti is 29°C. it may be that water temperatures are unfavourable on the lower Awash plain also. In this connection it is noteworthy that the few known localities for *B. pfeifferi* in Somalia all lie above 500 m. (Ayad, 1956). It seems unlikely that irrigation schemes in the middle and lower Awash zones will be extensively colonized by *B. pfeifferi*, if at all, and it should prove possible to destroy any pioneer populations by the local application of molluscicides.

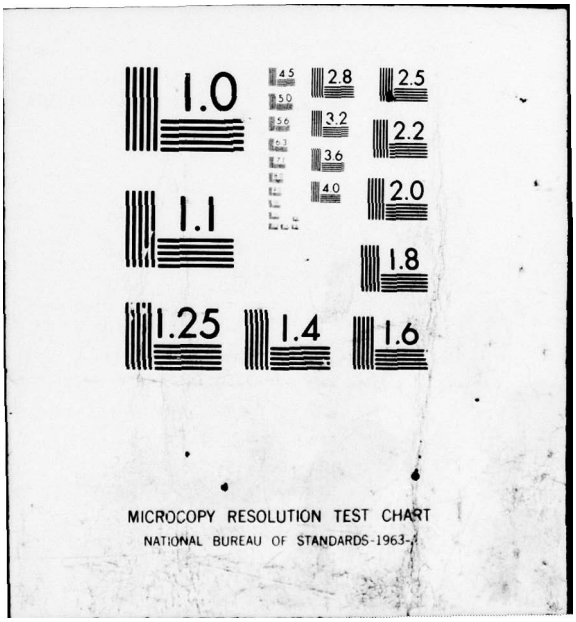
B. (*Physopsis*) *abyssinicus* has been found only in natural swamps in Ethiopia, but it is possible that irrigation systems will be colonized eventually as this snail occurs in canals in Somalia (Ayad, 1956). Members of the subgenus *Physopsis* in Ethiopia seem to belong to the tropical component of the fauna, as they do also in southern Africa (Brown, 1967*b*), and it seems likely that *B. abyssinicus* requires warm conditions. This species could perhaps be eradicated from the Awash valley by the application of molluscicides as it is comparatively rare and occurs in restricted colonies. Moreover, it seems unlikely that the Awash valley could be recolonized by snails transported from the nearest known localities in Somalia, some 700 km. to the south-east (text-fig.).

It is highly probable that the intermediate host of *S. haematobium* in the Awash valley is *B. abyssinicus*, but other species should not be excluded from investigation. *B. beccarii* (Paladilhe) and *B. reticulatus wrighti* Mandahl-Barth transmit urinary schistosomiasis in the former Western Aden Protectorate (Wright, 1963; Mandahl-Barth, 1965) and it would not be surprising if these snails are present also in eastern Ethiopia. Field surveys have provided no evidence that *S. haematobium* is transmitted in Ethiopia by members of the *B.* '*truncatus*' complex, yet snails resembling Egyptian *B. truncatus* in having a tetraploid chromosome complement may be potential intermediate hosts. If Ethiopian tetraploid populations are capable of carrying strains of the parasite from the Mediterranean region or Arabia, it is possible in view of rapidly developing human communications that introduced strains of *S. haematobium* could become established in the Awash valley.

In conclusion we suggest first that transmission of *S. mansoni* in the middle and lower Awash regions is limited or prevented by the rarity of *B. pfeifferi* and, secondly, that transmission of *S. haematobium* may be confined to the lowland plain by the restricted distribution of its presumptive intermediate host, *B. abyssinicus*. Although the development of irrigation may increase the density of snails within their existing ranges, it will probably not greatly alter the boundaries of the ranges because they seem to be determined basically by climatic temperature. Control of schistosomiasis in the Awash valley is feasible by the application of molluscicides, for colonies of snail hosts are at present restricted in area and widely separated.

AD-A080 030 CALIFORNIA UNIV SAN FRANCISCO F/G 6/5
STUDIES ON MOLLUSCICIDAL AND OTHER PROPERTIES OF THE ENDOD PLAN--ETC(U)
JUN 79 A LEMMA, D HEYNEMAN, H KLOOS N00014-76-C-0218
UNCLASSIFIED NL

5 OF 6
AD
A080030


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

SUMMARY

The Awash river, Ethiopia, flows from the cool highland plateau to the warm lowland plain, a descent of 2,500 m. The aquatic snail fauna shows zonation in relation to altitude. Irrigated cultivation is being developed in areas where intestinal and urinary schistosomiasis occur. *Schistosoma mansoni* is transmitted by *Biomphalaria pfeifferi*, which is apparently confined to the upper river basin. The presumptive intermediate host of *S. haematobium* is *Bulinus* (*Physopsis*) *abyssinicus*, found only in the middle valley and lower plains. Snails resembling *Bulinus truncatus* in their morphology and chromosome number are present in the upper basin, but they are not known to transmit schistosomiasis. Irrigation canals have been colonized by *Biomphalaria pfeifferi*, but not by *Bulinus abyssinicus*. Climatic temperature is thought to play an important part in determining the different geographical ranges of these two snails and accordingly their ranges should not be greatly increased by irrigation developments. Prospects for the control of schistosomiasis are favourable because host snails live in restricted colonies which could be destroyed by local application of molluscicides.

ACKNOWLEDGEMENTS.—The first author would like to thank Dr. Aklilu Lemma for accommodation in the Pathobiology Institute; Dr. J. Duncan for snail samples; Ato Worku Mekasha for transport and facilities at the Agricultural Research Station at Melka Werrer; Mitchell Cotts Co. (Ethiopia) Ltd. for making possible visits to Dubti and Assaita, and Dr. G. F. Santi for hospitality and valuable assistance. We are indebted to the British Medical Research Council and the Haile Selassie I University for support and to Dr. C. A. Wright for comments on the manuscript.

REFERENCES

AKLILU LEMMA. (*In press*). Bilharziasis in the Awash Valley, Ethiopia. *Ethiopian med. J.*

AYAD, N. (1956). Bilharziasis survey in British Somaliland, Eritrea, Ethiopia, Somalia, The Sudan, and Yemen. *Bull. Wld Hlth Org.*, **14**, 1.

BROWN, D. S. (1964). The distribution of the intermediate hosts of *Schistosoma* in Ethiopia. *Ethiopian med. J.*, **4**, 250.

——— (1965). Freshwater gastropod mollusca from Ethiopia. *Bull. Br. Mus. nat. Hist. Zoology*, **12**, 37.

——— (1967*a*). Records of Planorbidae new for Ethiopia. *Arch. Moll.*, **96**, 181.

——— (1967*b*). A review of the freshwater mollusca of Natal and their distribution. *Ann. Natal Mus.*, **18**, 477.

——— and BURCH, J. B. (1967). Distribution of cytologically different populations of the genus *Bulinus* (Basommatophora: Planorbidae) in Ethiopia. *Malacologia*, **6**, 189.

BURCH, J. B. (1967). Some species of the genus *Bulinus* in Ethiopia, possible intermediate hosts of *Schistosomiasis haematobia*. *Ethiopian med. J.*, **5**, 245.

MAFFI, M. (1960). Primo reperto nel Basso Oltregiuba, Somalia, dei molluschi d'acqua dolce. *Parassitologia*, **2**, 191.

MANDAHL-BARTH, G. (1965). The species of the genus *Bulinus*, intermediate hosts of Schistosoma. *Bull. Wld Hlth Org.*, **33**, 33.

RUSSELL, H. B. L. (1958). Final Report. Mobile Health Team, Ethiopia. W.H.O. Doc. EM/PHA/62. *Ethiopia* 13, 1958.

STURROCK, R. F. (1966). The influence of temperature on the biology of *Biomphalaria pfeifferi* (Krauss), an intermediate host of *Schistosoma mansoni*. *Ann. trop. Med. Parasit.*, **60**, 100.

WRIGHT, C. A. (1963). Schistosomiasis in the Western Aden Protectorate; A preliminary study. *Trans. R. Soc. trop. Med. Hyg.*, **57**, 142.

——— (*in press*). *Bulinus* on Aldabra and the sub-family Bulininae in the Indian Ocean area. *Proc. R. Soc.*

SNAIL INTERMEDIATE HOST OF *SCHISOTSOMA HAEMATOBIUM* IN ETHIOPIA

CHIN-TSONG LO, Ph.D.

Malacologist, Institute of Pathobiology,
Haile Selassie I. University, Addis Ababa (Ethiopia)

ABSTRACT

A survey was made in Gewani area in order to determine the snail host responsible for schistosomiasis haematobia in that area, where 61% positive infection among people had been reported. Gewani area becomes a huge marsh during the wet season, but it was completely dry except for a permanent lake in the marsh, and a small pond (20 × 20 m) which was probably the last remaining water hole. From this pond, located 1 km south-east of Galiela Dula Village, 65 *Bulinus Abyssinicus* were collected, and 60% of the 52 snails studied reliably were shedding cercariae of *Schistosoma haematobium*. Two other furcocercous and one non-furcocercous cercariae also emerged from this snail. *B. forskalii* were also present in the same pond, but they were negative for *S. haematobium*. *Bulinus* sp. (haploid chromosome number 36) and *B. forskalii* were also collected from the outlet of Lake Caddabasa near Gewani; they were not shedding any kind of cercariae.

The specimens of *B. abyssinicus* were mostly large, ranging from 4.0-15.5 mm high (av. 11.0 mm). In the laboratory, infected specimens shed from 290-1, 130 (av. 500) cercariae per snail in 2-4 hours. Infected snails died sooner and produced much fewer offspring than the uninfected. Offspring of *B. abyssinicus* were experimentally exposed to miracidia obtained from a patient: 13 out of 14 snail became infected when mass exposed to 10 miracidia per snail, and all of 20 snails became infected when the number of miracidia was increased to 20 per snail. Adults of *S. haematobium* were recovered from hamsters and mice infected with cercariae emerged from naturally and experimentally infected snails.

INTRODUCTION

Ayad (1956) reviewed the available information up to 1952 on schistosomiasis in Ethiopia and considered that several cases of schistosomiasis haematobia in Eritrea were imported or of undefined origin. The first endemic area for this disease was reported by Russell (1958) who found 91 (48%) out of 189 urine samples positive for the eggs of *Schistosoma haematobium* among the Danakil people in Gewani. Further investigation has been made since then, and it is now certain that the disease is widespread in the middle and lower Awash valleys starting from Melka Warer down to Assaita with the following

Reprinted by permission from
Proceedings of the Symposium
on Schistosomiasis by the
Organization of African Unity
(OAU). 1970.

prevalence: Melka Warer, 21%; Angelele, 58%; Cortume, 68%; Hertale, 41%; Gewani, 61%; Hadeleguera, 40% and Assaita, 4% (Aklilu, 1969).

The molluscan intermediate host for *S. haematobium* is still unknown in Ethiopia, although Bulinus abyssinicus has been suspected on circumstantial evidence (Brown, 1967). This snail is a known vector in Somalia and its occurrence in Ethiopia was reported by Brown (1967) from the Assaita area. The present report deals with a snail survey made from June 27 to July 1, 1970 in Gewani area for the purpose of finding the infected snails as well as to see what other freshwater snails are present in the vicinity.

DESCRIPTION OF THE AREA AND PEOPLE

Gewani (altitude 700 m) is located northeast of Addis Ababa, 390 km by road through Awash Station. Several hundreds of Danakil people, mostly of the Adele tribe, are living in Gewani itself, but there are also many temporary villages located along the Awash River which is 8 km away from Gewani (fig. 1). It is said that about 25,000 people are living in an area of 50 km in diameter. The Ministry of Community Development has a demonstration farm on the east side of the river in order to introduce farming practices among these nomadic people. There is a school in one of the villages, with about a hundred pupils. The Ministry also maintains a clinic. There is no medical doctor but a dresser who sees from 30-50 patients a day. The commonest diseases seem to be malaria, schistosomiasis, eye diseases, skin diseases, tuberculosis, wounds caused by accidental cut or crocodile bite.

It was at the end of the dry season when we visited, although in the highlands it had been raining for nearly a month. The air temperature ranged from 37-42 °C during the day; the lowest temperature recorded was 26 °C during the night. It did not rain and was extremely hot. The Awash River water measured 27.5-28 °C

The Gewani swamp along both sides of the river is aid to cover several hundred square km, but it was completely dry, with cracked mud and dust except for a place near the farm where a permanent lake was formed. The lake was surrounded by a thick floating matt of grasses and *Typha* sp., 2-3 m tall. Many hippopotami and crocodiles were present in and around the lake. It could only be seen from southwest side. When the river water overflows during the wet season, the lake becomes connected with the rest of the swamp. Many patches of *Typha* are present in the area, and the underground rhizomes of this plant are regularly consumed by the Adeles after they have been cut into small granules and baked. When leaves of the plant dry up during the dry season, the people burn the whole field of *Thypha* in order to make digging of rhizomes easy. The fire could be seen from a distance of many kilometers during the night. Besides the lake mentioned, there was a small pond about 1 km southeast of Galiela Dula Village. It was about 30 cm deep, T-shaped, measuring about 20 m each arm. There were many catfish in the pond, and several thousands of birds (about 10 species) were feeding in the area surrounding the pond. The

great aggregation of birds suggested that this was one of the few remaining water holes. The water level in the Awash River was said to be increasing rapidly, and the river was expected to overflow soon. The inundation of the whole swamp area in addition to the sudden increase of mosquito population make these people move twice a year to higher places near Gewani. The first move starts about 20th of July, and the return begins in early October; the second move starts at the end of February, and the return at the beginning of May. It takes only a matter of 3-4 days to complete the movement of the whole village. Both river and swamp water are extensively used for bathing and drinking.

SNAIL SURVEY

The edge of the Awash River was examined at three places, but no snails were found. An attempt to reach the lake was unsuccessful because of inadequate equipment. However, by examination of shallow waters around the lake in thick grasses and *Typha* plants the following species of snails were found in small numbers (less than 20 each): *Bulinus forskalii. Anisus natalensis, Segmentorbis angustus* and *Lymnaea natalensis.*

From the small T-shaped pond (water temperature 28 °C) we collected several dead shells and 65 living specimens of *Bulinus abyssinicus* after three hours of intensive search, in addition to the above mentioned species (less than 15 each). On examination, the *B. abyssinicus* were shedding four kinds of cercaria: three furcocercous including that of *S. haematobium*, and one non-furcocercous. *B. forskalii* were shedding one kind of non-furcocercous cercaria, and *L. natalensis* 1 kind of cercaria resembling that of *Fasciola.*

The outlet of Lake Caddabasa near Gewani was also examined; the following mollusks were found: *Bulinus* sp. (haploid chromosome number n = 36), *B. forskalii, Biomphalaris pfeifferi* (shell only), *Anisus natalensis, Cleopatra bulimoides, Melanoides tuberculata* and *Sphaerium* sp. (shell only). The two bulinid species (less than 10 specimens each) were not shedding any cercariae.

LABORATORY OBSERVATIONS ON *B. ABYSSINICUS*

Sixty-two *B. abyssinicus* were still alive on arrival at the laboratory in Addis Ababa. Twenty-one of them were shedding cercariae of *S. haematobium*, seven were shedding other types of cercariae and 34 were negative (July 3), but three days later seven more snails started to shed schistosome cercariae. When negative snails were examined again a month later (17 were still alive then), three were positive for *S. haematobium*, four positive for other cercariae and ten were negative. The total results for 52 snails (excluding ten dead snails) are as follows: 28 (54%) positive for *S. haematobium* only, three (6%) infected with both *S. haematobium* and other trematodes, 11 (21%) positive for other trematodes and 10 (19%) were negative. In other words, 31 specimens (60%) were infected by *S. haematobium*, and 10 of these were still in the prepatent

period when collected, indicating that those 10 snails had been infected within a month before being collected, i.e. during the month of June (cercarial incubation lasts about four weeks at 28 °C).

The snails were mostly large (av. 11.0 mm high), the smallest one being 4.0 mm high, uninfected; the largest specimen measured 15.5 mm and was infected with another trematode. When measured a week after collection, 35 snails infected with various cercariae had an average height of 12.0 mm, while 27 negatives (including some snails in prepatent period) had an average of 9.7 mm, showing that gigantism might be present in the infected snails.

Snails infected with *S. haematobium* were examined for shedding of cercariae seven times from July 4 to August 26. Each time five to 23 snails were used and the cercariae emerging in 2-4 hours were counted. Light intensity was slightly increased by placing a 75-watt lamp 30 cm away. The number of cercariae emerged from each snail ranged from 290-1, 130, with an average of 500.

Mortality among the infected specimens was higher than in negative snails. Within two months, 32 out of 42 infected snails were dead, while only 4 of 10 negative snails died. Fecundity was much lower in the infected than the uninfected snails. During the two month period, less than 10 offspring were obtained from aquaria containing the infected specimens, but with fewer snails the negative group produced about 200 young snails.

Offspring of *B. abyssinicus* were experimentally exposed to the miracidia obtained from a patient coming from Melka Warer. Fifteen snails (2-4 mm high) were mass exposed to 10 miracidia per snail; 14 survived and 13 were positive. When 20 snails were mass exposed to 20 miracidia per snail, all of them survived and all became positive. The cercariae obtained from naturally and experimentally infected snails produced adult *S. haematobium* in hamsters and mice, thus confirming the identity of cercariae.

LITERATURE CITED

AYAD, N. (1956). Bilharziasis survey in British Somaliland, Eritroa, Ethiopia, Somalia, the Sudan and Yemen. *Bull. Wld. Hlth Org.*, **14**, 1-117.

RUSSELL, H. B. L. (1958). Final Report. Pilot Mobile Health Team, Ethiopia. Wld Hlth Org. document EM/PHA/62.

BROWN, D. S. (1967). Records of Planorbidae new for Ethiopia (Gastropeda, Pulmonata). *Arch. Moll.*, **96**, 181-185.

LEMMA, A. (1969). Bilharziasis in the Awash Valley: I. An epidemiological study with special emphasis on its possible future economic and public health importance. *Eth. Med. J.*, **7**, 147-176.

Lo, C.T., Flemings, M. B. and Lemma, A. (1973) *Eth. med. J. 11*, 271.

SCHISTOSOMIASIS IN HARAR, ALEMAYA AND THE DAMOTA VALLEY, ETHIOPIA

C. T. Lo, Ph.D.,* M. B. Flemings, Ph.D.,** and Aklilu Lemma, Sc.D.

Institute of Pathobiology, Haile Sellassie I University, P.O. Box 1176, Addis Ababa

Kubasta (1964) examined the stools of 1,845 inpatients at the Ras Makonnen Hospital of Harar and found 13.2% positive for *Schistosoma mansoni* eggs. At about the same time, of 152 children from the Harar Moslem School whom he examined, 71.1% were positive for this parasite. In order to reconfirm these results the Institute of Pathobiology undertook the present study in 1970. Since the infection has been known to prevail in the nearby village of Alemaya, the 1970 study also covered examination of school children and snail surveys in Alemaya. In 1971 the Institute of Pathobiology made a further series of parasitological and malacological studies in the Alemaya area at the request of the Agricultural College which is located on the east short of Lake Alemaya. Results of these studies are reported in this paper.

Material and methods

In the 1970 study, fresh stools were collected from children in two different primary schools (a Moslem school and a Christian school) in Harar, and the Alemaya Elementary School. About 1 gramme of each of the samples were preserved in 7.5% formalin for concentration of parasite eggs with Ritchie's formol-ether concentration technique (Ritchie, 1948). In June 1971 some inhabitants of the Damota Valley were similarly examined for their parasitic infections.

Examination for the presence of snails in different localities was made by a scoop net. The places examined were streams flowing within the city limits of Harar, Lake Alemaya, a swamp on the southern edge of Alemaya, and streams in the Damota Valley. Detection of intramolluscan trematodes was accomplished by the standard technique of crushing and examination under the stereoscopic microscope.

Results

Stool examinations in Harar and Alemaya. The results of stool examination are shown in Table 1. The total figures show that 64% of school children at the Moslem School were positive for *S. mansoni* while in the Ras Makonnen School of Harar and the Alemaya Elementary School the infection rates were 20% and 19% respectively. The much higher infection rate among the Moslems might possibly be explained by their more frequent use of water associated with their religion and defecation disposal

* *Present adress*: Department of Parasitology, College of Medicine, National Taiwan Univesity, Taepei, Taiwan, China.

** *Present adress*: Department of Biology, C. W. Post Center, Long Island University, Greenvale, N.Y. 11548, U.S.A.

and some *Anisus sp.* As for *B. pfeifferi*, only 2 shells of dead snails were collected during the 1970 survey in front of the school. In June 1971 the lake was examined more extensively but we still failed to obtain any *Biomphalaria*, although Aram and Aweitu (1971) had obtained 5 living specimens in February near the buildings of the Electric Light and Power Authority. In that area, however, we noticed the presence of 5 waterholes dug by the farmers in order to get water from the lake for growing crops and vegetables. The holes were about 50 cm. in diameter, less than 30 cm. deep, and connected to the lake proper by a small ditch less than 10 m. long. In one of them there were about 300 adult *B. pfeifferi*. They were about 1 cm. in diameter, dark coloured and appeared very healthy. The ditch also contained some specimens, but they disappeared as soon as the ditch reached the lake, indicating that the lake conditions were unsuitable for their survival. We examined 100 snails for trematode infections and they were all negative. In the other 4 waterholes there were no *Biomphalaria*.

A swampy area adjacent to Alemaya (see Figure 1) had much water in April 1970. Many specimens of diploid *Bulinus sp.*, *Anisus sp.*, *Lymnaea truncatula* and *Succinea sp.* were collected but there were no *Biomphalaria*. In June 1971 this swampy area was dry, covered with tall grasses.

The Damota Valley. Conversations with Ato Legesse Zerihun, who was teaching at the Agricultural College and served as an assistant during our survey, revealed that *Biomphalaria* might be present in the Damota Valley located on the east side of the College (Figure 1).

At the village of Bati there are many houses, but above it the houses are scattered in the valley. Just outside of Bati on the east there is a pond about 10 m. long and 5 m. wide which was made to store water for irrigation purposes. It was connected to 3 small springs about 30 m. away by narrow canals. These small springs provide the drinking water for the people. Thousands of *Biomphalaria* snails of varying sizes were present in the pond as well as in the canals and springs. We examined 50 specimens by crushing. They were negative for schistosomes, but 3 of them had rediae of unknown trematodes.

In the Damota Valley there are 2 streams, the Yatu and the Damota. They join at a point about 50 m. east of the bridge on the highway south of Bati. The amount of water was small: at the bridge it was about 20 cm. deep and 1 m. wide. Both the streams are said to be perennial. In the Yatu stream there were no *Biomphalaria* up to about 1 km. from the bridge, presumably due to its sandy, and at places, muddy bottoms which were constantly being modified as the amounts of water fluctuated. In the upper stream where parts of the streambeds were covered with grasses the snails started to appear. In the Gandaberi area there were several pockets of water and some irrigation ditches along the stream and these contained many *Biomphalaria pfeifferi*. We examined 75 snails collected from an

habits. Children from the other 2 schools are predominantly Christians. The age distribution of the infected children in these three schools was very interesting. Dividing them into two groups of ages 7-11 and 12-17, the older age group had a prevalence 3.5 fold greater than the younger age groups in the Alemaya Elementary School, 2.9 times more in the Ras Makonnen School, and 1.7 times more in the Moslem School.

Snail surveys and epidemiological observations in Harar. Two small streams which flow through Harar from the north and from the south and join in the city, had been suspected to be the possible source of infection (Kubasta, 1964). We examined these streams at 7 places. The northern stream was polluted with human and domestic animal wastes. Riverbeds were generally rocky but in some places contained some muddy areas covered with weeds. Only one large *Biomphalaria pfeifferi* and some *Lymnaea natalensis* snails were collected. The specimen of *B. pfeifferi* was found among the weeds growing in a muddy place in the vicinity which was frequented by people for laundering and bathing. The southern stream flows at the southern edge of the town and contains relatively clean and cold water with sandy bottoms. No snails were found in this stream.

Snail surveys in Alemaya. Lake Alemaya (Figure 1) had numerous specimens of a diploid (n=18) population of *Bulinus sp.*, many *L. natalensis*

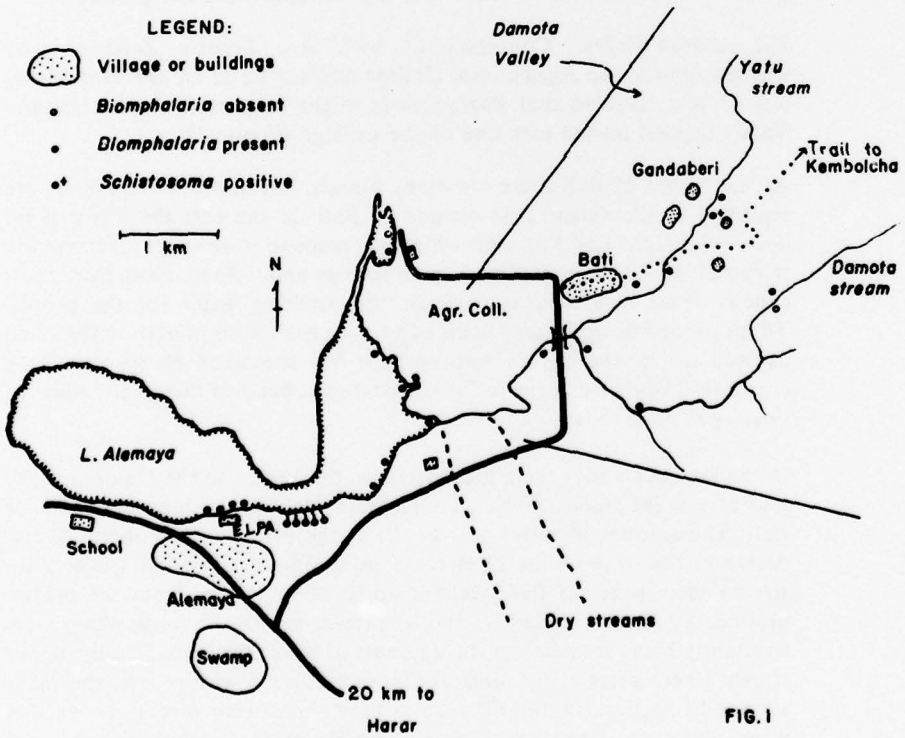


Figure 1. Map of the Damota Valley and Lake Alemaya.

expanded area of the stream and found 3 of them positive for *S. mansoni*. Two of the infected snails had very few mature cercariae when crushed, indicating that the infection had taken place recently, and the third snail had many cercariae. Human faeces were found along the stream. The Damota stream was examined up to about 3 km. from the bridge. No snails were found in the stream itself, but we found a waterhole a little above the stream on the hillside in which some *Biomphalaria* of small size were present. The examination of 5 specimens showed no infection.

The stream below the Damota — Yatu confluence had a sandy bottom and the water even sank into the ground at some sections. No live snails were found there. Where the stream joined with Lake Alemaya, the habitats seemed to be favourable but no *Biomphalaria* were found.

Stool examination of people living in the valley (Table 2) revealed that among 187 samples collected from various age groups, 8 (4%) had *S. mansoni* eggs. The positive cases were all below 30 years of age. A similar trend was also noticed in the age distribution of infection for other parasites.

Discussion

It is still unknown precisely where the school children in Harar get their *S. mansoni* infections. Although Kubasta (1964) suspected the 2 streams flowing through the city to be the only sources of infection, more snail are needed in order to confirm this view. In the vicinity of Alemaya one would suspect Lake Alemaya to be an important source of infection, but the snail studies seem to suggest the contrary. The presence of both positive snails and people in the Damota Valley has led us to believe that the waterholes and small streams may be the source of infections for the Alemaya region. It is interesting that loci like these, which appear unimportant, may play a greater role than the more conspicuous water bodies in the transmission of the parasite. Our studies also suggest that Lake Alemaya is unsuitable for colonisation by snails for some unknown reason. It would be very important to find the reasons for this.

Except for the big cities, piped drinking water is absent or very limited in most parts of Ethiopia. Thus the people spend much time and effort in securing water from natural sources for various purposes. Consequently their lives, like the molluscan fauna, are much influenced by seasonal variations. Therefore one must study an area at several points in time during both the dry and rainy seasons, in order to obtain a full picture. For example, *Biomphalaria* were very scarce in Lake Alemaya, but one might be able to find more snails during the rainy season when a large number of snails would be flushed down into the lake from the Damota Valley. Furthermore during this period the lake water might become infective, especially at the inlet area. The snails would probably reach

as far as the Electric Light and Power Authority buildings area, but eventually die out for some unknown limiting factors.

Brown (1965) reported the presence of *B. pfeifferi* in numerous small streams in Harar Province. Therefore, stool surveys coupled with snail examination in those areas might reveal more transmission sites. Between Alemaya and Harar there are several streams intersecting the highway. Most of them do not seem to be suitable for the breeding of snails, but careful examination is necessary to be certain.

Summary

The prevalence of *Schistosoma mansoni* infection in three groups of school children in Harar and Alemaya was determined and compared. Sixty-four percent of children in the Moslem School in Harar, twenty percent of the predominantly Christian children in Ras Makonnen School in Harar, and nineteen percent of the mixed Moslem and Christian children in the Alemaya Elementary School were infected. The reason for the significant difference in the infection rate amongst the Moslem and Christian children are not clearly known.

The epidemiological studies in the Alemaya area suggest that the infection is not transmitted in Lake Alemaya, which appears to be unfavourable to snail habitation. However in the Damota Valley, which carries water from the mountains to the lake, there are some isolated bodies of water which favour snail breeding and act as ideal disease transmission sites.

Acknowledgement

This work was supported in part by a research grant from the Rockefeller Foundation. We are grateful to Dr. M. Dow of Alemaya Agricultural College for providing and arranging all necessary assistance during the 1971 survey. The technical assistance of Miss I. Bystrom, Mr. G. Ericsson, Ato Legesse Zerihun, Ato Bahta Mazengia and Ato Bogale Assefa in the field and laboratory studies reported herein is greatly appreciated.

References

Aram, R. H. and Aweitu, F. (1971) A molluscan survey of the Lake Alemaya and Lake Adele area of Harar Province, with special reference to human schistosomiasis. Institute of Pathobiology Internal Publication.

Brown, D. S. (1965) Freshwater gastropod molluscs from Ethiopia. *Bull. Brit. Mus. Nat. Hist.* (Zool) *12*, 39.

Kubasta, M. (1964) Schistosomiasis mansoni in the Harar Province. *Eth. med. J. 2*, 260.

Ritchie, L. S. (1948) An ether sedimentation technique for routine stool examination. *Bull. U.S. Army Med. Dept. 8* (4), 444.

J. Zool., Lond. (1972) **167**, 97–132

On a polyploid complex of freshwater snails (Planorbidae: *Bulinus*) in Ethiopia

D. S. BROWN* AND C. A. WRIGHT

Experimental Taxonomy Unit, Zoology Department, British Museum (Natural History), London SW 7

(*Accepted 9 November 1971*)

(With 5 plates and 11 figures in the text)

In *Bulinus* as in other Planorbidae the basic haploid chromosome number is 18 and *B. tropicus* belongs to a group of diploid forms ($2n = 36$) distributed predominately in southern Africa. *B. truncatus* belongs to a group of tetraploid forms ($2n = 72$) occurring mainly in northern Africa and extending northwards beyond the limit of the diploid forms. Hexaploid ($2n = 108$) and octoploid ($2n = 144$) populations occur in Ethiopia. Observations were made on snails belonging to this polyploid complex collected from 69 localities in Ethiopia. Chromosome numbers were determined in gonad tissue and in embryos. Morphological features studied are the shell (spire length, columella shape, umbilicus, costulation), radula (size of the first lateral tooth and the shape of its mesocone), copulatory organ (presence or absence) and egg size. Biochemical features investigated are the body-surface mucus, egg-proteins and esterase iso-enzymes.

Certain diploid populations conform to *B. natalensis* and others to *B. tropicus* but these extremes are connected by intermediates preventing taxonomic subdivision and the entire diploid group is regarded as a northern part of the range of a *B. natalensis/tropicus* complex. Ethiopian tetraploid populations are identified as *B. truncatus* because of similarity to Egyptian snails in morphology and egg-proteins and the susceptibility of some Ethiopian snails to infection with Egyptian *Schistosoma haematobium*. The shell spire is comparatively long in many hexaploid and octoploid populations, and distinctive egg-protein patterns are given by octoploids but these groups do not provide clear-cut taxonomic species.

Hexaploids and octoploids are associated with streams at high altitudes while diploids and tetraploids occur in various habitats, most frequently at lower levels. The comparative rarity of the tetraploid *B. truncatus* probably is one of the factors that have apparently prevented the establishment of *truncatus*-borne strains of *S. haematobium* in Ethiopia, though present developments are likely to increase the abundance of tetraploid snails and provide opportunities for transmission of the parasite.

Contents

* External Scientific Staff, Medical Research Council. Fieldwork carried out at the Institute for Pathobiology, Haile Sellassie I University, Addis Ababa, Ethiopia.

Introduction

Snails of the genus *Bulinus* are a characteristic element of the freshwater fauna throughout continental Africa, in Arabia, on Madagascar and Mauritius and in the Mediterranean region eastwards to Khuzestan in Iran. They are found in a wide range of habitats from large lakes to temporary rain-pools but are most frequently encountered in small, slow-flowing or static waterbodies. In recent years the genus has attracted a great deal of attention because some of its members serve as intermediate hosts for blood-flukes of the genus *Schistosoma* parasitic in man and domestic animals.

Over 100 species of *Bulinus* have been named but Mandahl-Barth (1957) recognized only 20 species with a total of 10 subspecies, arranged in four groups, the *africanus*, *forskalii*, *tropicus* and *truncatus* groups. This revision, based on conchological and anatomical characters, has served as the foundation for all recent work on the genus. Subsequent changes have mostly resulted in the restoration to recognition of species previously relegated to synonymies and thus the number of accepted species has once again increased. Some species have been transferred from one group to another and a fifth species group has recently been segregated from the *forskalii* complex, the *reticulatus* group (Wright, 1971*a*). At the present time the species belonging to the *africanus*, *forskalii* and *reticulatus* groups are relatively well defined and they can be allocated to their species groups without much difficulty, but the members of the *tropicus* and *truncatus* groups are not readily identified at the specific level and the definitions of the groups themselves are subject to controversy. Attempts to resolve these difficulties are stimulated by the fact that members of the *truncatus* group serve as hosts for *Schistosoma haematobium* in West Africa, the Mediterranean region and the Middle East, while no member of the *tropicus* group has been found infected with the parasite in nature.

The morphological characters used by Mandahl-Barth (1957) to distinguish between the *B. truncatus* and *B. tropicus* groups are found in the radular teeth and in the copulatory organ. In *B. truncatus* the mesocone (middle cusp) of the tricuspid lateral teeth is arrow-head shaped (with angular sides) while in *B. tropicus* the mesocone is simply triangular (with straight sides). The copulatory organ is frequently absent (the aphallic condition) in *B. truncatus* but rarely so in *B. tropicus*. However, recent studies in South Africa (Brown, Oberholzer & Van Eeden, 1971*b*) have shown that the differences in the radula are completely bridged in *B. natalensis* (Küster), of which different individuals from the same population may have the shape of mesocone characteristic of *B. truncatus*, or of *B. tropicus*, while mesocones of intermediate shapes are found in other snails. Nor does the condition of the copulatory organ provide a reliable taxonomic character, as many populations of

B. truncatus are entirely or predominantly normal. Despite the presence of the angular type of mesocone and aphallic individuals in some populations, *B. natalensis* has not so far been found infected with *S. haematobium* under natural conditions.

In 1960 Burch showed that *B. truncatus* snails have 36 pairs of chromosomes ($2n=72$) in contrast to the other groups of *Bulinus* (and indeed most of the Planorbidae) which have $2n=36$. Wright & Ross (1965) found that there is a characteristic pattern in the egg-proteins separated by electrophoresis in *B. truncatus* which is distinct from the patterns given by other bulinids and Burch & Lindsay (1970) have reported immunological differences between the *truncatus* and *tropicus* groups. Snails having $2n=72$ (tetraploid) have a northern distribution, while *B. tropicus* and related forms (including *B. natalensis*) having $2n=36$ (diploid) predominate in southern Africa (Fig. 1). The ranges of these two assemblages overlap between latitudes 15°N and 5°S. Wherever it has been possible to check chromosome number against egg-proteins it has been found that the characteristic *truncatus* type of pattern occurs only in tetraploid snails, and in most cases where infection experiments have been carried out these snails have proved to be susceptible to "Mediterranean" strains of *S. haematobium*.

In Mandahl-Barth's (1957) revision of *Bulinus* a number of named forms from Ethiopia were grouped together in the single species *B. sericinus* (Jickeli) which was placed in the *tropicus* group. Mandahl-Barth mentioned that in some adjacent areas intermediates between *B. sericinus* and *B. tropicus* were found, while in other areas there were forms intermediate with *B. truncatus*. Wright & Brown (1962) studied radulae from snails obtained in the highland region near Debra Markos and as a result transferred *B. sericinus* to the *truncatus* group. Both Brown (1965) and Mandahl-Barth (1965) found high levels of inter-population variation among Ethiopian snails provisionally identified as *B. truncatus sericinus* and all of these forms were considered to be potential hosts for *S. haematobium* (Brown, 1964; Mandahl-Barth, 1965). Subsequently it was discovered that many of these populations had different chromosome numbers (Brown & Burch, 1967; Burch, 1967) indicating the existence of a complex of sibling species forming a polyploid series ($2n=36$, 72, 108 and 144). This paper describes the results of an investigation into some of the non-cytological characters of this complex.

Material and methods

Material from 69 localities in Ethiopia has been used for observations on the chromosome number, shell, radula and copulatory organ (Figs 2 and 3; Appendix). Laboratory colonies derived from 43 of these localities have provided material for the study of body-surface mucus (by paper chromatography), egg-proteins (by electrophoresis on cellulose acetate and gel-diffusion immunological tests), esterase iso-enzymes (by starch-gel electrophoresis) and egg-size. Snails from some localities were exposed to infection with *S. haematobium* originating from Cairo and *S. bovis* from Sardinia. Material was collected from 18 of the localities in 1965 (sample numbers prefixed by 65/) and the chromosome numbers of these samples have already been reported (Brown & Burch, 1967). Only material collected in 1969 (sample numbers prefixed by 69/) has been used for biochemical studies; 5 of these samples are from the same localities as those collected in 1965 and therefore morphological data for a total of 74 samples are given (Table 1). Comparative observations on *B. truncatus* from Cairo, Egypt have also been made and all material has been deposited in the Experimental Taxonomy Unit in the British Museum (Natural History).

Chromosome numbers for 70 of the samples (including the 18 collected in 1965) were deter-

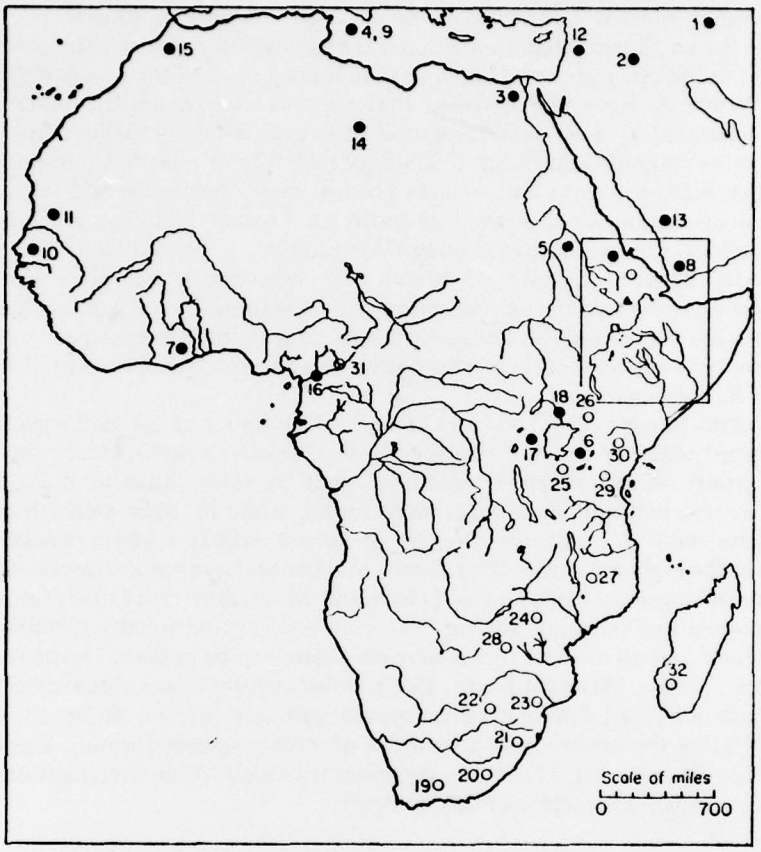


FIG. 1. Africa and the Arabian Peninsula showing the distribution of the various chromosome numbers known in the species groups of *Bulinus truncatus* and *B. tropicus*. Hexaploid (▯) and octoploid (▲) populations have a limited distribution within the area enclosed by the rectangle, shown in detail in Figs 2 and 3.

Tetraploid populations (●): 1, Iran, Teheran; 2, Iraq, Baghdad; 3, Egypt, Cairo; 4, Sardinia; 5, Sudan; 6, Tanzania, Mwanza; 7, Ghana, Kumasi; 8, Western Aden Protectorate, Belas; 9, Corsica; 10, Gambia, Kumbidja; 11, Mauretania, Tagant plateau; 12, Lebanon; 13, Yemen, Husseifa (near Taiz); 14, Libya, Sebha; 15, Morocco Gnafda and Goalmina; 16, Cameroon, Penja; 17, Uganda, Lake Mutanda; 18, Uganda, Jinja.

Diploid populations (O): 19–23, South Africa and Mozambique; 24, Rhodesia, Salisbury; 25, Tanzania, Mwanza; 26, Kenya, Kisumu; 27, Malawi, Lake Malawi; 28, Rhodesia, Bulowayo; 29, Tanzania, Lake Duluti; 30, Kenya Nairobi; 31, Cameroon, N'Dop Plain; 32, Madagascar, Basibasy.

Chromosome numbers for localities 12–18, 28–32 were determined in stocks of snails maintained in the British Museum (Natural History). Sources of previously published information are: localities 1–7, 24–26 (Burch, 1964) 8 (Natarajan *et al.*, 1965); 9–11 (Burch & Lindsay, 1970); 19–23; Brown *et al.*, 1971*a*); 27 (Wright *et al.*, 1967).

Note to Fig. 1. Natarajan *et al.* (1965) reported that specimens of *B. guernei* (Dautzenberg) collected in Senega were diploid. However, Burch & Lindsay (1970) found a tetraploid complement in material of *B. guernei* from the Gambia collected and identified by C. A. Wright. In our opinion the snails examined by Natarajan *et al.* may have been *B. jousseaumei* (Dautzenberg), a member of the *B. africanus* group in which other species are known to be diploid.

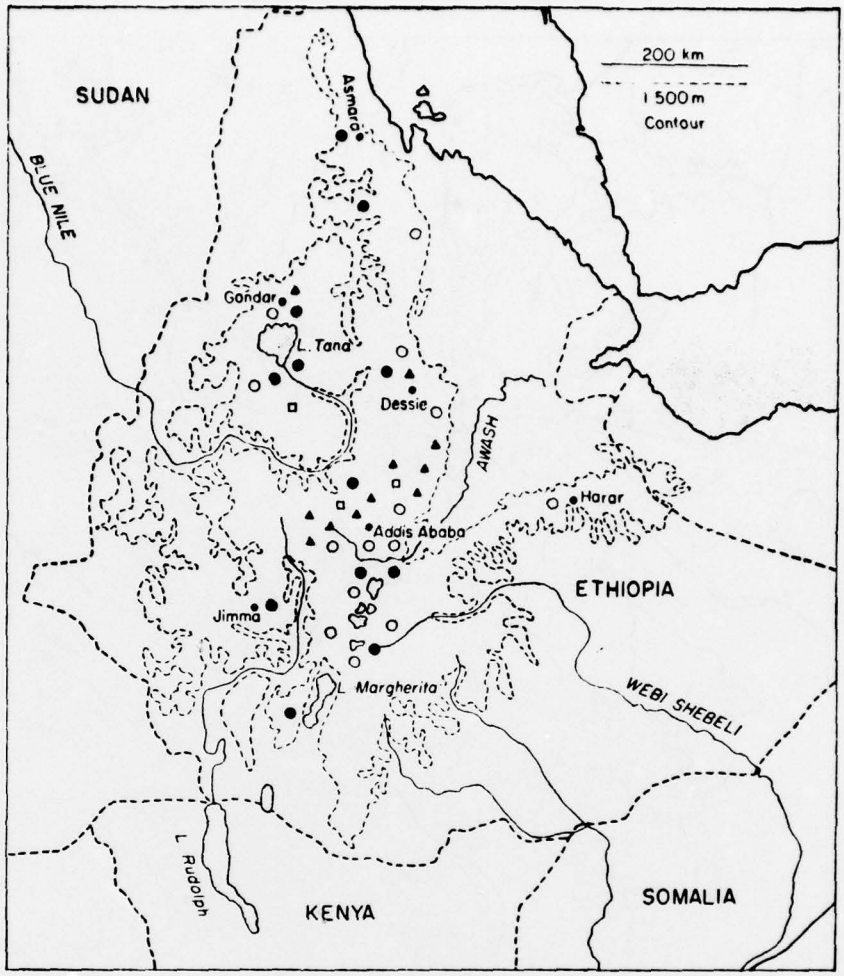


FIG. 2. The distribution of diploid and polyploid populations of *Bulinus* in Ethiopia. Diploid (O), tetraploid (●), hexaploid (□), octoploid (▲). The central area including Addis Ababa is shown in detail in Fig. 3.

mined from snails collected in the field and for 26 localities these results were confirmed in laboratory-bred material. For 4 localities only laboratory-bred snails were used. Results were obtained for 504 individuals in the 1969 material alone. Both meiotic and mitotic figures were examined. Meiotic figures were obtained from gonad tissue fixed in cold Carnoy's solution and subsequently crushed in acetic-orcein stain; the number of bivalents at diakinesis was counted. Mitotic metaphase figures were obtained from embryos about 48 h old exposed to colcemid solution and stained with acetic-orcein according to the method described by Claugher (in press).

Shell length (L) and aperture length (AL) (Fig. 4(a)) were measured with the aid of a camera lucida at a table-top magnification of ×12. The ratio L/AL, a measure of spire length, was determined for 1809 shells and mean values were calculated for each sample. The columella, umbilicus

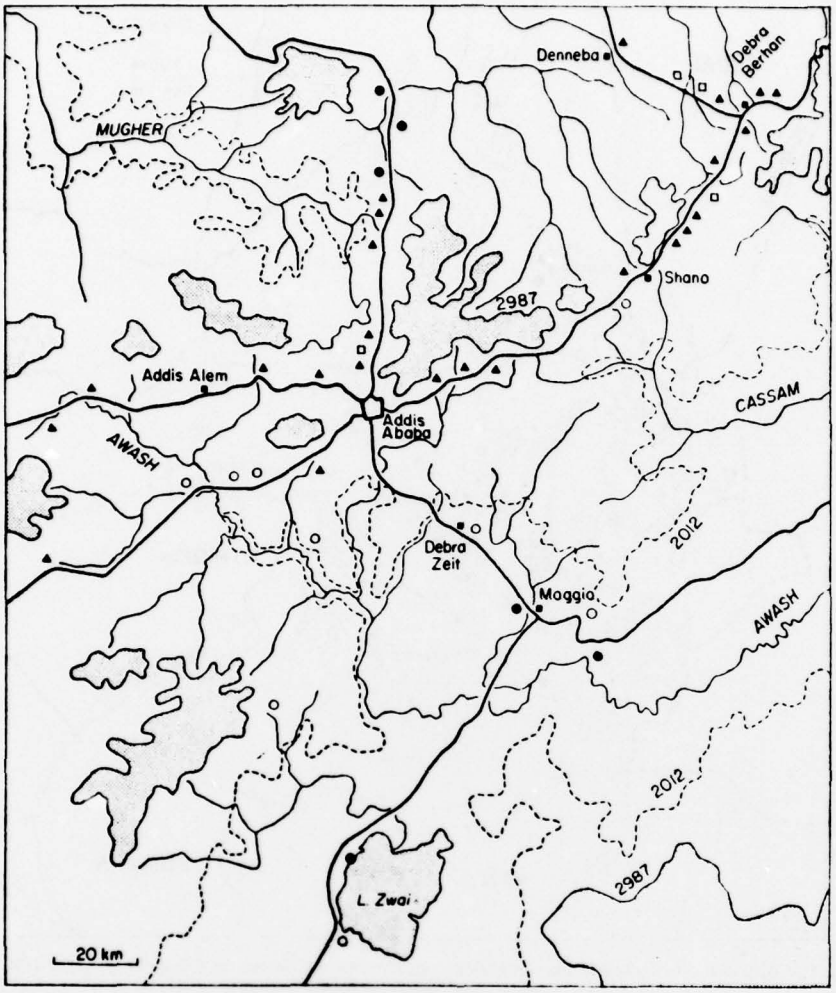


FIG. 3. The distribution of diploid and polyploid populations of *Bulinus* in central Ethiopia (symbols as in Fig 2). Major roads are indicated by bold lines and land over 2987 m is stippled. Lake Zwai and the lower course of the Awash river lie on the floor of the Rift Valley, on either side of which are escarpments indicated by the 2012 m contour (broken line).

and costulation of each shell were awarded values on the scale 1–4, according to the method of scoring used by Brown *et al.* (1971*a*) (summarized in Table II), and sample mean scores were calculated (Table I).

A total of 400 radulae from measured snails (usually 5 per sample) was examined. Radulae were removed from the buccal masses by maceration in warm 10% NaOH, rinsed in distilled water and stretched on slides in a drop of 5% acetic acid, which was allowed to evaporate. The preparations were stained for 2 minutes in Mallory's second staining solution and mounted in Canada balsam. In each radula the mesocones of the right and left first lateral teeth in 10 consecu-

infection of octoploid snails with an Egyptian strain of *S. haematobium*. Assessment of the significance of laboratory infection experiments is not easy because of the large number of variable factors involved (Wright, 1971*b*). It is sometimes possible to achieve infection of a few individuals of a snail which would not normally act as a host under natural conditions, and it is equally possible to fail to obtain infections in the normal host snail. At the present time it seems that susceptibility to *S. haematobium* and *S. bovis* is, within the species complex under consideration, primarily an attribute of tetraploid snails.

In summary, the morphological evidence indicates that of the diploid forms *B. natalensis* is most closely related to the tetraploid forms and it seems that the establishment of tetraploidy led to the acquisition of, or a considerable increase in, susceptibility to infection with *Schistosoma*. Tetraploidy probably led to the origin of hexaploids and octoploids through hybridization in various directions and chromosome doubling. It seems unlikely that octoploids could be derived from tetraploids by autopolyploidy because octoploids lack two of the most distinctive tetraploid characters, namely the aphallic condition and ready susceptibility to *Schistosoma* spp., and although octoploids show considerable immunological affinities with tetraploids they have additional antigens in common with diploids. It is possible that karyotype investigations will throw light on these problems, although the practical difficulties are considerable in these snails which have such small and numerous chromosomes (Claugher, in press).

Epidemiology of schistosomiasis

The existence of sibling species of *Bulinus* is relevant to the present epidemiology and possible future development of schistosomiasis in Ethiopia. *Schistosoma haematobium* is confined, so far as known, to a few localities near the lower Awash river, where the presumptive snail host is a member of the *Bulinus africanus* group (Brown & Lemma, 1970). It has puzzled parasitologists that so-called "*Bulinus truncatus*" is abundant in Ethiopia but does not seem to transmit the parasite, and poor communications, low climatic temperatures at high altitudes and a low human population density have been suggested as possible barriers to the spread of infection. In addition it is possible that differences in susceptibility between sibling species of *Bulinus* have played a part. The tetraploid *B. truncatus* appears to be the only, or at least the most suitable, potential intermediate host found in extensive areas but this species has a fragmented distribution and is uncommon in some areas that are climatically suitable for *S. haematobium*, notably the Awash valley and the lakes region of the Ethiopian Rift valley. However, modern developments are likely to favour the colonization of lowland Ethiopia by *B. truncatus* and *S. haematobium*; improving communications provide increasing opportunities for the introduction of suitable strains of the parasite, while antimalarial measures have allowed a great increase in the human populations on the shores of the Rift valley lakes, and the new irrigation schemes planned for the Awash valley will probably be colonized by the snail, which is already present in the Wonji sugar plantation. The possibility that the colonization of newly created habitats by *B. truncatus* might be forestalled by the artificial introduction of resistant diploid snails has been discussed by Brown & Burch (1967). It is regrettable that the present study has not revealed any characters of the shell or gross anatomy by which *B. truncatus* can be readily identified, and at present the chromosome number and biochemical tests provide indispensable characters.

Summary

Snails of the genus *Bulinus* occur in freshwaters in Africa and adjacent areas. Some species are intermediate hosts for blood-flukes of the genus *Schistosoma* parasitic in man and domestic animals. Taxonomic difficulties arise from extensive inter-population variation, particularly in the complex that includes *B. truncatus* and *B. tropicus*, the former being an intermediate host while the latter is not.

In *Bulinus* as in other Planorbidae the basic haploid chromosome number is 18 and *B. tropicus* belongs to a group of diploid forms ($2n=36$) distributed predominantly in southern Africa. *B. truncatus* belongs to a group of tetraploid forms ($2n=72$) occurring mainly in northern Africa and extending northwards beyond the limit for diploid forms. Hexaploid ($2n=108$) and octoploid ($2n=144$) populations occur in Ethiopia. Observations on this complex, aimed at determining the distributions of the various chromosome numbers and detecting non-cytological characters by which they might be recognized, were made on snails collected from 69 localities in Ethiopia in 1965 and 1969. Chromosome numbers were determined in meiotic figures in gonad tissues and mitotic figures in embryos. Morphological features studied are the shell (spire length, columella shape, umbilicus, costulation), radula (size of the first lateral tooth and the shape of its mesocone), copulatory organ (presence or absence) and egg size. Biochemical features investigated are the body-surface mucus (by paper chromatography), egg-proteins (by electrophoresis on cellulose acetate and gel-diffusion immunological tests) and esterase iso-enzymes (by starch-gel electrophoresis).

The shell varies widely between diploid populations, the spire may be depressed and the umbilicus nearly closed (lacustrine populations), or the spire may be exserted and the umbilicus widely open (temporary pools). The radular mesocone also varies greatly between diploid populations, being predominately angular or non-angular, or intermediate in shape. Octoploid and hexaploid populations vary less widely and achieve a comparatively large maximum individual size; the spire is generally long, the eggs (measured for octoploids only) are large and the angular type of mesocone has a generally high frequency. The maximum size in tetraploid samples was smaller, the shell is moderately depressed with a shouldered whorl, the columella is commonly twisted and many individuals are aphallic. Of the biochemical features studied, electrophoretic separation of egg-proteins yielded patterns which can be correlated with chromosome numbers; 2 pattern types were found in diploids, two in octoploids and one in tetraploids. The diploid and tetraploid patterns are similar to those that have been found for populations of corresponding chromosome number from outside Ethiopia.

Certain diploid populations conform, according to morphological characters, to *B. natalensis* and others to *B. tropicus*, but these extremes are connected by intermediate populations preventing taxonomic separation and the entire diploid group is regarded as a northern part of the range of a *B. natalensis/tropicus* complex, also widespread in southern Africa. Ethiopian tetraploid populations are identified as *B. truncatus* because of similarity to Egyptian snails in morphology and egg-protein pattern, and the susceptibility of some Ethiopian snails to infection with Egyptian *S. haematobium*. Although the shell spire is comparatively long in many hexaploid and octoploid populations, and distinctive egg-protein patterns are given by octoploids, these groups do not provide clear-cut taxonomic species.

Bulinus truncatus from an irrigation scheme in the Ethiopian Rift valley were susceptible to infection with *S. haematobium* from Cairo, and snails from the same locality and also from Lake Margherita were infected with *S. bovis* from Sardinia. No cercariae were obtained from diploid and octoploid snails exposed at the same time.

Diploid and tetraploid populations were found mostly at comparatively low altitudes (below about 2100 m) in a variety of habitats including lakes and temporary pools, whereas the higher polyploids are associated with streams at higher altitudes (about 2100 m). Although the ranges of the different groups of populations overlapped in some areas, only one chromosome number was observed in any particular locality.

Some examples of differences in chromosome number in other groups of animals are briefly reviewed. It is remarkable that polyploidy should not be more frequent in the self-fertilizing groups of Basommatophora. The polyploid complex of *Bulinus* has the geographical characteristics associated with young to mature polyploid complexes in higher plants. Extremes of climate have probably played an important part in the establishment of polyploidy in *Bulinus*, though higher polyploids have not been found in other areas of Africa with present climates more extreme than that of highland Ethiopia. Little is known of interrelations within the polyploid complex; according to morphological characters the diploid *B. natalensis* is placed closest to the origin of the tetraploid forms and apparently as a result of tetraploidy susceptibility to infection with *Schistosoma* was greatly increased. Immunological data indicate that octoploids possess antigens in common with tetraploids and with diploids.

The comparative rarity of the tetraploid *B. truncatus* may be one of the factors that have prevented the establishment of *truncatus*-borne strains of *S. haematobium* in much of Ethiopia, though present developments are likely to increase the abundance of tetraploid snails and provide opportunities for transmission of the parasite.

We thank colleagues in Ethiopia without whose aid this investigation would not have been possible; accommodation was provided by Dr Aklilu Lemma in the Institute for Pathobiology, Addis Ababa and assistance in field work was given by Ato Worku Mekasha and Drs J. Duncan, M. Largen and G. F. Santi. We also thank Mrs A. Gismann, Dr E. Demian and Dr G. Wernsdorffer for kind assistance in Cairo, Dr G. Mandahl-Barth for comments on this paper and G. C. Ross and D. Claugher for technical assistance.

REFERENCES

Berg, L. S. (1964). *Freshwater fishes of the U.S.S.R. and adjacent countries*. Translated from the Russian. Israel Program for Scientific Translations, Jerusalem.

Bondeson, P. (1950). A comparative morphological-biological analysis of the egg capsules of freshwater pulmonate gastropods. *Natura jutl.* **3**: 1–208.

Brown, D. S. (1964). The distribution of the intermediate hosts of *Schistosoma* in Ethiopia. *Ethiopian med. J.* **2**: 250–259.

Brown, D. S. (1965). Freshwater gastropod Mollusca from Ethiopia. *Bull. Br. Mus. (nat. Hist.)* (Zool.) **12**: 37–94.

Brown, D. S. & Burch, J. B. (1967). Distribution of cytologically different populations of the genus *Bulinus* (Basommatophora: Planorbidae) in Ethiopia. *Malacologia* **6**: 189–198.

Brown, D. S. & Lemma, A. (1970). The molluscan fauna of the Awash river, Ethiopia, in relation to the transmission of schistosomiasis. *Ann. trop. Med. Parasit.* **64**: 533–538.

Brown, D. S., Oberholzer, G. & Van Eeden, J. A. (1971*a*). The *Bulinus natalensis/tropicus* complex (Basommatophora: Planorbidae) in south-eastern Africa. 1. Shell, mantle, copulatory organ and chromosome number. *Malacologia* **11**: 141–170.

Brown, D. S., Oberholzer, G. & Van Eeden, J. A. (1971*b*). The *Bulinus natalensis/tropicus* complex (Basommatophora: Planorbidae) in south-eastern Africa. 2. Some biological observations, taxonomy and general discussion. *Malacologia* **11**: 171–198.

Burch, J. B. (1960). Chromosomes of *Gyraulus circumstriatus*, a freshwater snail. *Nature, Lond.* **186**: 497–498.

Burch, J. B. (1964). Cytological studies of Planorbidae (Gastropoda: Basommatophora). 1. The African subgenus *Bulinus* s.s. *Malacologia* **1**: 387–400.

Burch, J. B. (1965). Chromosome numbers and systematics in euthyneuran snails. In *Proc. Europ. Malacol. Congr.* **1**: 215–241. Cox, L. R. & Peake, J. F. (Eds). London: Malacological Society of London.

Burch, J. B. (1967). Some species of the genus *Bulinus* in Ethiopia, possible intermediate hosts of schistosomiasis haematobia. *Ethiopian med. J.* **5**: 245–257.

Burch, J. B. (1969). The chromosome numbers of *Bulinus sericinus* from Ethiopia. *Malac. Rev.* **2**: 113–114.

Burch, J. B. & Huber, J. M. (1966). Polyploidy in mollusks. *Malacologia* **5**: 41–43.

Burch, J. B. & Lindsay, G. K. (1970). An immuno-cytological study of *Bulinus* s.s. (Basommatophora: Planorbidae). *Malac. Rev.* **3**: 1–18.

Claugher, D. (in press). Karyotype analysis of Bulinid snails. *Bull. Wld Hlth Org.*

Colton, H. S. & Pennypacker, M. (1934). The results of twenty years of self-fertilization in the pond-snail *Lymnaea columella*. *Am. Nat.* **68**: 129–136.

Larambergue, M. de (1939). Étude de l'autofécondation chez les gastéropodes pulmonés et recherches sur l'aphallie et la fécondation chez *Bulinus* (*Isidora*) *contortus* Michaud. *Bull. biol. Fr. Belg.* **73**: 19–231.

Lo, C-T. (1969). Compatibility and host-parasite relationships between species of the genus *Bulinus* (Basommatophora: Planorbidae) and an Egyptian strain of *Schistosoma haematobium* (Trematoda: Digenea). *Malac. Rev.* **2**: 135–136.

Mandahl-Barth, G. (1957). Intermediate hosts of *Schistosoma*. African *Biomphalaria* and *Bulinus*. 2. *Bull. Wld Hlth Org.* **17**: 1–35.

Mandahl-Barth, G. (1965). The species of the genus *Bulinus*, intermediate hosts of *Schistosoma*. *Bull. Wld Hlth Org.* **33**: 33–44.

Manwell, C. & Baker, C. M. A. (1970). *Molecular biology and the origin of species*. London: Sidgwick & Jackson.

Marshall, N. B. (1953). Egg size in arctic, antarctic and deep-sea fishes. *Evolution* **7**: 328–341.

Morton, J. K. (1966). The role of polyploidy in the evolution of a tropical flora. In *Chromosomes today* **1**: 73–76. Darlington, C. D. & Lewis, K. R. (Eds). London: Oliver & Boyd.

Natarajan, R., Burch, J. B. & Gismann, A. (1965). Cytological studies of Planorbidae (Gastropoda: Basommatophora). II. Some African Planorbinae, Planorbininae and Bulininae. *Malacologia* **2**: 239–251.

Oberholzer, G., Brown, D. S. & Van Eeden, J. A. (1970). Taxonomic characters of the radula in the *Bulinus natalensis/tropicus* complex (Basommatophora: Planorbidae) in eastern southern Africa. *Wet. Bydr. Potchefstroom Univ.* (B) **10**: 1–58.

Paraense, W. L. (1955). Self and cross-fertilization in *Australorbis glabratus*. *Mems Inst. Oswaldo Cruz* **53**: 277–291.

Prinsloo, J. F. (1966). [*A comparative study of temperature as an ecological factor for the freshwater snail species* ***Lymnaea natalensis*** *Krauss and* ***Bulinus*** *(****Bulinus****)* ***tropicus*** *(Krauss).*] Thesis, Potchefstroom University, South Africa [in Afrikaans].

Shiff, C. J. (1964). Studies on *Bulinus* (*Physopsis*) *globosus* in Rhodesia. 1. The influence of temperature on the intrinsic rate of natural increase. *Ann. trop. Med. Parasit.* **58**: 94–105.

Staiger, H. (1955). Reziproke Translokationen in naturlichen Populations von *Purpura lapillus* (Prosobranchia). *Chromosoma* **7**: 181–197.

Staiger, H. (1957). Genetical and morphological variation in *Purpura lapillus* with respect to local and regional differentiation of population groups. *Année biol.* **33**: 251–258.

Stebbins, G. L. (1971). *Chromosomal evolution in higher plants*. London: Edward Arnold.

White, M. J. D. (1954). *Animal cytology and evolution* (2nd ed). Cambridge: University Press.

White, M. J. D. (1969). Chromosomal rearrangements and speciation in animals. *A. Rev. Genet.* **3**: 75–98.

Wright, C. A. (1964). Biochemical variation in *Lymnaea peregra* (Mollusca, Basommatophora). *Proc. zool. Soc. Lond.* **142**: 371–378.

Wright, C. A. (1966). Relationships between schistosomes and their molluscan hosts in Africa. *J. Helminth.* **40**: 403–412.

Wright, C. A. (1971*a*). *Bulinus* on Aldabra and the subfamily Bulininae in the Indian Ocean area. *Phil. Trans. R. Soc.* (B) **260**: 299–313.

Wright, C. A. (1971*b*). *Flukes and snails*. London: Allen & Unwin.

Kloos, H., Lemma, A. (1974) *Ethiop. Med. J. 12*, 157.

BILHARZIASIS IN THE AWASH VALLEY

II. MOLLUSCAN FAUNA IN IRRIGATION FARMS AND AGRICULTURAL DEVELOPMENT

Helmut Kloos* M.A., and Aklilu Lemma, D.Sc.

From the Institute of Pathobiology, Haile Sellassie I University, P. O. Box 1176, Addis Ababa

Foci of endemic schistosomiasis and the occurrence of its vectors in the Awash Valley, Ethiopia's most important agricultural region, were reported by Lemma (1969) in the first paper of this series.

He warned that the continuing large-scale agricultural development might lead to the spread of *Schistosoma mansoni* and *S. haematobium*. This paper presents the results of a malacological survey at all the valley's irrigation schemes.

Ayad (1956) and Lemma (1969) reviewed the literature on the distribution of schistosomiasis in Ethiopia. Brown (1964, 1965, 1967) and Brown and Lemma (1970) conducted extensive malacological studies. Burch (1967), Brown (1965), Brown and Burch (1967), and Brown and Wright (1972) contributed to the taxonomy of schistosomiasis vectors in Ethiopia. They concluded that only *Biomphalaria pfeifferi* transmits *S. mansoni* and *Bulinus abyssinicus* transmits *S. haematobium*, and that none of the still inadequately classified populations of *Bulinus truncatus* has been found naturally infected. However, *Bu. truncatus* (n=36) is a potential intermediate host of *S. haematobium*. Lo (1969) successfully infected this species with an Egyptian strain of *S. haematobium;* Brown and Wright (1972) infected a tetraploid population of *Bulinus sp.* from Wonji in the Awash Valley with Egyptian strains of *S. haematobium* and Sardinian strains of *S. bovis*. Brown (1973b) suggests that *Bu. truncatus* in Ethiopia be defined as a tetraploid species. Within the context of this paper, the *Bulinus sp.* found in the irrigation canals and reservoirs of all farms are assumed to have been tetraploid populations. Nevertheless, the possibility that they belong to diploid populations, such as *Bu. tropicus* or *Bu. natalensis* (and therefore probably not susceptible to *S. haematobium* infections) should not be ruled out.

The geographic distribution of both forms of schistosomiasis is highly localized in climatically and topographically complex Ethiopia. *S. mansoni* has been most frequently found at intermediate elevations (1,600-2,200 meters) and *S. haematobium* is generally thought to be limited in its distribution to several hot lowland areas, including the Awash Valley (Brown and Lemma, 1970). *Bu. abyssinicus* has not been found in irrigation canals in Ethiopia unlike in Somaliland from where it was reported by Maffi (1960).

* *Present address*, 2307 N. Backer, Fresno, California 93703, U.S.A.

Our knowledge of the endemicity of schistosomiasis in the Awash Valley comes from two types of evidence — the occurrence of naturally infected snails and the presence of the infection in the local human population. To date, *Bu. pfeifferi* shedding schistosome-type cercariae have been recovered from irrigation canals of Wonji and Metahara (Institute of Pathobiology, 1972) and Abadir (Goll and Aram, 1972). In Wonji, an estimated 10-15 percent of the farm labour population is thought to be infected with *S. mansoni* (Redda, 1973). The only other farm in the Awash Valley where both human cases of schistosomiasis and schistosome-infected snails have been found is Metahara (Institute of Pathobiology, 1971). This apparent localisation may be explained by the absence of longitudinal malacological and human epidemiological studies at the remaining schemes of the Awash Valley.

It appears that *S. mansoni* is endemic in the peripheral areas of the Awash Valley, the headwaters of the Awash River, and small, intermittent tributary water courses. Lo (1971a) recovered *Bu. pfeifferi* infected with *S. mansoni* from the Yatu River near Harar on the Somali Plateau. Kubasta (1964) reported 71.1 percent of 152 school children in Harar and Duncan and Hagos (1969) found 37.1 percent of 300 school children in Tensae Berhan infected with *S. mansoni*. More recently, the health officer of the Tensae Berhan Health Center reported 60 out of 100 children infected (Tensae Berhan Health Center, unpublished records).

S. haematobium seems to be endemic only in the middle and lower parts of the Awash Valley and farm labourers with this parasite in the upper part of the valley probably became infected elsewhere (Lemma, 1969). Lo (1971a) collected *Bu. abyssinicus* naturally infected with *S. haematobium* and *S. bovis* in the extensive swamps at Gewani, and Brown and Lemma (1970) found live specimens of *Bu. abyssinicus* in swamps near Asayita. That the disease transmission occurs in the flood plain near Gewani, which is permanently settled by Afar pastoralists, is indicated by the discovery of 165 out of 272 (60.7 percent) infected tribesmen by Lemma (1969). At the smaller swamp at Kortume, he found 26 of 38 local Afars to be infected with urinary schistosomiasis. From nearby Angelele, the site of a future agricultural scheme, and from Asayita he reported slightly lower infection rates. The Institute of Pathobiology in Addis Ababa has a continuing programme of epidemiological studies of schistosomiasis in the Awash Valley and these studies will be reported separately.

Aims of the present study

This study was undertaken in view of the continuous and rapid agricultural development in the Awash Valley and the concern of the Awash Valley Authority over the possible increase of schistosomiasis (Imperial Ethiopian Government, Awash Valley Authority, 1973). Efforts were made in the present study to provide comparable baseline data on snail distribution and ecology in all farms. Sixteen of the Awash Valley's 27 irrigation schemes had not been studied in earlier investigations. In addition to describing occurrences of snails at the local and regional levels, information on the cultural and physical environments was gathered to evaluate

the malacological data in the context of prevailing agricultural practices and physical conditions. This study is concerned primarily with geographical patterns and may serve as a base for future longitudinal studies on seasonal distributions of schistosome intermediate hosts.

Study design and methods used

All irrigation schemes in the Awash Valley were visited between March and August 1973.[1] This is the period of the small rains (March-April) and big rains (June-Sept.) in Ethiopia. Maps at the 1:10,000 scale were drawn of all farms showing irrigation canals and other potential snail habitats on and near farms. After reconnaissance surveys at the farms and natural water bodies, 197 snail collection points were designated on the basis of habitat type. These sites were then visited during monthly surveys and sampled for snails on a time-limited basis — normally one minute — to calculate approximate snail densities. Snail collections were made within the first week of each month at designated times during the day. All samples were taken with a hand dip net. Measurements were also made of pH, water temperature, and electrical conductivity. Specimens of dominant mesophytes and water plants, especially algae, were collected for identification. Molluscs were identified at the Institute of Pathobiology and schistosomiasis vectors shed for infectivity. Mice were injected subcutaneously with emerging brevifurcate cercariae and later sacrificed and perfused for recovery and examination of adult worms.

The earlier discoveries of *Bu. abyssinicus* prompted us to include some of the major natural water bodies of the Awash Valley to determine if they serve as reservoirs of schistosome-bearing snails. Thus the swamps near Gewani, Kortume, and Asayita, the Awash River and its major tributaries, and the lakes Galila, Koka II, and Hertale were visited.

Results

New Snail Habitats

B. pfeifferi was found in 9 and *Bu. truncatus* in 10 of the valley's 27 irrigation farms. Live *B. pfeifferi* were found for the first time at five schemes — Amibara, Melkasa Experimental Station, Nura Era, Mulugeta, and Takele. They are, with the exception of Amibara, located in the upper part of the Awash Valley. *Bu. abyssinicus* was not found in irrigation canals. Numerous shells of this species were noted at the drying margins of the swamps at Kortume, Gewani, and Asayita.

Infections of Snails

The infection rate of snails with schistosomes was surprisingly low. Only one specimen of *B. pfeifferi* collected at Wonji was found to shed *S. mansoni* cercariae and three *Bu. truncatus* from the Dukem River

1 The Wonji and Metahara Sugar Estates were visited only once because these farms pursue their own malacological studies in collaboration with the Institute of Pathobiology.

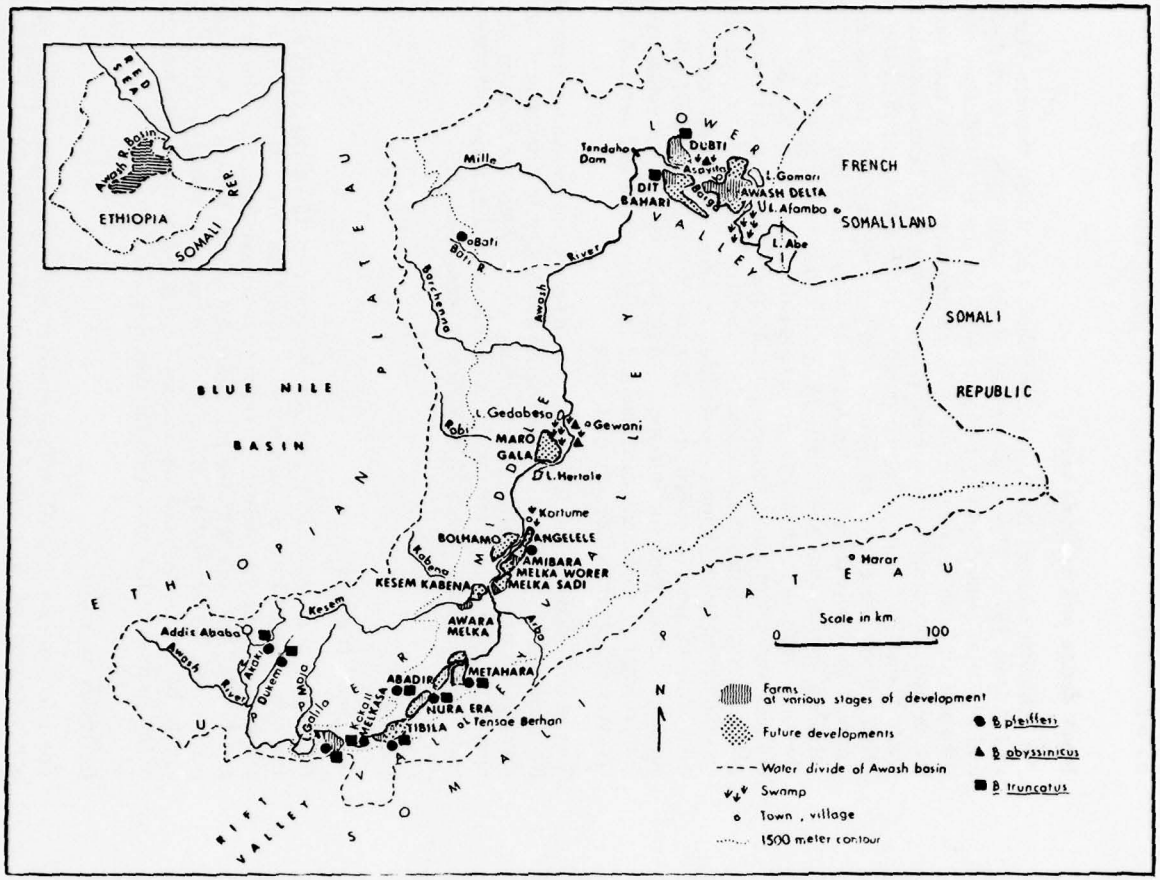


FIGURE 1: IRRIGATION DEVELOPMENT AND SCHISTOSOME INTERMEDIATE HOSTS IN THE AWASH RIVER BASIN

TABLE 1: Distribution and average density of molluscs in irrigation cannals, swamps, rivers, and lakes in the Awash Valley, March-September 1973 [a]

Habitat	No. of monthly surveys	No. of sampling points	Numbers of molluscs collected per one man, one minute of sampling [b]: *Biomphalaria pfeifferi*	*Bulinus truncatus*	*Bulinus abyssinicus*
Upper Valley					
Irrigation canals:					
Wonji	1	10	1.2	3.2	—
Melkasa	6	2	7.6	—	—
Tibila area [c]:					
Tesfa Hiwot	1	3	—	—	—
Tilota	1	4	—	—	—
Mulugeta	1	3	6.1	0.05	—
Takele	1	2	0.9	0.5	—
Nura Era	2	14	1.5	0.3	—
Abadir (cotton)	6	16	0.7	0.2	—
Abadir (fruit)	6	12	1.0	0.05	—
Metahara	1	10	1.3	0.2	—
Rivers:					
Akaki	6	1	1.7	0.7	—
Gogecha (Dukem R.)	6	3	6.1	3.0	—
Fanta (Dukem R.)	4	1	2.0	—	—
Mojo	6	2	—	—	—
Awash	6	2	—	—	—
Lakes:					
Galila (Koka I)	2	5	—	—	—
Koka II	6	2	—	1.1	—
Middle Valley					
Irrigation canals:					
Awara Melka	2	10	—	—	—
Kessem Kebena	1	2	—	—	—
Melka Sadi	6	16	e	—	—
Melka Worer	6	10	e	—	—
Amibara	6	10	1.2	—	—
Algheta	1	2	—	—	—
Ambash	1	1	—	—	—
Swamps:					
Andido (Kortume)	1	4	—	—	—
Gewani	2	2	e	—	e
Lakes:					
Hertale	1	3	—	0.02	—
Hertale defluent	1	4	—	—	—
Rivers:					
Awash	4	2	—	—	—

TABLE 1: Distribution and average density .... (continued)

Habitat	No. of monthly surveys	No. of sampling points	Numbers of molluscs collected per one man, one minute of sampling[b] *Biomphalaria pfeifferi*	*Bulinus truncatus*	*Bulinus abyssinicus*
Lower Valley					
(Irrigation canals:)					
Dubti	2	10	—	0.3	—
Dit Bahari	2	10	—	0.05	—
Barga	1	2	—	—	—
Awash Delta (Ausa Canal)	1	2	—	—	—
Swamps:					
Assayita[d]	2	2	e	—	e
Dubti	1	2	—	0.05	—
Dit Bahari	1	3	—	0.1	—
Rivers:					
Awash	2	3	—	—	—
Bati	2	1	1.3	—	—
Mille	2	1	—	—	—
Reservoirs:					
Dubti (Farm 2)	2	2	—	0.6	—
Dubti (A.V.A. Farm)	1	2	—	0.1	—

a In addition to the schistosomiasis vectors, *Lymnea natalensis*, *L. truncatula*, *Bulinus forskalii*, *M. tuberculatus*, *Cleopatra bulimoides*, *Anisus sp.*, *Gabiella senaariensis*, *Corbicula sp.*, and *Sphaerium sp.* were recovered.*

b The total number of snail specimens of each species collected in a farm or natural water body was divided by the amount of time spent sampling at such a locality during the six-month study period.

c No snails were found at H.S.I. Trust, Champel Kene, Bilata, Addis Hiwot, Halmesh or Abebe Farms.

d Sub-fossilised freshwater snails were found on the plateau at Asayita, 20 meters above the present water level. Shells of particular interest probably belong to an ancient, extinct species, found by Brown and Lemma (1970) in 1966 at this site and described and named as *Biomphalaria barthi sp. n.* by Brown (1973a).

e Only empty shells were recovered.

emitted unidentifiable mammalian schistosomes. However, up to 50 percent of the snails from the rivers and irrigation canals were found to be infected with trematodes other than schistosomes.

Seasonal Changes in Snail Populations

During the 6-month period of this study, a seasonal component in snail distribution could be discerned. The density of *B. pfeifferi* decreased after

* Information obtained in this study on the distribution of these snails is available on application to the Institute of Pathobiology, Haile Sellassie I University, P.O. Box 1176, Addis Ababa.

June in most habitats (Table 2). Similar declines were recorded for *Lymnea natalensis, Bulinus truncatus,* and *Bulinus forskalii.* These changes coincided with the onset of the big rains in July, unusually late for this season. The *Melanoides tuberculatus* and *Physa acuta* populations appear to be affected only slightly by these rains. A one-year malacological study is needed to determine a full seasonal cycle of population changes.

TABLE 2: Seasonal Changes in the Population Density of *B. pfeifferi* in Several Irrigation Farms and Rivers in the Awash Valley, March-August 1973

	Number of molluscs collected per one man, one minute of sampling					
	March	April	May	June	July	August
Akaki River	2.0	1.6	2.8	3.0	0	0
Gogecha R. (Dukem R.)	6.2	6.8	8.6	10.5	3.8	1.6
Fanta R. (Dukem R.)	1.8	1.1	2.4	2.2	4.0	0.5
Melkasa	5.1	3.5	15.0	10.1	5.0	7.5
Nura Era	a	2.9	a	0.4	a	a
Abadir (cotton farm)	1.2	1.6	1.5	0.9	0	0.4
Abadir (fruit farm)	0.8	0.8	1.6	1.7	0.8	0.5
Abadir (swamp)	0.1	0	0	0	0	0
Amibara	a	1.2	0	0	0	0

a No surveys were made during this month.

The Physical Environment

The study of water temperatures showed that values can reach 32°C. in Amibara canals during June, but that average values for the 6-month period under consideration are about 5-6 degrees lower at all farms of the middle valley. Temperatures did not reach maximum values until May and declined again after the June peak with the onset of the rains.

Snail density generally increased with decreases in water velocity and silt load. Both velocity and silt load are lower in the irrigation canals of the farms than in the Awash River. They decrease progressively with distance from the water intake sites and it is suggested here that the generally greater prevalence of *B. pfeifferi* and *Bu. truncatus* in canals at the distant end of the farms is in part due to this fact. Both species were found in some protected bays of eroded drainage canals at Abadir where midstream velocities were too high for their survival. Only *Melanoides tuberculatus* prefers fast flowing, silty waters and it is the only species found in the Awash River. Silt load of canal water has a strong seasonal component and increased up to over twenty-fold at different times due to the big rains in the highlands which wash large amounts of sediments into the rivers.

The markedly seasonal rainfall in the Awash Valley does affect irrigation practices because farmers tend to decrease the flow of irrigation water or temporarily stop irrigation according to amount and intensity of precipitation. The pH and conductivity data collected reveals considerable uniformity. Both generally increase downstream, but never reach the values recorded in some saltwater lakes (Baa-saa-ka) and hot springs (Bilen and at Gewani) which were devoid of snails.

TABLE 3: Irrigation Farms in the Awash Valley: History, Size, Crops Grown, Irrigation Type, Occurrence of Schistosomiasis Intermediate Hosts, and Human Population [1]

Farm	Date of start of irrigation	Size of farm in hectares (ha)		Crops grown	Irrigation type	Schistosomiasis vectors found (+ or —)		Average farm population, 1973	
		1973	Future[2]			Biom. pfeifferi	Bulinus truncatus	Permanent	Seasonal
Wonji	1951	7,000	7,000	Sugar cane	Furrow			30,000	2,000
Melkasa	1960	2.5	2.5	Various	"	+	+	40	—
Nura Era	1965	2,600	4,000	Cotton, fruit	"	+	+	3,000	4,000
Tibila area:						—	—		
Tesfa Hiwot	1967	350	640	Cotton	"	—	—	560	50
Tilota	1971	750	1,000	"	"	—	—	500	1,500
Mulugeta	1970	330	400	"	"	+	+	400	750
Takele	1971	250	400	"	"	+	+	400	750
Abadeska	1964	400	600	"	"	—	—	600	1,200
Halmesh	1969	200	300	"	"	—	—	250	500
Abebe	1971	120	280	"	"	—	—	150	400
H.S.I. Prize Trust	1955	370	2,400	Citrus, maize, beans	"	—	—	1,000	1,500
Champel Kene	1963	100	100	Teff, beans, maize	"	—	—	250	600
Addis Hiwot	1962	55	300	Maize, teff, beans	"	—	—	1,000	1,100
Bilata	1970	280	320	Maize, teff, beans	"	—	—	100	300
Abadir (cotton)	1965	2,000	3,000	Cotton, beans, maize	"	+	+	1,300	4,000
Abadir (fruit)	1965	300	300	Fruits	"	+	+	300	200
Metahara	1954	5,200	10,000	Sugar cane	"	+	+	9,000	1,000
Awara Melka	1953	1,200	200	Cotton, bananas, citrus, veget.	Various	—	—	500	2,000
Melka Sadi	1972	800	4,600	Bananas	Basin	—	—	800	—
Melka Worer	1967	200	1,350	Various	Various	—	—	300	—
Amibara	1967	1,300	10,000	Cotton, maize	Border, furrow	+	+	1,200	1,800
Algheta	1970	300	1,000	"	Border	—	—	400	600
Ambash	1970	300	1,200	"	Furrow	—	—	300	700
Kessem Kabena	1967	400	1,000	"	"	—	—	300	600
Dubti	1962	5,700	9,000	"	Basin	—	+	3,500	20,000
Dit Bahari	1964	3,600	16,300	"	"	—	+	1,500	10,000
Barga	1966	820	850	"	"	—	—	300	2,000
Awash Delta	Unknown but apparently very old	12,000	14,500	"	Flooding	—	—	Approx. 15,000 Afars and a similar number of migrant labourers from the highlands.	

1 Sources: I.E.G., A.V.A., 1973, p. 7.
Dorie and Ali, 1972 App. 1.
Personal data

2 The proposed farms of Maro Gala (23,000 ha) and Bolhamo-Angelele (8,000 ha) are not included here.

Irrigation Practices and Agricultural Methods

Great variations in size of farms, topography, water availability types of crops grown, and crop rotation exist in the Awash Valley. This, together with private ownership of farms and the still limited agricultural experience of commercial farmers, has led to greatly varying irrigation practices, canal outlay of schemes, and canal maintenance. Length of the irrigation season, daily irrigation schedules, and volumes of water applied per unit area of farm land are subject to the experience and knowledge of these new farmers.

Cotton, the most commonly raised crop in the Awash Valley, is generally irrigated from May or June to November and December. Labour demands at the farms are highest during the period of cotton picking, usually performed during January and February. The furrow type of irrigation is practiced at most farms. Daily irrigation is the rule during the dry season. After the harvest of cotton, it is a general practice among farmers to stop irrigating most of the land to use only certain canals to supply semi-permanent and permanent workers and their families with domestic water and to irrigate experimental and speciality crops on small plots. The larger the farm, the greater is the likelihood that certain irrigation canals are being used continuously.

The canals of the sugar cane and banana plantations are especially stable snail habitats. *B. pfeifferi* and *Bu. truncatus* were found in relatively more canals of the sugar estates Wonji and Metahara than in canals of any one of the cotton farms. Water may be found in most canals of the sugar estates throughout the year. Since 1972 bananas have been raised at Melka Sadi on a large scale. The irrigation networks of Melka Sadi and Metahara are still being extended. The drainage canals in Melka Sadi were put into use at the end of 1973, one year after the first irrigation. *Bu. forskalii, M. tuberculatus,* and *P. acuta* and the green algae *Spirogyra sp.* had already colonized the older canals at the southern end of the farm at the time of the present survey.

Irrigation inefficiency — the inability to control the flow of irrigation water — was noted in varying degrees at most farms in the form of water seepages, canal overflows and poor drainage water management. The permanent swamp in the depression of Abadir (cotton) farm, created by drainage water, is the most conspicuous form of irrigation inefficiency found. Apparently *B. pfeifferi* and *Bu. truncatus* occur seasonally in its shallow water. Smaller swamps have been created by overflowing canals and seepages at Nura Era. At nearly all other farms, inadequately cleaned canals constitute the most common sign of poor water management. Although they are generally being cleaned, operations are erratic. They are cleaned once or twice a year, usually after the cotton harvest. Lowered water velocity, abundant vegetation, and silt deposits are the most common visible signs of inefficient water use. Green algae were collected in nearly all farms and floating plants are common in those of the upper part of the valley, generally in slow flowing canals and standing water. The silting and vegetation problem is compounded by the location of most canal systems on alluvial plains with low gradients.

TABLE 4: Occurrence of Schistosomiasis Vectors in Different Habitats at Nura Era, Abadir, Amibara, Mulugeta and Takele Farms

Farm	Habitat type	No. of snail searches	No. of searches revealing		Human water contact and contact points
			Biom. pfeifferi	Bulinus truncatus	
Nura Era	Intake canal	4	2	2	Farm labourers and their families in canals bypassing the 11 labour camps
	Secondary supply canal	4	0	0	
	Secondary supply canal	4	3	1	
	Seepage pool	2	2	1	
Abadir (cotton farm)	Intake canal	30	0	0	Farm labourer and their families in canals bypassing the 3 labour camps. Also Kereyu Galla pastoralists with livestock in canals and the swamp
	Main drain	12	12	5	
	Secondary supply canal	12	3	1	
	Swamp	6	2	2	
Abadir (fruit farm)	Intake canal	6	0	0	Farm labourers and their families, in intake canal near the only camp, labourers also in field canals
	Secondary supply canal	12	5	1	
	Secondary supply canal	6	0	0	
Amibara	Intake canal	12	0	0	Farm labourers, their families, and Afar pastoralists in the 2 reservoirs, intake canals, and supply canals
	Secondary supply canal	12	0	0	
	End of secondary supply canal	6	1	0	
	Reservoirs	12	0	0	
	(Res. no. 1 and Res. no. 2)	12	0	0	
Mulugeta	Pond	1	1	1	Farm labourers and their families in canals near camps and in pond
Takele	Main canal	1	1	1	Farm labourers and their families in canals near camps

Discussion

The findings of this survey generally corroborate earlier malacological studies in the Awash Valley by Lemma (1969) and Brown and Lemma (1970). In addition, the finding of *B. pfeifferi* and *Bu. truncatus* for the first time indicates that these species are more wide spread than it was earlier thought. Of greatest interest is the discovery of *B. pfeifferi* in irrigation canals of Amibara in the middle part of the Awash Valley. It is not known whether this species is established in this habitat or whether the observed snail occurrence is of a transient nature and the result of unusual circumstances or environmental conditions. The presence of *B. pfeifferi* in the canals of nearby Metahara farm and the expansion of the Amibara-Melka Sadi-Bolhamo-Angelele agricultural complex suggest that this species may become part of the snail fauna of irrigation canals in the middle part of the Awash Valley. The total area and intensity of irrigation agriculture in this valley continue to be increased, representing the largest regional development project in Ethiopia. In 1972/73, its irrigation farms produced 20.16 million of Ethiopia's 26.40 million tons of cotton and practically all of its sugar cane. The fourth Five-Year Plan of the Awash Valley Authority calls for the cultivation of an additional 6,800 hectares of land for sugar cane, 11,000 hectares for cotton, and 1,400 hectares for bananas by 1979, or an almost 50 percent increase of the presently (1973) irrigated lands. An irrigation scheme is to be constructed at Bolhamo-Angelele and the existing cotton farm of Amibara will be enlarged. The World Bank is financing the feasibility study at Bolhamo-Angelele and the International Development Agency has agreed to forward a loan to pay for the Amibara irrigation project (Imperial Ethiopian Government, Awash Valley Authority, 1973). Thousands of farm labourers and their families have come from the highlands to work in the valley's farms and their number may be expected to increase in the future.

It was previously thought that *B. pfeifferi* could not exist below Metahara because of high temperatures and silt content of the river water (Brown and Lemma, 1970). Amibara is located over 60 kilometers northeast of Metahara in the hotter middle part of the valley. Mean water temperatures, corresponding in a general way with air temperatures, covered a range of about 6°C. among the farms located between Wonji and Dubti but never exceeded 32°C. Sturrock (1966) considered this value to represent the upper temperature limit of *B. pfeifferi* in Tanzania and attributed its seasonal occurrence in irrigation canals there to seasonal temperature fluctuations.

Little is known about the influence of current velocity and silt load on snail population dynamics (W.H.O., 1965), but the inverse relationship between water velocity and silt content of canal water and snail density noted here may be exploited in future snail control projects. A drop in *B. pfeifferi* and *Bu. truncatus* catches during and after heavy rains was observed not only in the canals but also in the Akaki, Gogecha, and Fanta rivers.

Although variations in water chemistry have been correlated in the past with survival rates of schistosomiasis vector populations in the Awash Valley, no such associations could be made in the irrigation schemes.

Kibron and Goll (1973) attributed the localisation of *Bu. abyssinicus* in swamps of the middle and lower parts of the valley to variations in ion concentrations. Gibb and associates (1973) undertook a longitudinal water analysis in the middle and lower parts of the valley, showing fairly uniform values, except for the lakes Gcmary, Afambo, and Abe, each of which is characterised by high boron, fluorine, salinity and alkalinity values. Not enough data exists on canal water to permit definite statements here on the effect of cation and anion concentrations on snail bionomics. Nevertheless it is suggested that the utilization of a common water source— the Awash River — at all farms except the Haile Sellassie I Trust Farm at Tibila (Worenso River) and Awara Melka (Kessem River) and the absence of greatly differing soil types among farms (F.A.O., U.N., 1965) minimize variations in water chemistry in irrigation canals.

The relationship between poor irrigation engineering practices and schistosome-bearing snails have repeatedly been pointed out (Lanoix, 1958, McJunkin, 1970, W.H.O., 1965). At most farms in the Awash Valley, especially the smaller ones, irrigation practices and agricultural methods are based on the experiences, financial resources, and inclinations of the individual farmers. Optimum growing conditions for crops in terms of water needs, irrigation intervals, and cultivation methods are seldom known, and the assistance and advice of the agricultural experiment stations at Melka Worer and Melkasa are rarely sought. Crop rotation and the timing of agricultural operations for the purpose of reducing snail habitats, as suggested by the World Health Organization (W.H.O., 1965), are not practiced at this time. Water is still plentiful and the adoption of water-saving irrigation practices does not seem urgent to farm concessionaires. Furrow irrigation is practiced as the least demanding type in terms of water at most farms. Basin irrigation, practiced at Melka Sadi, Dubti, Dit Bahari, and Barga, is most wasteful in this regard. Sugar cane requires about three times as much water as cotton and irrigation is nearly continuous during its 2-year growth period. Bananas demand about the same amount of water for optimum growth as sugar cane in the Awash Valley (Imperial Ethiopian Government, Awash Valley Authority, 1969). Most farmers grow cotton as a cash crop for which there exists a large market in Ethiopia. The climatic boundary for successful cotton cultivation passes through the western part of the Tibila area, west of which temperatures are too low, and dry farmed maize, teff, and vegetables become the major cash crop. Maize is grown on several cotton farms on a rotational basis. A considerable number of other crops, especially fruits and vegetables, are now being considered as market crops by cotton farmers as part of their efforts to diversify production. Crop diversification will prolong the irrigation season and probably improve the snail habitats.

It is recommended here that priority should be given to the Amibara area, Nura Era, and Abadir farms in future epidemiological studies. Live *B. pfeifferi* were found in each of these schemes, the largest cotton farms in the upper and middle valley. It is unlikely that schistosomiasis is presently transmitted at the remaining farms, with the possible exception of Mulugeta and Takele. The farms of the Tibila area, the Abadir fruit farm, and the Melkasa Agricultural Experiment Station are small in area and size of labour population. The single camp of Abadir fruit farm is

situated at the snail-free intake canal from which almost all domestic water is obtained. In the Tibila area, all schemes are located adjacent to the Awash River, the major water source for the labourers there. The hilly topography of this area necessitates the pumping of irrigation water at 6 farms. The higher cost of pumping water than gravity irrigation is a strong incentive to minimize water use at the Tibila farms. The continued absence of *B. pfeifferi* from the canals of Melka Worer, Ambash, Algheta, Awara Melka, Kessem Kebena, Dubti, Dit Bahari, and Barga seems to be due primarily to the severe hot and arid environment and the seasonality of the irrigations. It is unlikely that *Bu. abyssinicus* occupies the medieval canals built by the Afar farmers in the Ausa delta area, but this species may be present in the large, unexplored swamps of that area. The sporadic irrigations in this inland delta and a recent drop in the Awash River level, caused by the irrigation development in the upper and middle parts of the Awash Valley (Imperial Ethiopian Government, Awash Valley Authority, 1973), result in very unstable snail habitats. The small subsistence-type cotton and maize fields of the Afar and the increasing population of seasonal and squatter farmers from the highlands are infrequently flooded. The Ausa Canal, the largest canal in this area, was completely dry when visited in July 1973. The local farming and pastoral populations, a total of perhaps 30,000 people, largely depend on the Awash River and deep wells for domestic water needs. The only habitats in the Ausa delta suitable for *Bu. abyssinicus* may exist in the swamps bordering the lakes Gomari, Afambo, and Abe. A large proportion of the estimated 15,000 Afars are said to frequent these swamps during the dry season with their livestock.

The greater diversity and density of molluscs in the upper Awash Valley and the regionalisation of *B. pfeifferi* and *Bu. abyssinicus* in the upper and lower plains, respectively (Map 1), suggests that climatic variables, especially temperature, significantly influence snail occurrences. This is further supported by the history of irrigation development in this valley and the fact that water chemistry, silt load, and the physical dimensions of snail habitats in all the farms of the valley are similar. The continued absence of *B. pfeifferi* from all cotton schemes in the hot lower part of the valley and from nearly all farms in the middle valley, some of them with a history of over 10 years of irrigation, and the endemicity of this species at the new cotton farms in the upper valley, suggest that regional climatic differences significantly affect snail populations.

Several human aspects of schistosomiasis epidemiology warrant further studies in the Awash Valley as well. Domestic water sources are only slowly being improved at most farms, and irrigation canals and reservoirs continue to constitute the major water sources for large segments of the labour populations. Only at Wonji and Metahara are both seasonal and temporary workers supplied with piped water, obtained from deep wells, and are provided with latrines in the labour camps. Migratory labourers and pastoral nomads are still potential agents of schistosomiasis spread in the Awash Valley. All cotton schemes continue to rely largely on seasonal labourers in spite of increased diversification of crops grown. Approximately 95 percent of the labourers working on the farms in this valley originated in the bordering highland plateaus and in several more

distant labour source areas. The majority of the labourers in the middle and lower parts of the valley come from the vicinities of Bati, Harar, Tensae Berhan, and from Tigre Province. Lemma (1965) reported high prevalence rates of *S. mansoni* from Adua, Tigre Province. Lemma (1969) and the health officers of Dubti (Bogale, 1973) and Melka Worere (Asfaw, 1973) found infected farm labourers from Bati and Harar. Most itinerant workers in the upper part of the valley are from the provinces of Shoa, Hararghe, and Gamu Gofa. The magnitude of the labour movements is indicated by the data in Table 3. The prevalence of schistosomiasis at 22 of the Awash Valley's 27 irrigation schemes is unknown due to the absence of laboratory facilities and epidemiological surveys at most farms.

Summary

The molluscan fauna of all irrigation schemes in the Awash Valley was studied between March and September 1973. This valley is characterised by large-scale development of water resources and a corresponding increase in the number of potential snail habitats. *Biomphalaria pfeifferi*, the intermediate host of *Schistosoma mansoni* in Ethiopia, was found for the first time in the irrigation canals of Melkasa, Nura Era, Mulugeta, Takele, and Amibara. *Bulinus abyssinicus*, the intermediate host of *Schistosoma haematobium* in the Awash Valley, was not found at the farms.

The discontinuous geographic distribution of *B. pfeifferi* and the observed seasonal changes in the occurrence of this species in several rivers and farms, indicate that a combination of environmental factors and agricultural practices affect the stability of snail habitats. High temperatures and silt content of water probably cause marked variations in the survival rates of *B. pfeifferi* and *Bu. truncatus*. The limited data on water chemistry does not permit conclusions here concerning its effects on snail population dynamics, but it is suggested the quality of irrigation water among farms is quite uniform. Agricultural practices, particularly those relating to irrigation type, season and intervals, and type of crops grown may cause variations in snail survival rates. The irrigation canals and reservoirs in the sugar cane farms of Wonji and Metahara are very suitable for *B. pfeifferi*. Most cotton farms are less likely to become transmission sites of schistosomiasis. Attention is directed to the new banana plantation Melka Sadi and the Amibara-Melka Sadi-Bolhamo-Angelele agricultural complex.

Several potential schistosomiasis transmission sites were pointed out. *B. pfeifferi* are most common in the medium size canals bypassing labour camps. Most farms lack adequate sanitary facilities and safe water supplies for the majority of their workers. Repeated water contacts and numerous water contact points were noted at labour camps and in the fields. Swamps created by irrigation water and some other types of irrigation inefficiencies exist at several farms. Parasitological data on the human populations of the farms are needed to better evaluate the public health importance of *B. pfeifferi* in the Awash Valley.

Acknowledgements

We are grateful to Harry G. Lee, M.D., for his assistance in snail shedding and to Abraham Kibron, Bahta Mazengia, Tewodros Eskinder, Fasil

Aweitu, and Peter Goll, M.S., for their help with snail collection and identification. We are also thankful to the Awash Valley Authority, particularly to A.B. Paltrinieri, M.D., for providing transportation and accommodation at Melka Worere and Dubti for the senior author. Batista Montenari of Awara Melka and Nura Era, W.M.M. Rijnberg, M.D., of Metahara, and Pier G. Cerri, Ph.D., of Melka Sadi, provided accommodation at these farms and made certain data available. Amare Retta of Melka Worer and E.D.W. Rogers, consultant to Sir Alexander Gibb & Associates, supplied hydrological and climatological data. We are indebted to Tewolde Berhan, Ph.D., Mike Gilbert, M.S., and Tekle Hawariat Hagos, M.S., for assistance with plant identification. Our thanks also to D.S. Brown, Ph.D., Donald Heyneman, Ph.D., and Peter Goll for reading the manuscript and for forwarding valuable suggestions.

Résumé

La faune de mollusques présente dans tous les modes d'irrigation dans la vallée de l'Awash a été étudiée pendant la période Mars-Septembre 1973. Cette vallée est caracterisée par un riche développement de sources d'eau et un potentiel élargi correspondant au nombre d'habitats des escargots. *Biomphalaria pfeifferi*, qui est l'hôte intermédiaire du *Shistosoma mansoni* en Ethiopie a été trouvé pour la première fois dans les canaux d'irrigation de Melkasa, Nura Era, Mulugeta et Amibara. Le *Bulinus abbysinicus* hôte intermédiaire du *Shistosoma hematobium* dans la vallée de l'Awash, n'a pu être retrouvé dans les fermes.

La distribution geographique inégale de *B. pfeifferi*, ainsi que celle due au changement de saison, dans l'apparition de ces éspèces dans plusieurs rivières et fermes indiquent qu'une combination de facteurs d'environnement et de pratiques dans l'agriculture affectent la stabilité des habitats des escargots. Les températures élevées et le contenu minéral de l'eau causent aussi des variations marquées dans le pourcentage de survie des *B. pfeifferi* et *B. truncatus*. Les données très limitées dont nous disposons sur le contenu chimique de l'eau ne nous permettent pas de tirer des conclusions sur son effet sur les escargots, mais il apparait que la qualité de l'eau d'irrigation dans toutes les fermes est assez uniforme — les pratiques employées en agriculture, spécialement ceux concernant le type d'irrigation, les saisons et les intervalles ainsi que le type de grains cultivés provoquent des variations dans la survie des escargots. Les canaux d'irrigation et les reservoirs dans les fermes de canne à sucre du Wonji et Metahara se prêtent très bien au *B. pfeifferi*. La plupart des fermes de cotton sont moins aptés à devenir un lieu de trasmission pour la shistosomiase. L'attention est en ce moment dirigée vers la nouvelle plantation de bananes Melka Sadi et du complexe d'agriculture d'Amibara-Melka Sadi Bolhamo-Angelele. Plusieurs lieux de transmission de shistosomiase sont cités. *B. pfeifferi* sont le plus souvent trouvés dans les canaux de grandeur moyenne qui longent les champs de travail. La plupart des fermes ne disposent pas de facilités sanitaires et d'eau potable pour la majorité de leur ouvriers. Il est à noter que de nombreux contacts aver l'eau et avec des centres d'eau ont été observés. Il est nécessaire d'avoir plus de données parasitologiques concernant la population humaine des fermes pour pouvoir évaluer l'importance des *B. pfeifferi* en termes de santé publique dans la vallée de l'Awash.

References

Asfaw, M. (1973). Personal communication.

Ayad, N. (1956). Bilharziasis survey in British Somaliland, Eritrea, Ethiopia, Somalia, the Sudan, and Yemen. *Bull. Wld. Hlth. Org. 14*, 1.

Bogale, A. (1973). Personal communication.

Brown, D.S. (1964). The distribution of intermediate hosts of *Schistosoma* in Ethiopia. *Eth. med. J. 2*, 250.

Brown, D.S. (1965). Freshwater Gastropod mollusca from Ethiopia. *Bull. Brit. Mus. (Nat. Hist.). 12*, 39.

Brown, D.S. and Burch, J.B. (1967). Distribution of cytologically different populations of the genus *Bulinus* in Ethiopia. *Malacologia. 6*, 189.

Brown D.S. and Lemma, A. (1970). The molluscan fauna of the Awash river, Ethiopia, in relation to the transmission of schistosomiasis. *Ann. Trop. Med. Parasit. 57*, 533.

Brown, D.S. and Wright, C.A. (1972). On a polyploid complex of freshwater snails Planorbidae: *Bulinus in Ethiopia. J. Zool. 1967*, 97.

Brown, D.S. (1973a). New species of freshwater Pulmonata from Ethiopia. *Proc. Malac. Soc. London. 40*, 369.

Brown D.S. (1973b). Personal communication.

Burch, J.B. (1967). Some species of the genus *Bulinus* in Ethiopia, possible intermediate host of *Schistosomiasis haematobium. Eth. med. J. 5*, 245.

Dori, B.S. and Ali, M. (1972). General information on the development of the Awash Valley. Unpubl. report, Awash Valley Authority, Addis Ababa. 18 pp.

Duncan, J. and Hagos, H. (1969). Report on a visit to Tensae Berhan Unpubl. report, Instit. of Pathob., Addis Ababa. 5 pp.

Duncan, J. and Lemma, A. (1970). First annual report on the bilharzia control project at HVA Wonji-Shoa with some remarks concerning HVA Metahara. Unpubl. report, Instit. of Pathob., Addis Ababa. 69 pp.

F.A.O./U.N. (1965). *Report on survey on Awash River basin.* Vol. 2. *Soils and agronomy*. Rome: F.A.O. 298 pp.

Goll, P. and Aram, R.H. (1972). Unpubl. data.

Gibb, Sir Alexander & Associates. (1973). Unpubl. data.

Imperial Ethiopian Government, Awash Valley Authority. (1969). Melka Sadi proposed irrigation project: Feasibility study. Vol. 4. Irrigation and Engineering. Unpublished report by Italconsult, Rome. 116 pp.

Imperial Ethiopian Government, Awash Valley Authority. (1971). *Awash Valley development 1966-1971*. Addis Ababa: Artistic Printers. 58 pp.

Imperial Ethiopian Government, Awash Valley Authority. (1973). Proposed A.V.A. development programme during the fourth five-year plan (E.C. 1967-1971). Mimeographed draft. Addis Ababa, 34 pp.

Institute of Pathobiology. (1971). Molluscan survey of H.V.A. Metahara from 23-27 August, 1971. Unpubl. report 6 pp.

Institute of Pathobiology. (1972). Third annual report on the bilharzia control project, H.V.A. Wonji-Shoa Sugar Estate, 1971-1972. Unpubl. report. 11 pp.

Kibron, A. and Goll, P.H. (1973). Mollusc survey at Dubti and Dit Bahari cotton plantations and in the environs of Assait, 31 Jan. — 7 Feb. 1973. Unpubl. report, Instit. of Pathob. 15 pp.

Kubasta, M. (1964). *Schistosoma mansoni* in the Province. *Eth. med. J. 2,* 260.

Lanoix, J.N. (1958). Relation between irrigation, engineering, and bilharziasis. *Bull. Wld. Hlth. Org. 18,* 1011.

Lemma, A. (1965). Schistosomiasis in Adwa — A report on an ecological pilot study. *Eth. med. J. 3,* 84.

Lemma, A. (1969). Bilharziasis in the Awash Valley. I. An epidemiological study with special emphasis on its possible future, economic & public health importance, *Eth. med. J. 7,* 147.

Lo, T.S. (1969). Combatibility and host-parasite relationships between species of the genus *Bulinus* and Egyptian strains of *S. haematobium*. *Malac. Rev. 2,* 135.

Lo, T.S. (1971a). Snail survey in lake Alemaya and Damota valley. Unpubl. report, Instit. of Pathob, 4 pp.

Lo, T.S. (1971b). Studies on *Schistosoma haematobium* and *bovis* in Ethiopia. Unpubl. report, Instit. of Pathob., 2 pp.

Maffi, M. (1960). Primo reperto nel basso Oltregiuba, Somalia, dei mollusci d'acqua dolce. *Parassitologia, 2,* 191.

McJunkin, F.E. (1970). *Engineering measures for control of schistosomiasis.* A report for the Agency of International Development. Chapel Hill, N. Carol., 69 pp.

Sturrock, R.F. (1966). The influence of temperature on the biology of *Biomphalaria pfeifferi* (Krauss), an intermediate host of *Schistosoma mansoni*. *Ann. Trop. Med. Paras. 60,* 100.

W.H.O. (1965). *Snail control in the prevention of bilharziasis.* Wld. Hlth. Org. Monograph Series No. 50, Geneva, 255 pp.

INTESTINAL SCHISTOSOMIASIS NORTH AND WEST OF LAKE TANA, ETHIOPIA

A. M. POLDERMAN
Institute of Pathobiology, Haile Selassie I University, Addis Abbaba, Ethiopia

Received August 22nd, 1973

Summary. The shores of Lake Tana, and the hills, the plains, and the lowlands north and west of Gondar, Ethiopia, were surveyed for the prevalence of intestinal schistosomiasis and its intermediate host. The little streams in the hills around Lake Tana appeared to be the main transmission sites for *S. mansoni.* In some communities in this area infection rates of up to 80% were found among children between 7 and 15 years of age. Transmission at the shores of Lake Tana seems to be very localized. The risk of increasing transmission of *S. mansoni* in the economically important lowlands along the Sudan border is discussed.

INTRODUCTION

Relatively little is known about the distribution of schistosomiasis in Ethiopia. Ayad reviewed the older Italian literature which includes little systematic data (Ayad, 1956). More recently haematobium schistosomiasis has been shown to be endemic in Gewani (Russell, 1958) and other parts of the middle Awash Valley (Lemma, 1969). High infection rates for *S. mansoni* were found in Harrar (Kubasta, 1964), Adwa (Lemma, 1965a), and other parts of Tigrae Province (NAMRU unit 3, pers. comm.).

Two very limited surveys have been performed in the Lake Tana area (Chang, 1961; Zaphiropoulos, 1963). These surveys indicate a fair transmission rate of *S. mansoni* in Gorgora, and it was assumed, and partly proved that the infection was transmitted by *Biomphalaria pfeifferi* (Krauss) which was present though not abundant along the shores of Lake Tana at Gorgora. Using direct smears Chang found an infection rate of 22.8% among 202 schoolchildren. Zaphiropoulos, probably also using direct smears, found 19.9% of 241 schoolchildren to be positive for *S. mansoni.* The files over 1968–1969 of the Public Health Training Centre in Gorgora showed that about 7% of the direct smears that were performed on outpatients with abdominal complaints contained *S. mansoni* eggs (Polderman, unpublished data).

The aim of the present study was to investigate the geographical distribution of *S. mansoni* infections and its transmission in the area north and west of Lake Tana.

MATERIALS AND METHODS

Parasitological surveys were performed in a number of towns, villages, and rural areas. If a school was present in a particular community, pupils from the 1st, 3rd, and 5th or 6th grades who were born in that town were selected for parasitological examination. If no school was present, house to house surveys were performed, and in some cases children who happened to present themselves were examined. Thus no random sampling was achieved.

Plastic cups in six different colours were distributed and faeces were produced instantly in almost

all cases. From each child about two grams of faeces were collected and stored in 7.5% formaline. Most faecal samples were examined with Ritchie's concentration method (Ritchie *et al.*, 1960); in some cases, however, due to shortage of chemicals, a simple water sedimentation method was used. From each sample one slide was examined. If no *S. mansoni* eggs were found, another slide was examined. No information on the quantitative egg-output was gathered in this way.

In the areas where parasitological data were collected, streams, pools, or the shores of Lake Tana were checked for the presence of *B. pfeifferi*. A simple sweep net was used in searching snails. Since in most cases only one or a few snail surveys were performed in a particular area, the information on presence or absence of *B. pfeifferi* should be interpreted with great care.

DESCRIPTION OF THE AREA *(Figures 1–6)*

The area under investigation consists of four different zones:
(1) the shores of Lake Tana (altitude ± 1840 m),
(2) the alluvial Dembia plains north of Lake Tana (alt. ± 1840 m),
(3) the hills around Lake Tana and the Dembia plains (alt. ± 1840–2200 m),
(4) the lowlands towards the Sudan border (alt. ± 700–1200 m).

In the following descriptions the distinction town-rural area (including villages) will be made according to local tradition. In general a town is built along a motor road, and it is a centre for trade and often for education and government administration. Most inhabitants of the rural areas are traditionally or recently converted Coptic Christians. A good deal of a towns trade, however, is in the hands of Muslims. In general, the roadside towns have a population of about 1000–4000, 20% of which may be Muslim.

(1) The shores of Lake Tana are rocky *(Fig. 2)* or swampy with bushes of papyrus and reeds. In the swampy areas it is very difficult to get to the water. The Christian Amhara farmers are the largest and in a sociological way also the most important group of inhabitants of the area. They despise fish as unclean food. A fewWaito fishermen and farmers, however, go regularly into the Lake for fishing. Children, mainly boys, often swim in the Lake in some places near the towns. Villagers and townsfolk who live close to the Lake (the inhabitants of Gorgora, Delghi, Sedeber, Kunzila) are completely dependent on the lake for their water supply. There is no piped water and there are no protected wells.

(2) The densily populated and fertile Dembia plains *(Fig. 6)* are traversed by a few big streams (Dirma, Megatch). The area is inhabited by farmers. They sell their crops in the weekly markets of the roadside towns, so they have a lot of contact with the area decribed under(3). Agriculture is of a primitive type. There are no irrigation schemes and no functional agricultural cooperatives. Each group of huts has a partly protected well of its own. New wells are dug every few years; they contain relatively clean water. During the rains (July, August, beginning of September) vast areas of the plains are transformed into virtual swamps.

(3) The low hills around Lake Tana and the Dembia plains *(Fig. 5)* consist of a mainly agricultural area with many old villages. To the east and north those hills are bordered by a mountain massif of over 2500–3000 m. To the west a narrow mountain ridge of 2100–2200 m. separates the Lake Tana basin from the lowlands which descend to the Sudan border. Numerous little streams are flowing in the area. Most of them drain into Lake Tana, while others are tributaries of the Atbara that joins the Nile north of Khartoum. They are the sole source of water for the villagers. For obvious technical reasons most roads in this part of the province cross the hilly area rather than the marshy plains or the higher mountains. Most towns along these roads were established after the construction of the roads during the Italian occupation (1936–1941). In many of these towns pumps were constructed some 15 years ago, but they are no longer functioning.

(4) A steep escarpment separates the north-western Ethiopian highlands from the lowlands of the Sudan border. The border area between Metemma and Settit-Humera has been rapidly developed in recent years thanks to the introduction of cash crops (sesame, cottom, sorghum). During the harvest season many labourers from Tigrae and Begemder provinces migrate into this area. The water supply and the health and sanitary facilities are completely inadequate for this influx of labourers. The crops are grown without irrigation, and, apart from some big rivers, the amount of available water is very limited. A few big lowland rivers (Atbara, Tekazze) cross the savannah-type land, and many farmers have to collect water at those rivers in drums at considerable distances from their farmlands *(Fig. 3)*. In contrast to the areas mentioned above which have mean yearly temperatures varying between about 15° and 20° the mean yearly temperature here is around 30°, though no accurate data are available.

TABLE

GEOGRAPHICAL DISTRIBUTION OF *S.MANSONI* INFECTIONS
(children of 7–15 years. Positive cases in brackets)

Locality	*Sample*	*Method*	*No examined and positives*			
			Female		*Male*	
Gondar						
T. Work school	schoolchildren	R	2	(1)	20	(2)
Meseret school	schoolchildren	R	26	(1)	17	(1)
Addis Alem	schoolchildren	S	18	(16)	45	(34)
Shores of Lake Tana						
Gorgora	schoolchildren	R	29	(18)	28	(26)
Mangi	house to house	S	4	(0)	7	(1)
Delghi	schoolchildren	R	9	(0)	19	(2)
Sedeber	house to house	S	7	(0)	5	(1)
Kunzila	schoolchildren	S	5	(1)	19	(10)
Dembia plains						
Guramba Bahta	house to house	R	4	(1)	3	(0)
Acherra Mariam	house to house	R	13	(0)	12	(2)
Northern hills						
Azozo	schoolchildren	S	14	(8)	25	(17)
Tadda	schoolchildren	S	21	(13)	20	(11)
Emfraz	schoolchildren	R	14	(7)	21	(15)
Sakalt	schoolchildren	R	17	(7)	19	(7)
Behona, Chinchai	available	R	4	(1)	18	(3)
Gella Duba	schoolchildren	R	15	(10)	15	(8)
Aykel	schoolchildren	R	14	(1)	23	(2)
Chenkela Abo, Yessus	house to house	R	17	(10)	18	(13)
Kolla Duba	schoolchildren	S	25	(15)	31	(16)
Jenda	house to house	R	25	(23)	19	(16)
Chiwahit	available	R	21	(15)	43	(36)
Aberdja	house to house	R	3	(3)	2	(1)
Chenker	house to house	S	8	(6)	18	(11)
Southern hills						
Chewa Duba	house to house	R	10	(1)	13	(3)
Chach	shepherd	R	0	(0)	17	(3)
Maconta Yessus	available	R	3	(1)	7	(0)
Dengelbar	available	S	12	(0)	18	(0)
Aleffa	available	S	13	(0)	11	(0)
Zana Abo, Mariam	available	S	5	(0)	8	(0)
Quara	available	S	12	(0)	12	(0)
Lowlands near the Sudan Border						
Metemma	schoolchildren	R	6	(0)	18	(2)
Settit Humera	schoolchildren	R	13	(0)	21	(0)

Method: R= Ritchie's concentration method
S= Simple Sedimentation method.

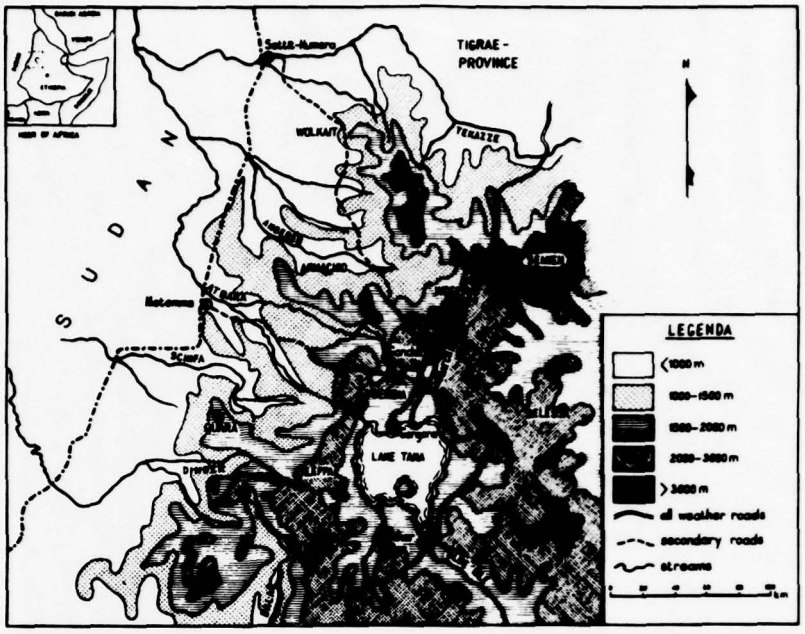


Fig. 1. North west Ethiopia; altitudes and districts

Fig. 2. Lake Tana near Gorgora.

Fig. 3. Tekazze river in Settit Humera.

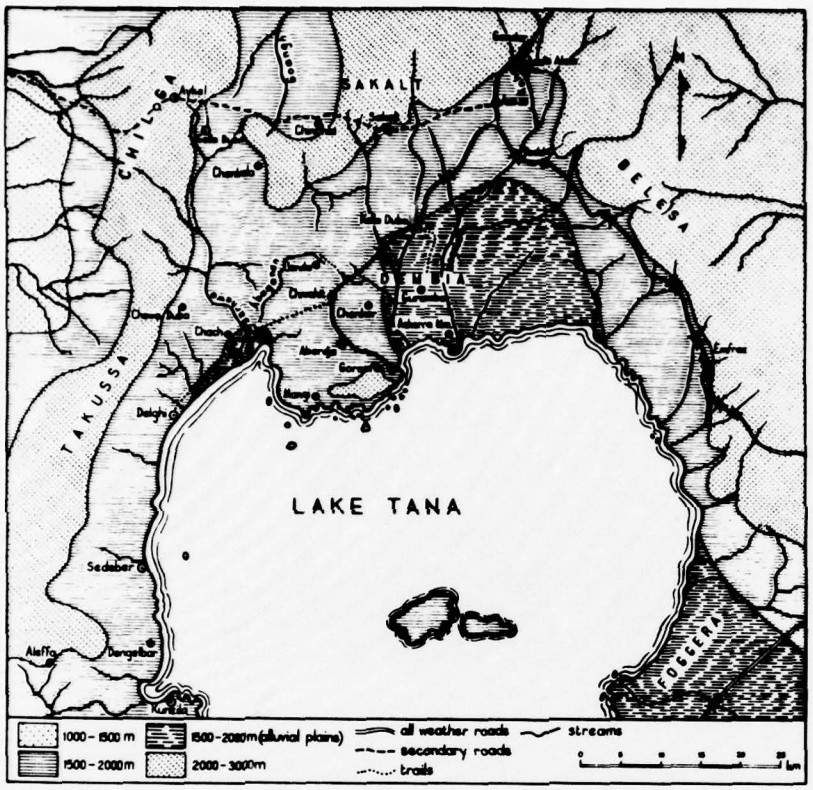


Fig. 4. The area north of Lake Tana; altitudes and localities.

Fig. 5. The hilly area near Azozo.

Fig. 6. The Dembia plains near Guramba.

RESULTS

Occurrence of the parasite

In the period between April 1971 and November 1972 a number of stool specimens were collected in 32 communities. Out of the 1495 collected samples, *S. mansoni*-eggs were found in 684. In the *Table* the infection rates for different communities are summarized. Apparently there is great variation. Of 13 localities in the northern hills the per cent infected was only 8% in one, and over 80% in four others. For the purpose of a rough localization of the transmission sites, only children who were born in that community or its immediate surroundings are considered in the table.

Occurrence of the intermediate host snail

Biomphalaria pfeifferi was most abundant in the very small streams in the hilly area just beyond the springs from which these streams originate *(Fig. 5).* These little streams contain water throughout the year, though they may be reduced to merely a series of little, isolated pools during the second half of the dry season. The flow rate of these streams is negligible except during the heavy rains. The vegetation is often abundant. Medium sized rivers (Demazza in Azozo, a Dirma tributary in Kolla Duba, Keha river near Addis Alem) show very great seasonal fluctuations in snail populations. The bigger rivers (Dirma and Megatch in the Dembia plains and Sarweha and Abaganen in the marshes west of the Dembia plains) are muddy and almost without vegetation. During three surveys (in December 1971, June 1972, Oktober 1972) only one little *B. pfeifferi* was found.

Only in the beginning of the rainy season a fair number of *B. pfeifferi* was found on the shores of Lake Tana at Gorgora. During the rest of the year no or only very few *B. pfeifferi* could be found. In January 1972 (in the middle of the dry season) no *B. pfeifferi* were found in any of the other communities along the shores of Lake Tana that are mentioned in this study. Only a couple of juvenile *B. pfeifferi* were discovered near Mangi in March 1972. In general, rocky parts of the shores were surveyed. Often there is considerable wave action at those places. In October 1971 a more abundant mollusc fauna including numerous *B. pfeifferi* was found along the swampy shores with a dense vegetation of reads near Wawa, a few kilometers to the east of Gorgora.

A few small *B. pfeifferi* were found in the rivers (mainly in the Genet river) halfway down the escarpment of Settit-Humera (July 1972). No snails were found in the lowland rivers Tekazze (in Settit-Humera) or Atbara (7 km north of Metemma). However, *B. pfeifferi* might be present in these rivers, particularly in the Atbara, at other times of the year.

Furcate cercariae that were shed by *B. pfeifferi* from streams near Chiwahit, Jenda, Sakalt and Addis Alem were proved to be *S. mansoni* cercariae by mouse exposures in the laboratory.

DISCUSSION

Generally small number of *B. pfeifferi* were found along the shores of Lake Tana and in most of the lake side communities low prevalences of *S. mansoni* were recorded in children. In Gorgora and Kunzila, however, a high prevalence of intestinal schistosomiasis was found. Probably the infectivity of the lake water is low except during short periods in some foci. The far lower prevalence that was found by both Chang and Zaphiropoulos

(1963) may be caused by the use of less sensitive techniques. However, there may be other causes as well: the transmission dynamics in Gorgora (and Kunzila) are not well understood. Many boys swim in the lake very often; girls do so only rarely, which may explain the significant difference in prevalence between boys and girls in Gorgora and Kunzila ($p<0,05$).

Probably no transmission takes place in the Dembia plains. It is very likely that the three infected children got their infection in nearby highly endemic areas. The reasons for the absence of endemic schistosomiasis are the practice of digging new and partly protected wells every few years and the unfavourable habitat for snails in the rivers.

Even though the altitude of the hilly area seems very high for *S. mansoni* transmission, this area appears to be highly endemic. The high prevalence of *S. mansoni* in the roadside towns may suggest a recent introduction of the disease. This, however, does not seem very likely for two reasons. Firstly, many parts of the roads along which the towns are located are built on a watershed with streams draining to either side of the road. So, many of the most favourable snail habitats happen to be situated close to the roads and the roadside towns. Secondly, for several centuries the area has been the centre of the Ethiopian Empire. Many armies used to cross the area in all directions, there was an influx of Galla tribesmen from the south, and Muslim merchants entered it from the north.

The great difference between the prevalence among school children from Gondar city (about 6%) and Gondar's Muslim subtown Addis Alem (about 80%) is probably caused by the presence of a well organized system of piped water in Gondar and the complete dependence of the population of Addis Alem on the Keha and Angereb rivers which were shown to be infective. In Aykel, which is only some 5 km from the highly endemic market town Gella Duba, the transmission seems to be almost or completely absent. Yet, the streams around Aykel town look very similar to the ones around Chiwahit for instance. Unfortunately, no climatic observations are available, but the low temperatures caused by the high altitude (± 2200 m) and its situation on the top of a narrow ridge of mountains may well prevent *S. mansoni* from becoming established in Aykel streams, even though the intermediate host snails are present.

The prevalence of *S. mansoni* in the lowland centres Metemma and Settit-Humera is very low. However, 6 out of 20 (30%) students at Settit-Humera school who were born in the nearby lowland district Wolkait were positive for *S. mansoni*. In the Metemma area the aboriginal Gumis tribesmen can easily be distinguished from the recent highland immigrants. Two out of eight Gumis schoolboys (25%) who were born in the Metemma area, and who had almost certainly never been on the highland plateau, had *S. mansoni* eggs in their stools. According to the files of the Health Centre in Settit-Humera, approximately 7% of the outpatients of the Health Centre was positive for *S. mansoni* (direct smear). This indicates a very high prevalence among seasonal labourers (the Health Centre in the highly endemic Gorgora area, using the same techniques, found a similar infection rate of 7% among its outpatients). This high infection rate among older persons should probably be explained by the fact that the great majority of the outpatients were seasonal labourers from Tigrae and Begemder provinces. In parts of both provincies *S. mansoni* is known to be highly endemic. The situation in the lowland area can be summarized as follows; endemic schistosomiasis is likely to exist in the lowlands to the west and north-west of Gondar, and in recent years many thousands of infected seasonal labourers have migrated and still continue to migrate into the formerly sparsely populated area. So, in the near future, with a further development of the area, great attention should be paid to the possible spread and the impacts of intestinal schistosomiases.

The author wishes to acknowledge the invaluable assistance given in the field work by Addis Teshome, Aberra Kumsa, and Tadesse Desta, and the facilities made available by the Public Health College in Gondar. This study was funded by the Netherlands University Foundation for International Cooperation, and by a grant from the W.H.O.

REFERENCES

Ayad, N., Bull. Wld. Hlth. Org., 14 (1956) 1.
Chang, W. P., *Gondar Health series*, 1 (1961) 5.
Kubasta, M., *Ethiop. med. J.*, 2 (1964) 260.
Lemma, A., *Ethiop. med. J.*, 3 (1965) 84.
Lemma, A., *Ethiop. med. J.*, 7 (1969) 147.
Ritchie, L. S., et al, *Amer. J. trop. Med. Hyg.*, 9 (1960) 444.
Russell, H. B. L., *Wld. Hlth. Org. Document*, EM/PHA/62 (1958).
Zaphiropoulos, H., *Gondar Health series*, 3 (1963).

Present address:
Laboratory of Parasitology
University of Leiden, Netherlands

Annals of Tropical Medicine and Parasitology, 1975, Vol. 69, No. 3

Studies on *Schistosoma bovis* in Ethiopia

BY C. T. LO* AND AKLILU LEMMA

Institute of Pathobiology,
Haile Sellassie I University,
Addis Ababa, Ethiopia

Received 5 August 1974

During a study on human schistosomiasis in Ethiopia, we noticed cercariae that were indistinguishable from those of *Schistosoma haematobium* emerging from bulinid snails collected in some localities. The cercariae were subsequently identified as those of *Schistosoma bovis* by recovering adult worms from experimental animals. The present paper reports some aspects of this parasite in Ethiopia.

Endemic Areas and Infection Rates

Adwa

This is a town in northern Ethiopia (see map) where 61·4% of the residents were infected with *Schistosoma mansoni* (Buck *et al.*, 1965), and where the molluscicidal property of the berries of the endod plant (*Phytolacca dodecandra*) was first discovered (Lemma, 1965*b*). While studying some ecological problems of *S. mansoni* infection in Adwa, Lemma (1965*a*) noticed eggs resembling those of *S. bovis* in cattle faeces, and found many live and mature worms in the mesenteric veins of a slaughtered cow.

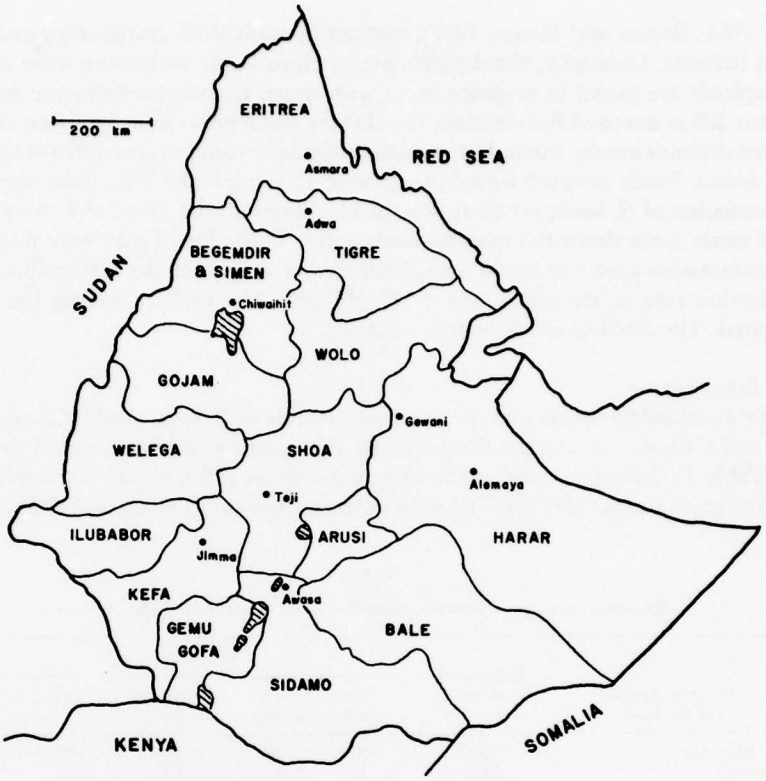
There are two main river systems in Adwa: the Assem, which flows through the centre of the town, and the Guagua to the west, joining just outside the town. In both streams *Biomphalaria pfeifferi* and a tetraploid (n = 36) species of *Bulinus* are present. In October 1969, 26 specimens of *Bulinus* were collected from the Assem river, nine of which were found to be infected with *S. bovis*. In January 1971, one snail was found infected out of 50 bulinids collected from the Edaga river, a tributary of the Guagua.

In order to estimate the infection rate among the cattle, 200 faecal specimens, each of about 1 g were examined by Ritchie's (1948) concentration method. Unexpectedly, *S. bovis* eggs were not found, but 57 (29%) had eggs of *Fasciola* sp.

Gewani

During the survey of *S. haematobium* in Gewani in the middle Awash valley, *Bulinus abyssinicus* was found to be infected with schistosome cercariae. Experimental exposures of animals revealed that the cercariae had included both *S. haematobium* and *S. bovis*, with a combined infection rate of 60% (Lo, 1970*b*). Later, 197 samples of cattle faeces were collected from several villages along the Awash river in the vicinity of Gewani. By the same concentration method, *S. bovis* eggs were found in three samples (1·5%). The *Fasciola* infection rate was 78%.

* Present address: Department of Parasitology, College of Medicine, National Taiwan University, Taipei, Taiwan, China.



MAP. Distribution of *S. bovis* in Ethiopia (solid dots).

Lake Awassa

In February 1971, Dr. W. P. Chang of the World Health Organization collected 35 bulinid snails from the eastern shore of Lake Awassa near the Shell petrol station. One specimen was shedding schistosome cercariae, later identified as *S. bovis*. Before the parasites were identified, the following water birds from Lake Awassa were examined *post mortem* for avian schistosomes with negative results: one each of Spurwing Plover (*moplopterus spinosus*), European Shoveler (*Spatula clypeata*), Garganey Teal (*Anas querquedula*), Hammerkop (*Scopus umbretta*), African Jacana (*Actophilornis africanas*), Black Crake (*Limnocorax flavirostra*), Heron (*Ardea* sp.), Cattle Egret (*Bubulcus ibis*), Greenshank (*Tringa nebularia*); two each of White-faced Tree Duck (*Dendrocygna viduata*), and Egyptian Goose (*Alopochen aegyptiaca*). Rat traps set along the lake shore caught nothing.

In March 1971, 715 specimens of *Bulinus* spp. were collected from the lake, mostly in the area between the Shell petrol station to the south and the Black River to the north. When examined individually, 132 were found to be infected with one or two of some seven types of cercariae, including 22 infected with *S. bovis*. It is known that Lake Awassa contains two cytologically different forms of *Bulinus*, diploid (n = 18) and tetraploid (n = 36)

(Burch, 1964; Brown and Burch, 1967), distinct in adult shell morphology and occupying different habitats. Generally, the diploids prefer clean water with more wave action, while the tetraploids are found in stagnant areas with much decomposed organic matter. In an area about 200 m north of Bekele Mola Hotel there was a heavy concentration of tetraploids with some diploids among them, but as most snails were young it was difficult to distinguish the two forms. Snails infected with *S. bovis* were all tetraploids. This place seems ideal for the transmission of *S. bovis*, for there is a road leading into the town, and twice daily many herds of cattle come down the road to drink water. Cattle faecal pats were numerous, and this contamination probably made the habitat more suitable for the susceptible tetraploids. The infection rate in the cattle was 5·5% (11/200), the highest among the three areas investigated. The *Fasciola* infection rate was 60%.

Animal Infections

Laboratory and wild animals were exposed to cercariae of *S. bovis* shed by snails from Lake Awassa and Gewani. Among the three species of laboratory rodents exposed to the Awassa strain (Table I), hamsters were apparently better hosts (38% worm recovery) than mice (15·4%) or guinea pigs (13·7%). The wild rodents *Arvicanthis niloticus* and *Praomys albipes*

TABLE I

Exposure of some rodents to cercariae of *S. bovis* from Lake Awassa

No. and species of animals	Estimated total no. of cercariae	Worm recovery				
		No. pairs	No. males	Separate females	Total	%
1 hamster	500	81	28	0	190	38·0
6 mice	1200	51	83	0	185	15·4
2 guinea pigs	2000	26	222	0	274	13·7
2 *Arvicanthis niloticus*	600	26	66	0	118	19·7
3 *Praomys albipes*	900	83	202	0	368	40·9
1 *Tachyoryctes* sp.*	800	0	4	0	*	*

* Received cercariae, possibly all males, of the Gewani strain. By perfusion four male worms were recovered, but there were hundreds of dead and partly degenerated male worms in the liver causing an extensive liver abscess.

were susceptible, and *Praomys albipes* was as good a host as the hamster, with 40·9% worm recovery. A single mole rat (*Tachyorcytes* sp.) was exposed to 800 cercariae and died three and a half months after infection. Four male worms were obtained by perfusion, and were alive but not well developed. The liver of the host was severely damaged by an extensive abscess, and hundreds of dead worms, all males, were embedded in the liver tissue. The mole rat needs further investigation, for only one animal was studied and the cercariae used might possibly have been all males.

For the Gewani strain (Table II), only qualitative statements can be made because the cercariae were a mixture of *S. haematobium* and *S. bovis*, the proportions and numbers of which were unknown. Hamsters, mice, *Arvicanthis niloticus*, *Mastomys coucha*, and *Theropithecus gelada* (the Gelada baboon) were susceptible to both species of schistosome, as proved by the recovery of mature paired worms. Rabbits, guinea pigs and *Rattus rattus*

TABLE II

Animal exposures to mixture of *S. haematobium* (*S.h.*) and *S. bovis* (*S.b.*) cercariae obtained from naturally infected *B. abyssinicus*

No. and species of animals	Estimated total no. of cercariae	Worm recovery				
		S.h.• pairs	*S.b.*• pairs	Others†	Total	%
6 hamsters	2200	43	26	93	231	10·5
11 mice	4400	25	52	73	227	5·2
3 rabbits	3000	0	52	87	191	6·4
3 guinea pigs	1500	0	17	0	34	2·3
9 *Rattus rattus*	3500	0	29	48	106	3·0
3 *Praomys albipes*	600	0	0	58	58	9·7
1 *Arvicanthis niloticus*	500	2	2	10	18	3·6
2 *Mastomys coucha*	600	2	15	3	37	6·2
1 *Lophuromys flavopunctatus*	200	0	3	3	9	4·5
1 goat	600	0	0	49‡	49	8·2
1 sheep	600	0	0	26‡	26	4·3
1 dog	500	0	0	0	0	0
1 *Theropithecus gelada*	500	4	10	9	37	7·4

• Identification is based on female worms.
† Includes all unidentifiable worms.
‡ Immature female worms; some had abnormal eggs resembling those of *S. bovis*.

were susceptible to *S. bovis* but not to *S. haematobium* (we had previously found three rabbits, two guinea pigs and four *R. rattus* refractory to infection by cercariae of *S. haematobium* from the middle Awash valley). *Lophuromys flavopunctatus* was susceptible to *S. bovis* and *S. haematobium*, giving 4·5% *S. bovis* worm recovery when exposed to a mixture of *S. bovis* and *S. haematobium* cercariae from naturally infected *B. abyssinicus*. A 2·5% worm recovery was recorded when *L. flavopunctatus* was exposed to *S. haematobium* alone (unpublished data). A total of 58 worms (51 female, one male, three pairs) was recovered from three *P. albipes* three and a half to five and a half months after infection. All the male worms were mature but all the females were immature. The reason for the inability of paired females to mature might be that the males were of a different species. Goats and sheep are receptive to *S. bovis* and refractory to *S. haematobium*. The immature females recovered from the goat and sheep contained some abnormal elongated eggs somewhat resembling those of *S. bovis*, and again perhaps unisexual infection had taken place. No worms of any stage were obtained from a dog, indicating that it is refractory to both parasites.

The combined results for two strains of *S. bovis* show that at least five species of wild rodents in Ethiopia are susceptible. Infectivity of the Adwa strain was not tested, but hamsters used for recovering adult worms were highly susceptible. Our data are not sufficient to indicate if there are any strain differences in the infectivity of cercariae.

Snail Susceptibility

S. bovis originating from three endemic areas was established in the laboratory using the golden hamster. The eggs of *S. bovis* were recovered from the infected liver, and snails exposed to newly hatched miracidia (Table III).

TABLE III

Exposure of *Bulinus* spp. to miracidia of *S. bovis*

Origin of *S. bovis*	Snails			Number of miracidia per snail	Number of snails exposed	Number surviving	Number positive	% positive
	Species	Origin	•n=					
Gewani	*B. abyssinicus*	Gewani	18	5	20	8	5	62·5
	B. globosus	Rhodesia	18	5	13	13	0	0
	± *B. natalensis*	Nelspruit	18	15–20	20	16	1	6·3
	B. sp.	L. Chalalaka	18	5	20	14	0	0
	B. sp.	285 km Jimma Rd.	36	5–20	20	20	0	0
	B. sp.	304 km Jimma Rd.	36	10–15	20	20	1	5·0
	B. sp.	Adwa	36	10–15	20	20	5	25·0
	B. octoploidus	14 km N. Shano	72	10–15	30	15	0	0
Adwa	*B. natalensis*	Nelspruit	18	5	50	44	0	0
	B. sp.	Adwa	36	10	15	14	7	50·0
	B. sp.	285 km Jimma Rd.	36	5	50	46	6	13·0
	B. guernei	Gambia	36	5	50	45	1	2·2
	B. truncatus	Egypt	36	10	20	20	1	5·0
Lake Awassa	*B. africanus*	288 km Jimma Rd.	18	20	20	20	0	0
	B. sp.	Regabu River	18	20	20	2	0	0
	B. sp.	304 km Jimma Rd.	36	20	50	50	27	54·0
	B. sp.	Adwa	36	20	20	15	1	6·7
	B. sp.	Lake Awassa	36	20	20	20	10	50·0
	B. sp. (albino)	Lake Awassa	36	20	50	12	12	100·0
	B. truncatus	Egypt	36	20	50	40	6	15·0
	B. octoploidus	14 km N. Shano	72	20	50	48	4	8·3

• Haploid chromosome number = 18.

Eight populations of bulinids including members of the *tropicus*, *truncatus* and *africanus* groups were tested with the Gewani strain. *Bulinus natalensis* from Nelspruit, South Africa, were susceptible but *tropicus* group snails from Lake Chalalaka, Ethiopia, were refractory. In the *truncatus* group, the tetraploids showed positive and negative results, while *B. octoploidus* (Burch, 1972) (n = 72) were negative. *B. globosus* in the *africanus* group from Rhodesia was not susceptible, but *B. abyssinicus*, which is of the same group and is the natural host, had an infection rate of 62·5%.

The Adwa and Lake Awassa strains gave more uniform results than the Gewani strain in their infectivity, for members of the *truncatus* group were susceptible, whereas the *tropicus* and *africanus* groups were refractory. These two geographical strains of parasite seemed to be more closely related to each other than to the Gewani strain which was capable of infecting a wider variety of bulinids. Since these studies were done only once, further confirmation is needed, especially of the negative results.

DISCUSSION

In addition to the three endemic areas reported above, *S. bovis* has been found in other parts of the country. Dr. T. Yamaguchi (personal communication, 1970) collected many large adults from the portal veins of cattle at the slaughter house in Asmara, 150 km north of Adwa. Mr. A.M. Polderman (personal communication, 1971) obtained some adult *S. bovis* by immersion of mice in a stream in Chiwaihit, 15 km north of Gorgora, while studying

S. mansoni. Yasuraoka (1972) found that cattle in Teji, Alemaya and Jimma were passing eggs resembling those of *S. bovis*. Summarizing this information, it is clear that *S. bovis* occurs in at least seven out of 14 widely separated provinces in Ethiopia (Map). Sheep and cattle are raised all over the country, and bulinids are widely distributed. There might, therefore, be numerous transmission sites scattered over large areas.

There is no doubt that *S. bovis* occurs in Adwa, yet 200 faecal specimens revealed no *S. bovis* eggs. The large size and elongated form of the eggs may require a modification of the concentration technique, or the infection may be so light that repeated examinations are necessary to detect eggs.

Animal reservoirs of African schistosomes have been reviewed by Nelson *et al.* (1962), who also reported the results of examination of more than 1 000 rodents in Kenya: *S. bovis* was found in only two (*Mastomys natalensis* and *Lophuromys flavopunctatus*) of 15 species. In Ethiopia there is no report of *S. bovis* infection in wild rodents, but experimentally we have shown that in at least five species the parasite could grow to maturity. Their importance as reservoir hosts is, however, unknown.

There are reports from several countries that *S. bovis* and *S. haematobium* have a common intermediate host, e.g. in Iran (Arfaa *et al.*, 1965), Mauritania (Marill, 1961*a*, *b*), and Somalia (Moffi, 1960). In Corsica *B. truncatus* is the host for *S. bovis* (Doby *et al.*, 1966), and although *S. haematobium* is not present there, the same snail can be infected with *S. haematobium* originating from Morocco and Algeria (Capron *et al.*, 1965). Mutual susceptibility also holds true for *B. abyssinicus* as demonstrated here, while the only other Ethiopian bulinid to show susceptibility to either parasite of Ethiopian origin is *Bulinus* sp. of the *truncatus* group (n = 36), which is susceptible to *S. bovis* only. The finding of Yasuraoka (1972) was therefore surprising as so far only diploid *Bulinus* has been found near Alemaya, and these were shown by Lo (1970*a*) to be refractory to *S. haematobium*. It was to be expected, therefore, that the Alemaya *Bulinus* sp. also would be refractory to *S. bovis*. Of hundreds of *Bulinus* sp. (n = 18) collected in the area, none were infected with *Schistosoma* spp., which suggests either the presence of undetected colonies of tetraploid bulinids, or that the *S. bovis* infection was not autochthonous.

SUMMARY

Schistosoma bovis occurs in at least seven of the 14 provinces of Ethiopia. Results of faecal and snail surveys in three foci are reported.

Adwa. One collection showed that nine out of 26 bulinids were infected with *S. bovis*. The snail host was a tetraploid form of *Bulinus* (n = 36). The examination of 200 specimens of cattle faeces revealed no *S. bovis* eggs, which was attributed to poor technique or light infection.

Gewani. The snail host was *Bulinus abyssinicus*, which was also infected with *S. haematobium*, the combined infection rate being 60%. *S. bovis* eggs were seen in 1·5% (3/197) of specimens of cattle faeces.

Lake Awassa. Among 715 bulinids (a mixture of diploid (n = 18) and tetraploid (n = 36) forms), 22 were infected with *S. bovis*. Infected snails all belonged to the tetraploid form. Infection in cattle faeces was 5·5% (11/200).

The *Fasciola* infection rates in these three areas were 29%, 78% and 60% respectively.

Susceptibility of laboratory and wild animals to the Gewani and Lake Awassa strains of *S. bovis* was investigated. Combined results show that there are at least five species of wild rodents in Ethiopia which are susceptible to *S. bovis*: ***Arvicanthis niloticus***, ***Praomys albipes***, ***Rattus rattus***, ***Mastomys coucha*** and ***Lophuromys flavopunctatus***, in addition to hamsters, white mice, rabbits and guinea pigs. Immature female worms resembling *S. bovis* were recovered from a goat and a sheep exposed to a mixture of *S. bovis* and *S. haematobium* cercariae shed by naturally infected snails. Using the same mixture of cercariae, a Gelada baboon (*Theropithecus gelada*) could be infected by both schistosomes, but a dog was completely refractory. A mole rat (*Tachyoryctes* sp.) exposed to the Awassa strain had hundreds of dead worms in the liver, which contained an extensive abscess. Some of these inconclusive results are thought to be due to a unisexual infection.

The Gewani strain of *S. bovis* had a wider range of snail hosts than the Adwa and Awassa strains, covering the *tropicus*, *truncatus* and *africanus* groups of *Bulinus*. The Adwa and Awassa strains could infect only members of the *truncatus* group.

ACKNOWLEDGEMENTS. This work was supported by a research grant from the Rockefeller Foundation, New York. We extend our gratitude to the following persons at the Institute of Pathobiology, Haile Sellassie I University, Addis Ababa, Ethiopia, for their technical assistance: Ato Bogale Assefa, Ato Bahta Mazengia, Ato Getachew Assefa and Ato Wondimagnehu Gebre/Michael. We are grateful to our colleagues at the University of Michigan for loaning some of the laboratory equipment (Dr. J. B. Burch; U.S. Public Health Service Grant AI 07279), and providing bulinid snails for susceptibility experiments (Dr. E. G. Berry and Dr. H. van der Schalie; U.S. Army Medical Research Grant DA-49-193-MD-2651).

REFERENCES

ARFAA, F., SABBAGHIAN, H. & BIJAN, H. (1965). Studies on *Schistosoma bovis* in Iran. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **59**, 681–683.

BROWN, D. S. & BURCH, J. B. (1967). Distribution of cytologically different populations of the genus *Bulinus* (Basommatophora: Planorbidae) in Ethiopia. *Malacologia*, **6**, 189–198.

BUCK, A. A., SPRUYT, D. J., WADE, M. K., DERESSA, A. & FEYSSA, E. (1965). Schistosomiasis in Adwa—A report on an epidemiological pilot study. *Ethiopian Medical Journal*, **3**, 93–106.

BURCH, J. B. (1964). Cytological studies of Planorbidae (Gastropoda: Basommatophora). I: The African subgenus *Bulinus*, s.s. *Malacologia*, **1**, 387–400.

BURCH, J. B. (1972). Names for two polyploid species of African *Bulinus* (Basommatophora: Planorbidae). *Malacological Review*, **5**, 7–8.

CAPRON, A., DEBLOCK, S., BIQUET, J., CLAY, A., ADENIS, L. & VERNES, A. (1965). Contribution à l'étude expérimentale de la bilharziose à *Schistosoma haematobium*. *Bulletin World Health Organisation*, **32**, 755–778.

DOBY, J. M., RAULT, B., DEBLOCK, S. & CHABAUD, A. (1966). Bulinus et bilharzioses en Corse. Répartition, fréquence et biologie de *Bulinus truncatus*. *Annales Parasitologie Comparée*, **41**, 337–349.

LEMMA, A. (1965*a*). Schistosomiasis in Adwa—A report on an ecological pilot study, *Ethiopian Medical Journal*, **3**, 84–92.

LEMMA, A. (1965*b*). A preliminary report on the molluscicidal property of endod (*Phytolacca dodecandra*). *Ethiopian Medical Journal*, **3**, 187–190.

LO, C. T. (1970*a*). Experimental exposure of *Bulinus* spp. to miracidia of *Schistosoma haematobium* from Ethiopia. *Organisation of African Unity, Health Bureau Publication No.* 1, *Symposium on Schistosomiasis*, 75–81.

LO, C. T. (1970*b*). Snail intermediate host of *Schistosoma haematobium* in Ethiopia. *Organisation of African Unity, Health Bureau Publication No.* 1, *Symposium on Schistosomiasis*, 85–88.

MARILL, F. G. (1961*a*). Diffusion de la bilharziose chez les bovins, ovins et caprins en Mauritanie et dans la vallée du Sénégal. *Bulletin de l'academie Nationale de Medecin Paris*. **145**, 147–150.

MARILL, G. G. (1961*b*). Première enquête sur l'épidémiologie des bilharzioses en Mauritanie. *Bulletin de l'academie Nationale de Medecin Paris*. **145**, 436–438.

Unpublished Research Note No. 17

CLINICAL SURVEY IN TENSAE BERHAN

Giuseppe DeSole (M.D.), Institute of Pathobiology, 1976

A clinical survey of Tensae Berhan was carried out in late June 1975, late October and early November 1975, and late May 1976. The aims of the clinical survey: 1) To obtain an impression of the general health status of the population in Tensae Berhan and 2) To correlate the clinical symptoms with the results of the parasitological survey.

The town of Tensae Berhan, being the capital of the Arba Gugu Awraja, has a new and well-equipped health center. Part of the drugs and equipment are supplied by UNICEF. The health staff is very active not only in the routine job of the clinic but also in health education in the schools. We are very grateful to them for their competent and friendly collaboration.

Neither marasmus nor kwashiorkor cases were found in the 820 people examined. Fifty-eight per cent were in good general health, 34% fair and 7% poor. The general health of the people was estimated by their appearance and correlation of age, weight and height. It must be considered that the main survey was conducted about one and a half months after the harvest. Food in the market was abundant, varied (barley, teff, goat, sheep, chicken and beef meat, butter, fruits, spices, etc.) and the prices was significantly lower than the average in Ethiopia.

Five hundred and eighty-seven thin and thick blood smears were done for the detection of blood parasites. Only two cases of P. *falciparum* were found during the late June survey, both in farmers working in the Awash Valley, where malaria is known to be endemic. During the survey period, about 45 days in all, and the various short visits to the town in different months of the year, few mosquitoes were seen and none of them were Anopheles. The intensive pesticide spraying on the plantations in the Awash Valley probably has a big impact on mosquito breeding in the area. Dr. P. Neri reported during the previous year (near the end of and just after the big rainy season) the absence of malaria vectors from the town area.

Seventy heparinized capillary tubes were centrifuged in a hematocrit hand centrifuge for 5 - 10 minutes at 100 - 200 rpm. The hematocrit value was 45-50% in 95% of the people studied and approximately 40% in the remaining 5% of the people.

Splenomegaly was found in only 0.5% of the 820 people examined. The hepatomegaly rate in the same group of people was 2.5%.

Chronic eye lesions were found in 31% and acute eye lesions in 10% of the 820 cases. Acute lesions were found more commonly in infants, 29%, followed by 16% in older children and 3% in adults.

No difference in symptoms by sex was noted. The high rate of acute lesions compared with the rate of choronic lesions can be explained by the peculiar age distribution in a town where children and old people predominate.

Lung disease, with the emphasis on tuberculosis, will be studied later with the help of the T.B. Center of Addis Ababa.

Venereal diseases were not surveyed, but from the data of the Health Center and considering the peculiar age distribution of the town where children and old people predominate, it does not seem to be a prominant problem.

The correlation between the symptoms and egg output as determined with Kato's method has been plotted in a histogram (Lemma et al.,unpubl.). A previous work on 200 people showed a low correlation but the clinical survey was done two months after the stool examination.

The 620 related to the histogram were examined both clinically and parasitologically at the same time. The clear-cut correlation should be considered with prudence because of the small number of cases with more than 200 eggs per gram of stool. There were 52 cases (8%). All but two of the heavy egg excretors were children between the ages of 6 months and 18 years, most of them school children.

No correlation between hepatomegaly and S. mansoni was found. Professor C.S. Leithead, in a survey of the Lake Tana area in Begemder, arrived at the same conclusion regarding hepatomegaly and S. mansoni.

From the data collected, it seems evident that S. mansoni is the greatest health problem in the Tensae Berhan area. The nutritional status of the Tensae Berhan population appears to be higher than that of the average Woynadega subsistence farming community of Ethiopia. Malaria does not seem to be present in the town as indicated by the entomological and parasitological findings. This is also indicated by the extremely low incidence of splenomegaly and the absence of anemia. The eye lesion data, although it indicates a need for improved hygiene of the eye in the population, does not exceed and may be slightly lower than the average in the country. The less heavily infected people with S. mansoni have only minor complaints and many of them are asymptomatic. The few heavily infected people usually experience moderate and occasionally severe discomfort but are not incapacitated in their normal life activities.

REFERENCE

Lemma, A., DeSole, G., Polderman, A.M., Mazengia, G., Redda, A. and Kloos, H. 1979. The epidemiology of Schistosoma mansoni infection in Tensae Berhan, Ethiopia. I. Prevalence of schistosomiasis. Unpublished report. Institute of Pathobiology.

THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE
Vol. 26, No. 3

Printed in U.S.A.

MORBIDITY FROM *SCHISTOSOMA MANSONI* INFECTIONS: AN EPIDEMIOLOGIC STUDY BASED ON QUANTITATIVE ANALYSIS OF EGG EXCRETION IN ETHIOPIAN CHILDREN

ROBERT A. HIATT* AND MEHARI GEBRE-MEDHIN†
George Williams Hooper Foundation, University of California, San Francisco, California 94143, Institute of Pathology, Addis Ababa University, Addis Ababa, Ethiopia, and The Ethiopian Nutrition Institute, Addis Ababa, Ethiopia

Abstract. Morbidity from infection with *Schistosoma mansoni* was investigated in a population of school children, aged 7 to 16 years, from the town of Chwahit, located north of Lake Tana in Highland Ethiopia. Malaria was under control in the area, and *S. haematobium* was not endemic. Quantitative fecal egg counts were determined for 336 children (94% of those in the school) by the Kato thick-smear method; other intestinal parasites were determined by the Ritchie formol-ether concentration method. Morbidity was measured by a standardized medical examination of 272 of these children and was analyzed in four categories of egg count intensity: 0 (12%), 1–100 (19%), 101–500 (40%), and 501+ (29%) eggs per gram (epg). Prevalence was 88%, and the geometric mean egg count was 259 epg. The highest prevalence and intensity were found in 12-year-olds (95% and 300 epg, respectively). No schistosomiasis-associated symptoms were significantly related to the intensity of *S. mansoni* infection, although the complaint of blood in the stool was more frequent in groups with higher egg counts. There was a trend for hepatomegaly (especially of the left lobe) to increase in frequency with increasing egg count, but this trend was not significant. These children had no splenic enlargement. Anthropometric weight-for-height measurements, average school grade, and days absent from school were not related to the intensity of *S. mansoni* infections. Physical performance in a standardized 12-minute walk-run was better in uninfected boys, but there was no correlation with the intensity of infection. The minimal morbidity found in this population is compatible with the moderately low intensity of *S. mansoni* infection and with the findings of a previous population-based study in a neighboring village.

In recent years, population-based studies of schistosomiasis mansoni have begun to provide sound information on the clinical significance and the public health impact of this infection in man. Earlier studies of morbidity based on hospital and clinical patients or other types of selected individuals tended to bias observations towards the severe forms of the disease. The World Health Organization emphasized the need for community-based studies of schistosomiasis and made detailed recommendations for assessing the impact of the infection on public health.[1] Kloetzel was the first to employ fecal egg counts in a population-based morbidity study in Brazil.[2] Ongom and Bradley expanded this approach in their morbidity study in Uganda,[3] and, more recently, Lehman et al.[4] and Siongok et al.[5] gave reports on very complete morbidity studies based on quantitative egg output in endemic communities in Brazil and Kenya, respectively.

The use of standard quantitative parasitologic techniques in these studies has been an essential factor in allowing comparison between these diverse endemic areas. Jordan has proposed that if standardized, comparable techniques are used, populations with similar prevalence rates and intensities of infection may be shown to have similar levels of clinical manifestations.[6]

In this study, we examined the clinical manifestations of *Schistosoma mansoni* infections in a Highland Ethiopia school population. This population was chosen so that we could enlarge upon and complement a study conducted in the same year on a random sample of the inhabitants of the nearby village of Twawuzgi-Chenker where school-age children were the most intensely infected.[7] In both studies, on the basis of a single

Accepted 6 November 1976.

* Present address: Epidemiology Program, School of Public Health, University of California, Berkeley, California 94720.
† Present address: Institute of Nutrition, Medical Faculty, University of Uppsala, Uppsala, Sweden.

fecal egg count, individuals were divided into groups differing in level of egg output. The hypothesis was that signs and symptoms compatible with *S. mansoni* infection have a direct relationship with fecal egg output. We could not give valid consideration to the duration of infection in these studies. The data from the school children were analyzed in a manner similar to that used by Cook et al. in their study of children in St. Lucia.[8] The present study and that from Twawuzgi describe population-based clinical and quantitative parasitological parameters of *S. mansoni* infections in Highland Ethiopia, and comparisons may be made with similar studies in other endemic areas of the world.

POPULATION

The study was conducted in the town of Chwahit, north of Lake Tana in Begemdir Province, on the road between Gondar and Gorgora. The town has a population of about 2,200. The prevalence of *S. mansoni* infection was approximately 42%; it was transmitted seasonally in three small streams at the outskirts of the village.[9] Messing described the historical and anthropologic characteristics of Chwahit and its people,[10] and Polderman studied the nonclinical aspects of schistosomiasis epidemiology in some depth.[9] *S. haematobium* has never been found in Highland Ethiopia, and 100 urine specimens from the population of Chwahit and Twawuzgi examined by a sedimentation technique during the present study were negative. Malaria was under control in the region as a result of the efforts of the Malaria Eradication Service. Kala-azar, although endemic in the Ethiopian lowlands, has not been found in the study area.

Children between the ages of 7 and 17 who lived in or near the village attended a mud and wattle school in Chwahit. Seven rooms accommodated 358 students in six grades. Previous surveys in the school by Polderman showed a prevalence of *S. mansoni* infection of about 75% in 314 students examined by the Ritchie formol-ether concentration technique.[9]

Seventy-eight percent of the children professed to be Coptic Christians, and 20% were Muslims. Forty-three percent were born in Chwahit. Most of the others came from towns in the same province.

METHODS

A stool specimen was requested from each student on the first visit to the school. As a rule, children could produce a stool on demand. Precautions were taken to avoid mislabeling and loss of stool cups. For quantitative evaluation of the intensity of infection, stools were examined by the Kato thick-smear technique.[11] As previously described,[7] a disposable measuring device modified from that suggested by Katz et al.[12] was used to obtain a consistent sample of 25 mg; one thick-smear preparation from each student was examined. For qualitative determination of other intestinal parasites, an additional sample of 1 gram from the same specimen was preserved in 10% formalin for later examination by the formol-ether concentration technique.[13]

On a second visit to the school, clinical histories were obtained, and physical examinations were performed on children in all classes except one of the two first grades. That first grade was not included because of time limitations. Histories were obtained by means of a standardized, precoded questionnaire which emphasized symptoms commonly associated with *S. mansoni* infections. Only symptoms present approximately within a week of the examination were included in the analysis. Questions were also asked about previous infection with malaria, the presence of jaundice, or the use of the hepatotoxic herb, *kosso*.[14] Physical examinations included general assessment of health status and anthropometric measurements. Abdominal findings and signs of malnutrition were emphasized. The anthropometric and nutritional data will be dealt with in more detail in a forthcoming publication. The liver was palpated at the costal margin in the midclavicular line (MCL) and in the midsternal line (MSL). Hepatomegaly was defined as equal to or greater than 2 cm below the costal margin. The spleen was palpated with students in the right lateral decubitus position. Children in minimal dress were weighed to the nearest 0.1 kg on a carefully adjusted bathroom scale. They were measured for height (without shoes) to the nearest 0.1 cm.

Skinfold thickness was measured by standard techniques with the Harpenden caliper.[15] The authors performed the clinical examinations (RAH) and made the anthropometric measure-

TABLE 1

Prevalence and intensity of S. mansoni *infection in 272 Ethiopian school children*

Age	Males			Females			Both sexes		
	No. exam.	Prevalence	Eggs/gram feces*	No. exam.	Prevalence	Eggs/gram feces	No. exam.	Prevalence	Eggs/gram feces
7	6	50.0	370	5	40.0	310	11	45.5	345
8	7	85.7	281	6	83.3	136	13	84.6	201
9	18	88.9	277	11	72.7	244	29	82.8	266
10	15	100.0	267	20	80.0	182	35	88.6	219
11	26	88.5	303	27	92.6	270	53	90.6	286
12	25	96.0	376	23	95.7	235	48	95.8	300
13	12	91.7	244	19	89.5	282	31	90.3	267
14	20	100.0	171	13	76.9	438	33	90.9	232
15	11	90.9	271	1	100.0	145	12	91.7	228
16	5	80.0	162	2	100.0		7	85.7	194
Total	145	91.0	270	127	85.0	246	272	88.2	259

* Geometric mean of those infected.

ments (MGM) without knowledge of the students' parasitologic status.

After the clinical examination, venous blood was drawn for hematology and blood chemistry studies. Hematocrits were determined by the MSE microhematocrit method. Hemoglobin concentration was measured by the cyanmethemoglobin method. Total serum protein and serum albumin were determined on sera shipped in wet ice to the Ethiopian Nutrition Institute in Addis Ababa.

Results of the clinical evaluations were analyzed by dividing the students into four arbitrary categories of *S. mansoni* egg counts: 0, 1–100, 101–500, and 501 or more eggs per gram (epg).

From school records of those children who attended in the previous year, numerical scores representing their average grade and days absent from school were obtained. Finally, a standardized test of physical endurance was performed on all boys 11 to 17 years of age. Boys were told to cover as much distance as possible on a measured ¼-mile course within a 12-minute time limit.[16]

RESULTS

From the total school population of 358, 336 (94%) responded by providing a stool for examination. Overall prevalence of *S. mansoni* from one thick-smear determination was 86%. Of 289 students in the classes where clinical evaluations were done, 272 (94%) were actually examined. All subsequent analyses were done on these 272 children.

Schistosoma mansoni infection by age and sex is presented in Table 1. The overall prevalence in this group was 88%. Males had a higher prevalence of infection (91%) than did females (85%). Likewise, the geometric mean egg count was slightly higher for males (270 epg) than for females (246 epg). Determinations of age-specific prevalence of infection revealed that, except for the 7-year-olds, all age groups had a very high prevalence (83–96%). Age-specific mean intensities showed a gradual increase until age 12, and then an equally gradual decrease. In almost all age groups males had heavier intensities of infection.

Muslims were more commonly infected than Copts (94% vs. 87%), and males in both religious groups had higher prevalence rates than females. None of these differences was significant, however. Those born in Chwahit had a slightly higher prevalence rate than those born elsewhere (92% vs. 86%).

The frequency of other intestinal parasites and their distribution relative to the levels of *S. mansoni* infection are presented in Table 2. Ascariasis and trichuriasis were common, reaching prevalence rates of 82% and 58%, respectively. All parasites were found at similar frequencies in all four groups categorized according to the level of intensity of infection with *S. mansoni*.

The frequencies of signs and symptoms are shown by intensity of infection with *S. mansoni* (Table 3). No significant differences between the groups were noted for any of the symptoms

TABLE 2

Percentage of individuals with intestinal parasites in groups differing in intensity of infection with S. mansoni *among students whose stool specimens were examined by both the concentration and thick-smear techniques*

Other parasites*	*S. mansoni* egg count per gram†				
	0	1–100	101–500	501+	Total
Ascaris lumbricoides	84	86	82	79	82
Trichuris trichiura	63	59	53	62	58
Hookworm	13	16	17	17	12
Taenia sp.	0	4	2	1	2
E. histolytica	22	27	25	14	22
No. examined	32	51	109	78	270

* Determined by concentration technique.
† Determined by thick-smear technique.

(χ^2, goodness of fit). There was a trend toward higher frequencies of the complaint of blood in the stool with increasing egg count, but this trend was not significant (at $P < .05$) by the *t*-test for linear trend.[17]

The frequency of hepatomegaly (≥2 cm below the costal margin) of both lobes of the liver increased with intensity of infection (Table 3). The left lobe was enlarged almost twice as frequently as the right (36% vs. 19%). Both lobes of the liver tended to be more frequently enlarged as intensity of infection increased, although this trend was irregular for the right lobe. These trends were not statistically significant for either lobe. The frequency of tenderness of the liver increased with intensity of infection, and the differences between groups were significant ($P < .02$, χ^2, goodness of fit). Most livers felt firm upon palpation, but we did not attempt to quantify this finding. Splenic enlargement was not observed.

Other information gathered on the students to help clarify the etiology of enlarged livers in the population is presented in Table 4. A past history of malaria seemed to be inversely related to the intensity of *S. mansoni* infection. The same tendency was noted for those who said they had never had jaundice. Those who admitted to using the hepatotoxic herb, *kosso*, were found in comparable frequency in all egg count groups.

No relationship was noted between academic performance, judged by average grade in the previous academic year, and the intensity of infection (Table 5). Those with heavier infections were, in fact, absent from school fewer days than those with lighter infections. However, a direct relationship between physical endurance and intensity of infection was suggested by the data. The mean distance covered in the 12-minute walk-run was farthest for boys without *S. mansoni* eggs in their stools and was less in those with infections. Although the differences were significant by the χ^2 goodness of fit ($P < .05$), the decrease in distance covered was not related as a gradient to intensity of infection (that is, those with 1–100 epg did slightly worse than those with over 501 epg).

TABLE 3

Percentage of individuals with symptoms and signs associated with chronic schistosomiasis at the time of examination in groups differing in intensity of infection with S. mansoni

Symptoms and signs	*S. mansoni* egg counts per gram of feces				
	0	1–100	101–500	501+	Total
Complaint of:					
Cramping abdominal pain	19	14	19	9	15
Diarrhea	16	8	7	10	9
Blood in the stool	6	8	13	13	11
Mucus in the stool	6	2	6	6	5
Physical finding of:					
Right lobe liver 2 cm below MCL	6	25	17	24*	19
Left lobe liver 2 cm below MSL	28	32	38	41*	36
Tender liver	6	12	17†	20‡	15
No. examined	32	52	109	79	272

* n = 108.
† n = 78.
‡ n = 77.

TABLE 4

Percentage of individuals with a history of factors possibly significant in the etiology of hepatomegaly in groups differing in intensity of infection with S. mansoni

History of	*S. mansoni* egg counts per gram of feces				
	0	1–100	101–500	501+	Total
Malaria	16	10	2	6	6
Jaundice	9	10	6	3	6
Use of *kosso*	34	60	41	48	46
No. questioned	32	52	109	79	272

TABLE 5
Measures of performance and nutritional status in groups differing in intensity of S. mansoni *infection*

Measure	*S. mansoni* egg count per gram of feces				
	0	1–100	101–500	501+	Total
Mean grade for previous year of school work	73.7 (15)*	67.7 (44)	67.5 (95)	71.3 (73)	69.3 (227)
Mean number of days absent from school in previous year	11.8 (14)	5.1 (43)	5.2 (77)	4.8 (64)	5.5 (198)
Mean distance covered in 12-minute walk-run (males ages 11–17)	8,829 (7)	7,909 (16)	8,376 (36)	8,132 (29)	8,247 (88)
Weight for height as mean percent of median wt/ht	98.6 (32)	102.7 (52)	99.5 (109)	100.0 (79)	100.0 (272)
Weight for height as mean percentile	42.3 (32)	52.5 (52)	47.3 (109)	47.6 (79)	47.6 (272)

* Arithmetic mean (number examined).

Weight-for-height measurements (Table 5) showed that the nutritional state of these children (by this measure) was close to the standard,[18] and that there was no relationship between mean weight for height and egg count. In fact, the children in the lowest egg count group had the lowest weight for height.

To assess the relationship between nutritional status and hepatomegaly, irrespective of *S. mansoni* egg count, the students were separated into two groups—165 with livers nonpalpable or palpable only on deep inspiration and 109 who had at least one lobe of the liver palpable 2 cm or more below the costal margin. The difference in mean weight/height percentiles between these two groups (48.8% vs. 45.2%) was not significant.

The results of hematologic and serum protein determinations are presented in Table 6. No differences were noted in mean values for either hematocrit or hemoglobin. Total serum protein rose with increasing egg count, and this was accounted for solely by the globulin fraction.

DISCUSSION

Previous epidemiological studies of schistosomiasis in Ethiopia have been reviewed elsewhere.[9, 19] *S. mansoni* is the predominant species and is endemic over most of the highlands, whereas *S. haematobium* is endemic only in the Awash Valley.[19] Relatively little is known about intensity and the clinical impact of the infection. Polderman previously noted hepatomegaly in people in Chwahit and the nearby village of Jenda, but did not find any relationship with *S. mansoni* infection.[9] The results of the present school study and a previous investigation in the nearby village of Twawuzgi[7] are the first population-based data on clinical manifestations of schistosomiasis in Ethiopia related to quantitative fecal egg counts. The results of these studies are compatible with the impression of local clinicians that only minimal morbidity is associated with *S. mansoni* infection. Polderman has speculated that this minimal morbidity is due to the presence of a *S. mansoni* strain

TABLE 6
Group mean values for laboratory results in groups differing in intensity of infection with S. mansoni

Values for	*S. mansoni* egg count per gram of feces				
	0	1–100	101–500	501+	Total
Hematocrit (%)	43.9 (27)*	42.8 (45)	43.3 (93)	43.2 (63)	43.2 (228)
Hemoglobin (g/100 ml)	14.7 (20)	14.2 (35)	14.4 (75)	14.2 (52)	14.3 (182)
Total serum protein (g/100 ml)	8.2 (31)	8.5 (50)	8.5 (104)	8.6 (77)	8.5 (262)
Globulin (g/100 ml)	3.8 (31)	4.0 (50)	4.0 (104)	4.1 (77)	4.1 (262)

* Arithmetic mean (number examined).

of low virulence or to acquired immunity, enhanced by the seasonal pattern of transmission.[9] The present study suggests that the relatively low intensity of infection plays some role.

To date, quantitative stool techniques have been used in large groups in only a few studies. Kloetzel, in the Pernambuco State of Brazil,[2] and Ongom and Bradley, in West Nile Uganda,[3] have studied communities where *S. mansoni* is hyperendemic and intensities of infection are very high. Lehman et al.[4] have recently reported on a population-based study in Bahia, Brazil, where intensities of infection were moderately high. Intensity of infection in the Kenyan population studied by Siongok et al.[4] was also high. In all of these studies, there has been a direct relationship between the intensity of infection as measured by egg counts and the frequency of clinical manifestations of schistosomiasis. A recent population based study in Puerto Rico, where intensity of infection is low, failed to show any striking difference between infected people and age and sex matched controls.[20]

In the study of the village of Twawuzgi[7] the highest prevalence and the heaviest infections were found in 49 children in the 10- to 14-year age group (80% and 213 epg respectively). By examining a larger group in Chwahit, which has an epidemiological pattern and levels of intensity similar to Twawuzgi,[9] we believed that we would find the possible relationships between *S. mansoni* egg counts and clinical manifestations more evident. In Chwahit 200 children in the 10- to 14-year age group had a prevalence of 92% and a geometric mean egg count of 266 epg. This difference was entirely due to the difference in the intensity of infection in females, since the prevalence and intensity were the same for males in both villages (93% vs. 93%, and 272 epg vs. 270 epg for Chwahit and Twawuzgi, respectively). This discrepancy may be explained by school girls in Chwahit having more prolonged contact with water.[9]

Gastrointestinal symptomatology was common but not related to the magnitude of *S. mansoni* egg counts. However, the complaint of blood in the stool was more common as the intensity of infection increased. Occult blood in the stool was significantly related to the intensity of infection in St. Lucian children,[8] and direct associations between the symptom and egg counts have also been noted in Uganda[3] and Kenya.[5] The association between chronic schistosomiasis mansoni and gastrointestinal blood loss as a phenomenon dependent on intensity of infection seems fairly well established by these studies.

The effect of this blood in terms of producing anemia is apparently minimal. In the present study mean hematocrit levels were normal in all egg count groups. The lack of an appreciable anemia producing effect from gastrointestinal blood loss was also observed in St. Lucian children.[8] In Ethiopia, however, a more serious problem could have been masked, since dietary iron intake is high and anemia is not a common public health problem.[21]

The lack of correlation between anthropometric measurements as a reflection of nutritional status and the intensity of infection with *S. mansoni* has been noted in previous studies.[4,7,8] This should not be interpreted to mean, however, that there is no relationship between nutritional status and the manifestations of infection with *S. mansoni*. Error in anthropometric measurements can be appreciable if the age variable is employed in a culture where exact age is not important and there is no formal record keeping system. In our present study we judged age reliable only to the nearest year, and used only weight for height (independent of age) for analysis.

Hepatomegaly (2 cm below the right costal margin) was common. The enlargement was usually of a mild degree, and the left lobe was palpated more frequently than the right (36% vs. 19%, respectively). An etiologic role for *S. mansoni* infection was suggested by the direct association of frequency of hepatomegaly and the intensity of infection. Stronger direct associations between hepatomegaly and the intensity of *S. mansoni* infection have been noted in other studies in St. Lucia,[8] Brazil,[4] and Kenya.[5] Prata and Bina observed the predominance of the left lobe on the liver over the right in most individuals with hepatomegaly and schistosomiasis,[22] and Siongok et al. reported that the left lobe was more prominent than the right, especially in their younger age group.[5]

The significance of this pattern of mild hepatomegaly without splenomegaly remains elusive. In Ethiopia the unlikely contribution of other possible confounding environmental factors has been discussed.[7] Malaria was under control, and

nutritional status was judged not sufficiently poor to result in enlarged fatty livers. Infectious hepatitis and hepatic toxins, although common, would probably cause a small postnecrotic type of liver pathology after the acute illness and are not likely etiologies. Other endemic diseases (for example, amoebiasis and kala-azar) did not occur frequently enough to explain the observations. Nevertheless, the possibility of other etiologic factors remains, since an appreciable degree of hepatomegaly was found in the group with stools negative for *S. mansoni*. This might be due to *S. mansoni* infections missed by our relatively insensitive stool technique. Hepatomegaly in presumably uninfected St. Lucian children has been noted,[8] however, and one of us (RAH) found hepatomegaly just as frequently in individuals in an Ethiopian village population with a low prevalence of *S. mansoni* infection (10.7%) as in individuals in a comparison village population with a moderately high prevalence (43.3%). In commenting on the findings in the St. Lucian children, Cook et al.[8] referred to a study in Jamaica where schistosomiasis is not endemic and yet 26% of 194 children were found to have otherwise unexplained liver enlargement.[23]

Even if *S. mansoni* infection is responsible for the observed hepatomegaly, the long-term clinical significance of this sign is unknown. On the basis of the absence of severe clinical manifestations in adults in Twawuzgi and the fact that clinically apparent schistosomiasis is not recognized by physicians at the nearby Gondar Public Health College,[7] we would judge that this finding is at most a transient manifestation of *S. mansoni* infection and that it does not carry an unfavorable prognosis.

Mean levels of serum protein, especially globulins, were shown to increase slightly with egg count in the Ethiopian children. This also is consistent with the findings of Cook et al.,[8] and Kellermeyer et al.[24] have found the IgG and IgM levels elevated in individuals (especially children) infected with *S. mansoni* as compared to noninfected controls.

With respect to performance criteria, use of the average grade for the previous academic year and the number of days absent from school were admittedly crude measurements and only showed an unexplained inverse relationship between days absent and intensity of infection. The 12-minute walk-run test of endurance was based on the test Cooper standardized for adults.[16] This test was used for boys, ages 11 to 17 years, on a purely empirical basis and for lack of a better standard. The results were only suggestive of a decline in performance with increasing egg count. Walker et al.[25] studied running performance in a 600-yard race in South African children, and noted no differences between infected and uninfected individuals.

In summary, we were able to document only minimal morbidity associated with *S. mansoni* infection in this group of Highland Ethiopian school children. The findings are compatible with their moderately low intensity of infection as a group. Jordan has suggested that "if it can be shown that, irrespective of region, the physical findings are the same for the same load of eggs, collection of quantitative egg output data may be sufficient to indicate the importance of disease in different endemic areas."[6] In general, the results from the present study are similar to those of Cook et al.,[8] which is what would be expected from the similarities in age structure and intensity of infection in the two study populations. More data from other endemic areas based on standard quantitative techniques, clinical format, and methods of analysis are still needed, but the comparison between these two studies would tend to support his hypothesis.

ACKNOWLEDGMENTS

This study was done while the principal author was in a training program under the Bureau of Medical Services, Health Services Administration, United States Department of Health, Education, and Welfare, and the School of Public Health, University of California, Berkeley. He was assigned to the George Williams Hooper Foundation, University of California, San Francisco, and the Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia. The data were analyzed at the San Juan Laboratories, Center for Disease Control, United States Public Health Service, Department of Health, Education, and Welfare (HEW). The work was supported by Addis Ababa University (formerly Haile Selassie I University) project no. F/65/IP-13. We are grateful for the direction and support of Dr. Aklilu Lemma, Director, Institute of Pathobiol-

ogy, Addis Ababa University. Data analysis was partially supported by a grant from the Bowie Fund, George Williams Hooper Foundation, University of California, San Francisco. Anthropometric data were analyzed with the assistance of the Special Projects Branch, Bureau of Smallpox Eradication, Center for Disease Control, U.S. Public Health Service, HEW, Atlanta. The assistance of Dr. A. M. Polderman was essential in our introduction to this population. The authors acknowledge the assistance of Ato Bahta Mazengia, Chief Laboratory Technician of the Institute of Pathobiology, and S. Amsale Gebre-Yohannes, Ato Yilma Hableyes, and Miss Berit Borg of the Ethiopian Nutrition Institute. Gondar Public Health College assisted with laboratory work, and NAMRU-5 Addis Ababa, helped transport the samples. We also thank Ato Mosus Tiruneh, Ato Aberra Kumsa, and Ato Mitiku Gebeyu for their work in the laboratory and in the field, and Ato Ambachew Abate, principal of Chwahit School, for his cooperation in the study.

REFERENCES

1. World Health Organization, 1967. Measurement of the public health importance of bilharziasis. *Tech. Rep. Ser. No. 349.*
2. Kloetzel, K., 1962. Splenomegaly in schistosomiasis mansoni. *Am. J. Trop. Med. Hyg., 11:* 472–476.
3. Ongom, V. L., and Bradley, D. J., 1972. The epidemiology and consequences of *Schistosoma mansoni* infection in West Nile, Uganda. I. Field studies of a community at Panyagoro. *Trans. R. Soc. Trop. Med. Hyg., 66:* 835–851.
4. Lehman, J. S., Jr., Mott, K. E., Morrow, R. H., Jr., Muniz, T. M., and Boyer, M. H., 1976. The intensity and effects of infection with *Schistosoma mansoni* in a rural community in northeast Brazil. *Am. J. Trop. Med. Hyg., 25:* 285–294.
5. Siongok, T. K. A., Mahmoud, A. A. F., Ouma, J. H., Warren, K. S., Muller, A. S., Handa, A. K., and Houser, H. B. 1976. Morbidity in schistosomiasis mansoni in relation to intensity of infection: Study of a community in Machakos, Kenya. *Am. J. Trop. Med. Hyg., 25:* 273–284.
6. Jordan, P., 1972. Schistosomiasis and disease. Pages 17–23 *in* M. J. Miller, ed., *Proceedings of a Symposium on the Future of Schistosomiasis Control.* Tulane University, New Orleans.
7. Hiatt, R. A., 1976. Morbidity from *Schistosoma mansoni* infections: An epidemiologic study based on quantitative analysis of egg excretion in two Highland Ethiopian villages. *Am. J. Trop. Med. Hyg., 25:* 808–817.
8. Cook, J. A., Baker, S. T., Warren, K. S., and Jordan, P., 1974. A controlled study of morbidity of schistosomiasis mansoni in St. Lucian children, based on quantitative egg excretion. *Am. J. Trop. Med. Hyg., 23:* 625–633.
9. Polderman, A. M., 1975. The transmission of intestinal schistosomiasis in Begemdir Province, Ethiopia. Thesis, Rijksuniversiteit, Leiden, Holland. (Published in *Acta Leidensia, Volume 42*, 1975.)
10. Messing, S. D., 1972. The community of Chwahit. *In The Target of Health in Ethiopia.* MSS Information Corporation, New York.
11. Martin, L. K., and Beaver, P. C., 1968. Evaluation of the Kato thick-smear technique for quantitative diagnosis of helminth infections. *Am. J. Trop. Med. Hyg., 17:* 382–391.
12. Katz, N., Chaves, A., and Pellegrino, J., 1972. A simple device for quantitative stool thick smear technique in schistosomiasis mansoni. *Rev. Inst. Med. Trop. S. Paulo, 14:* 397–400.
13. Ritchie, L. S., Lin, S., Moon, A. P., Frick, L. P., Williams, J. E., Asakura, S., and Hishinuma, Y., 1960. The possible effects of pH and specific gravity of the ether-sedimentation procedure in concentrating eggs and cysts. *Am. J. Trop. Med. Hyg., 9:* 444–449.
14. Coady, A., 1965. The possibility of factors of plant (particularly fungal) origin in Ethiopian liver disease. Parts I and II. *Ethiopian Med. J., 3:* 173–185.
15. Jelliffe, D. B., 1966. *The Assessment of the Nutritional Status of the Community.* WHO Monograph Series, No. 53, Geneva.
16. Cooper, K. H., 1968. A means of assessing maximal oxygen uptake; correlation between field and treadmill testing. *J. Am. Med. Assoc., 203:* 201–204.
17. Snedecor, G. W., and Cochran, W. G., 1967. *Statistical Methods*, 6th edition. Iowa State University Press, Ames, Iowa, pp. 243–246.
18. Committee on Nutrition Advisory to Center for Disease Control Food and Nutrition Board, 1974. *Comparison of Body Weights and Lengths or Heights of Groups of Children.* U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Atlanta, Georgia.
19. Lemma, A., 1969. Bilharziasis in the Awash Valley. I. An epidemiological study with special emphasis on its possible future economic and public health importance. *Ethiopian Med. J., 7:* 147–176.
20. Cline, B. L., Rymzo, W. T., Hiatt, R. A., Knight, W. B., and Berríos-Duran, L. A., 1977. Morbidity from *Schistosoma mansoni* in a Puerto Rican community: A population-based study. *Am. J. Trop. Med. Hyg., 26:* 109–117.
21. Hofvander, Y., 1968. Hematological investigations in Ethiopia with special reference to a

high iron intake. *Acta Med. Scand., Suppl. 494.*

22. Prata, A., and Bina, J. C., 1968. Development of the hepatosplenic form of schistosomiasis. *Gaz. Med. Bahia, 68:* 49–60.
23. Miller, C., 1960. Observation on liver size in healthy Jamaican children. Hermitage survey. *West Indies Med. J., 9:* 124–130.
24. Kellermeyer, R. W., Warren, K. S., Waldermann, T. S., Cook, J. A., and Jordan, P., 1973. Concentration of serum immunoglobulins in St. Lucians with schistosomiasis mansoni compared with matched uninfected St. Vincentians. *J. Infect. Dis., 127:* 557–562.
25. Walker, A. R. P., Walker, B. F., and Richardson, B. D., 1970. Studies on schistosomiasis in a South African Bantu schoolchild population. *Am. J. Trop. Med. Hyg., 19:* 791–814.

SCHISTOSOMIASIS AND IRRIGATION IN THE AWASH VALLEY OF ETHIOPIA

KLOOS, Helmut, Ph.D.
University of California, Davis, 1977

Schistosomiasis is endemic in irrigation schemes and natural waters in the Awash Valley. Schistosomiasis mansoni is most common among some migrant farm labor populations, indigenous farmers, and pastoralists. Schistosomiasis haematobium only afflicts large numbers of Afar pastoralists but is increasingly becoming a disease of migrant farm laborers. The large-scale development of irrigation agriculture in the Awash Valley since the 1950's resulted in substantial changes in the size and composition of surface waters, the distribution of the human population, the occurrence of schistosome-transmitting snails, and the prevalence and incidence of schistosomiasis. Four hypotheses were tested to determine the influence of several physico-environmental and human-ecological factors and the impact of regional irrigation development on the distribution of infection.

Extreme temperatures in the Awash Valley and the Ethiopian highlands are the main factors in the low Schistosoma mansoni infection rates in most irrigation schemes. Water temperatures are too low in the labor source areas above 2,200 meters altitude, or beyond the 16°C isotherm, for the completion of the transmission cycle. Most migrant farm laborers from the central and southern parts of the Ethiopian highlands were free of infection upon arrival in the irrigation schemes. Many migrants from the eastern and northern parts of Ethiopia, however, contracted schistosomiasis in their home areas. In the middle and lower parts of the Awash Valley, below 800 meters and beyond the 26°C isotherm, temperatures are too high for the survival of Biomphalaria pfeifferi, the snail transmitting S. mansoni in the study area. Lesser factors in the absence of this snail from the lowlands include high silt concentration, water chemistry, and irrigation practices.

Labor migrations are more conducive to schistosomiasis mansoni spread in the irrigated areas than any other type of population movement, resulting in high infection rates among migrants in the lower plains of the Awash Valley. Seasonal migrations of some pastoralists may also contribute to the diffusion of the parasite, but the travels of merchants, tourists, and pilgrims appear to be of little relevance.

The absence of appropriate host snails of S. haematobium from the irrigation systems prevents this parasite from becoming established on the farms. Schistosomiasis haematobium is highly prevalent only among pastoral Afar inhabiting the areas of the freshwater swamps and lakes below 750 meters altitude. These habitats are apparently the only ecological niche of Bulinus abyssinicus, the sole intermediate host of S. haematobium in the Awash Valley.

Water resources development caused increases in schistosomiasis mansoni and decreases in schistosomiasis haemato-

Reprinted from
DISSERTATION ABSTRACTS INTERNATIONAL

Volume XXXVIII, Number 6, 1977

bium prevalence in the Awash Valley. Evidence that S. mansoni became more common includes the spatial and temporal distribution of surface waters, host snails, and infections. Physico-environmental and human-ecological changes associated with irrigation development disfavor S. haematobium transmission. The construction of Koka High Dam, development of the flood plains to agriculture, and increased withdrawal of Awash River water for irrigation reduced the seasonal river floods and the B. abyssinicus-containing swamps. Increased settlement of Afar pastoralists in irrigated areas is reducing the amount of exposure they have with the remaining infested swamps.

Although temperature and other environmental factors prevent the spread of endemic schistosomiasis in the farms in the hot lowlands, the proposed enlargement of the irrigated areas may break down some of these barriers, through the creation of new snail habitats and increased population density.

Order No. 77-27,384, 307 pages.

THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE
Vol. 26, No. 5

Printed in U.S.A.

SCHISTOSOMIASIS IN IRRIGATION SCHEMES IN THE AWASH VALLEY, ETHIOPIA

HELMUT KLOOS* AND AKLILU LEMMA

The George Williams Hooper Foundation, University of California, San Francisco, California 94122, and the Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia

Abstract. Surveys were made of the prevalence of schistosomiasis in 16 irrigation farms in the Awash Valley, some nearby villages of indigenous pastoralists and subsistence farmers, and 21 towns and villages in the main labor source areas in the Ethiopian highlands. The results indicate that schistosomiasis mansoni is endemic only in the upper part of the Awash Valley, that it is absent from most home areas of the migrant farm laborers but highly prevalent in some localities, and that schistosomiasis haematobium is endemic only in the swamps and some lakes in the middle and lower parts of this valley. *Schistosoma mansoni* infection rates ranged from 0.8% to 15.2% among migrant farm populations, from 0% to 33.9% among indigenous pastoralists and farmers, and from 0% to 72.0% among school children in the main labor source areas. *S. haematobium* infection rates were lower than 2% in all migrant farm populations except one (20.0%) located in swamps where the parasite is endemic. Among indigenous pastoralists, rates varied between 0% and 26.9% in different parts of the valley. Snails were collected from the habitats visited and were identified, and susceptibility of *Bulinus* (*Bulinus*) *truncatus sericinus* to *S. haematobium* was studied. The role of *Bulinus* (*Physopsis*) *abyssinicus* in the transmission of *S. haematobium*, as well as the influence of some environmental factors on the distribution of *Biomphalaria pfeifferi* and of labor migrations on schistosomiasis occurrence are discussed.

The only surveys on the prevalence of schistosomiasis in the irrigation farms in the Awash Valley were made by Lemma.[1] Little is known about possible changes in prevalence and distribution of *Schistosoma haematobium* and *S. mansoni* infections since his investigations in the mid-1960s. Moreover, it has not been established in which farms schistosomiasis has become endemic. Considerable change in irrigation and settlement has occurred during the past 10 years. This paper presents data collected on schistosomiasis prevalence and host snail occurrence during a 2-year study (1973 and 1975–76) of the 16 largest irrigation farms in the Awash Valley. Malacological data collected during the first year of the study and schistosomiasis haematobium prevalence data on the pastoral Afar people have been published elsewhere.[2,3]

AREA OF STUDY AND POPULATION

The Awash Valley covers much of the larger Awash Basin, the 120,000 km² large area located between 8°N to 12°N and 38°E to 43°E that drains the watershed of the Awash River. All 16 irrigation farms are located on the Awash flood plains, at altitudes between 350 and 1,540 m. These farms were expanded during recent years and in 1976 they ranged in size from 250 to 10,000 ha (Fig. 1); the total irrigated area was 58,500 ha. Most farms in the upper section of the valley have reached their maximum size, but those in the middle valley and lower plains are still being expanded and new ones are being planned.[4,5] Fifteen of the 16 schemes studied obtain all their irrigation water from the Awash, only Awara Melka depending on the Kessem River.

The Awash Valley study area was divided into three parts to reflect altitudinal and corresponding climatic variations. The upper valley has a semi-arid, warm upland climate, the middle valley and lower plains have hot steppe and desert climates. The farms in the upper valley are located at altitudes between 1,540 m (Wonji) and 960 m (Metahara). Corresponding mean annual temperatures at these two farms are 20.7 and 24.9°C and annual rainfall 122 and 61 cm, respectively. The irrigation systems in the middle valley are located between 750 m (Melka Sadi) and 620 m

Accepted 5 March 1977.

* Present address: 2307 N. Backer, Fresno, California 93703.

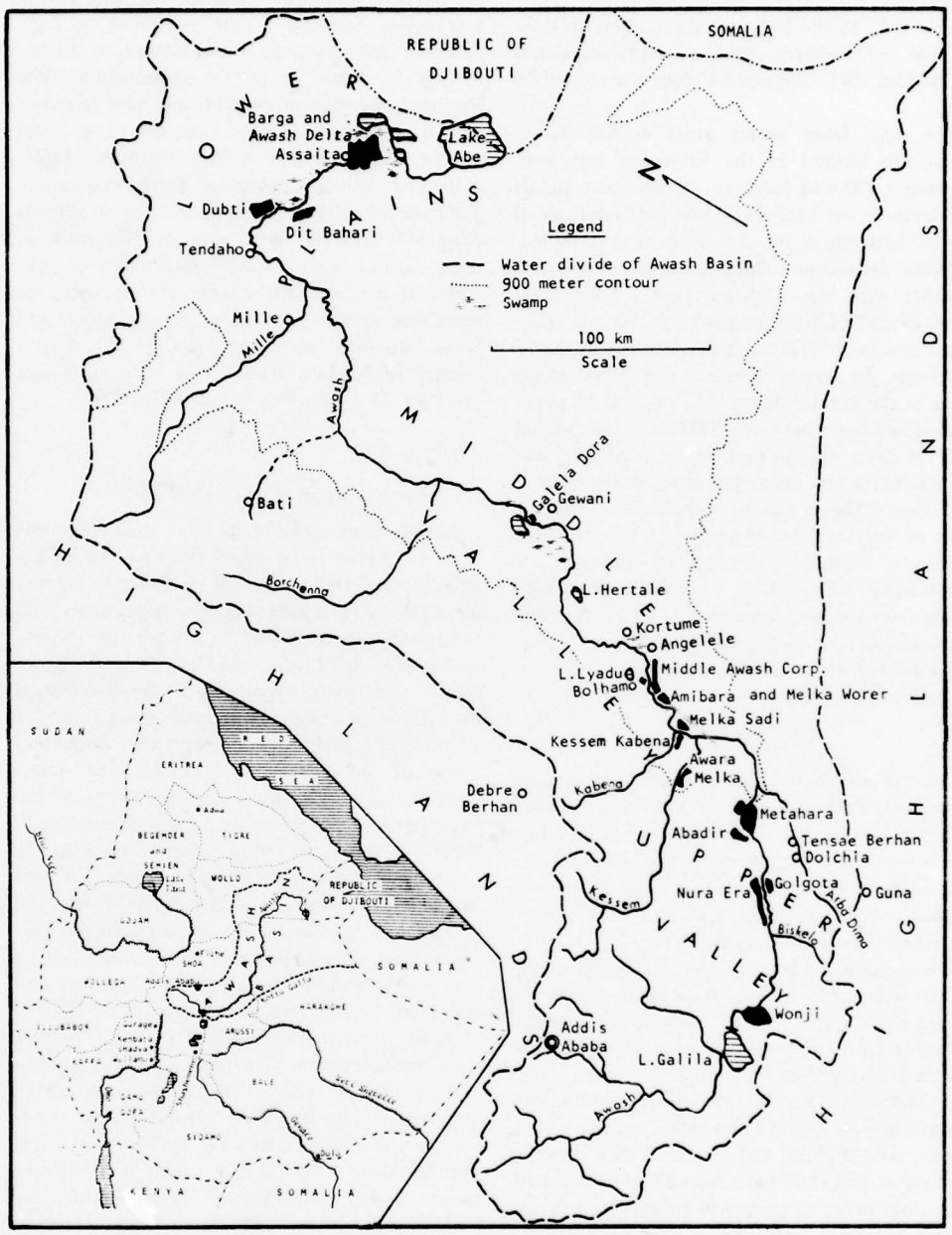


FIGURE 1. Location of the irrigation farms, towns, and villages studied in the Awash Basin, and of villages, towns, and labor source areas in the Ethiopian Highlands.

(Galela Dora). Corresponding mean annual temperatures are 25.9 and 27.0°C and rainfall is 57 and 25 cm. In the lower plains, located between 380 and 350 m altitude, mean annual temperatures are around 29°C and rainfall does not exceed 20 cm.

The main labor source areas of the Awash farms are located in the Ethiopian highlands, between 1,400 and 3,000 m, where mean annual temperatures are between 25 and 13°C and annual rainfall between 75 and 200 cm in most localities.

Farm populations have gradually increased, together with the irrigated areas. There were approximately 150,000 people in the Awash irrigation farms in 1976, nearly twice as many as during the 1960s. In 1976 between 800 and 32,000 people lived in the various farms, but most farm populations numbered between 3,000 and 6,000. About 75% of farm people were migrant laborers and their families who originated in the overpopulated highlands. The remaining people were children born of migrant laborer parents in the irrigation farms, or indigenous subsistence farmers and pastoralists. About 60% of the total population in the schemes were permanent residents and 40% seasonal laborers, mostly cotton pickers. Increasing numbers of indigenous pastoralists are now settling in these farms.[5]

METHODS

The epidemiological data were obtained between December 1972 and August 1973 and between October 1975 and July 1976. All large irrigation farms in the Awash Valley were included to better assess the effects of various physical environmental and epidemiological factors on schistosomiasis and host snail occurrence.

Ten percent random surveys of the total populations were made in all labor camps of nine farms (Nura Era, Golgota, Abadir, Melka Sadi, Amibara, Awara Melka, Kessem Kabena, Bolhamo and Galela Dora) and in some labor camps in Wonji and Metahara (Fig. 1). In the remaining five farms (Middle Awash Corporation, Melka Worer, Dubti, Dit Bahari and Barga), only laborer, pastoralist, and child volunteers could be included.

A total of 2,782 people in irrigated areas and 910 in the labor source areas were examined for *S. mansoni* and 2,205 people in the farms and near swamps, as well as 183 in the labor source areas for *S. haematobium*. Ten ml of urine and 2 g of stool were collected from persons randomly selected in the farms the morning after distribution of specimen containers, and on the spot among volunteers. Specimens were preserved in 7.5% formalin and taken to the Institute of Pathobiology in Addis Ababa for examination. The Ritchie concentration method[6] was used to examine all specimens. Urines were centrifuged and sediment was examined without filtration. Information on age, sex, place of birth, occupation, and duration of stay was obtained from all persons examined. Monthly snail surveys were made in most farms where parasitological material was collected, and one-time surveys were made in the remaining farms and in some swamps, rivers and lakes. Bulinine snails were sent to the British Council of Medical Research in Kisumu, Kenya (to Prof. D. S. Brown) for identification.

RESULTS

Prevalence of S. mansoni *infections*

Results of examination of 2,782 stool specimens from 15 migrant farm populations and 10 indigenous groups are summarized in Table 1. Overall infection rates among migrant populations and indigenous peoples were 9% in the upper valley, 2% in the middle valley and 11.3% in the lower plains. Of greater importance is the distribution of infections among indigenous groups, all of whom live around the farms where they frequently come into contact with canal and reservoir water. These people ordinarily do not move among the three parts of the valley or outside of the Awash Basin. In the upper valley, infection rates among these people were almost twice as high as among migrant farm populations, but in the middle and lower parts of the valley no infected samples were found. The highest rate of infection (33.4%) was among indigenous subsistence farmers who frequently come into contact with the Wonji irrigation system in the upper valley. The highest rates among migrant farm populations occurred in the same sugar cane estate (11.3%) and among children of migrant labor populations in Dubti (12.6%) and Dit Bahari (15.2%) cotton farms and in Assaita town (16.3%), all in the lower plains. Lowest rates among migrant populations were recorded in the middle valley farms. Whereas in the upper valley nearly all cases were children and young adults who either had been born in and around the farms or who had lived there for several years, in the middle valley only one and in

TABLE 1

Schistosomiasis mansoni in migrant farm populations and indigenous peoples in and around the Awash irrigation schemes

Farm surveyed	Sample	Male		Female		Total	
		No. exam.	% pos.	No. exam.	% pos.	No. exam.	% pos.
Upper valley							
1. Wonji	Migrant labor popul.	180	14.4	111	6.3	291	11.3
	Indigenous farmers	44	43.2	12	0	56	33.9
2. Nura Era	Migrant labor popul.	163	6.1	89	6.7	252	6.3
	Indigenous pastoralists	47	17.0	41	12.2	88	14.8
3. Golgota	Migrant labor popul.	146	5.5	50	4.0	196	5.1
4. Abadir	Migrant labor popul.	186	8.6	78	5.1	264	7.6
	Indigenous pastoralists	22	22.7	26	7.7	48	14.6
5. Metahara	Migrant labor popul.	162	9.3	106	1.9	268	6.3
	Indigenous pastoralists	25	4.0	28	3.6	53	3.8
	TOTAL	975	11.1	541	5.4	1,516	9.0
	Total migrant labor popul.	837	9.1	434	4.8	1,271	7.4
	Total indigenous popul.	138	15.1	107	7.5	245	13.2
Middle valley							
1. Melka Sadi	Migrant labor popul.	100	2.0	92	2.2	192	2.1
	Indigenous pastoralists	22	0	6	0	28	0
2. Awara Melka	Migrant labor popul.	171	0.6	61	4.9	232	1.7
	Indigenous pastoralists	24	0	5	0	29	0
3. Kessem Kabena	Migrant labor popul.	59	1.7	19	5.3	78	2.6
	Indigenous pastoralists	6	0	2	0	8	0
4. Amibara	Migrant labor popul.	114	3.5	47	4.3	161	3.7
	Indigenous pastoralists	12	0	7	0	19	0
5. Middle Awash Corp.	Migrant labor popul.	98	1.0	35	0	133	<1.0
6. Bolhamo	Migrant labor popul.	22	4.5	19	0	41	2.4
7. Galela Dora	Migrant labor popul.	26	7.7	4	0	30	6.7
	Indigenous settlers	29	0	12	0	41	0
	TOTAL	683	1.8	309	2.6	992	2.0
	Total migrant labor popul.	590	2.0	277	2.9	867	2.3
	Total indigenous popul.	93	0	32	0	125	0
Lower plains							
1. Dubti	Migrant labor popul.	15	0	0	0	15	0
	School children of the migrant labor popul.	79	17.7	72	6.9	151	12.6
2. Dit Bahari	School children of the migrant labor popul.	13	38.5	20	0	33	15.2
3. Assaita	School children of the migrant labor popul.	34	20.6	9	0	43	16.3
	School children of the indigenous pastoralists	29	0	3	0	32	0
	TOTAL	170	15.3	104	4.8	274	11.3
	Total migrant labor popul.	141	18.3	101	5.0	242	9.7
	Total indigenous popul.	29	0	3	0	32	0
GRAND TOTAL		1,828	8.0	954	4.9	2,782	6.8

TABLE 2

Schistosomiasis mansoni in school and farm children aged 6 to 16 years in labor source areas of the Awash irrigation farms

Area or locality surveyed, by province	No. of settlements surveyed	Altitude of settlement (m)	No. of people examined	% positive
Shoa				
Kembata/ Hadya area	6	1,900–2,800	316	1.3
Gurage area	2	2,100 & 2,500	33	0
Central Shoa	4	2,200–3,000	207	0.5
Sidamo				
Wollamo area	2	1,900 & 2,000	27	0
Wollo				
Bati	1	1,700	50	72.0
Hararghe				
Kottu Galla area	2	1,400 & 1,800	68	1.5
Arussi				
Tensae Berhan	1	1,600	143	59.1
Dolchia	1	1,800	31	45.2
Guna	1	2,800	35	0
Total	20		910	

TABLE 3

Altitude, mean annual air temperatures, mean maximum water temperatures, and B. pfeifferi *density in the Awash irrigation farms*

Farm	Altitude	Mean annual air temp. (°C)	Mean maximum water temp. (°C)	*B. pfeifferi* density*
Upper valley				
Wonji	1,540	20.7	24.2	1.5
Nura Era	1,100	23.6	25.2	3.3
Golgota	1,100	23.6	No data	0.9
Abadir	960	24.9	26.2	0.7
Metahara	960	24.9	26.8	1.1
Middle valley				
Melka Sadi	740	25.9	27.9	0.4
Awara Melka	750	25.9	28.0	0
Amibara	735	25.9	28.0	0.1
Middle Awash Corp.	730	26.0	No data	Negligible
Kessem Kabena	740	25.9	No data	0
Bolhamo	730	26.0	No data	0
Galela Dora	620	27.0	No data	0
Lower plains				
Dubti	380	29.0	31.2	0
Dit Bahari	360	29.1	31.5	0
Barga	350	29.1	No data	0

* Figures are mean monthly density expressed as the number of snails collected by 1 man per 1 minute of sampling.

the lower plains none of the infected persons had been born locally. All 31 cases in Dubti, Dit Bahari and Assaita appear to have originated in the labor source areas in the highlands of Wollo, Tigre and Eritrea provinces, as determined from place of birth and travel histories.

Results of examination of stools from 910 children in villages and towns in the main labor source areas are summarized in Table 2, showing that schistosomiasis mansoni is absent or nearly absent from 17 of the 20 communities surveyed. Of particular interest are the low infection rates in the Kembata Hadya, Wollamo, Gurage and central Shoa areas (0–1.3%), which together supply about 80% of all farm laborers in the upper and middle Awash Valley, and the high rates in Bati (72.0%), the center of an area that supplies most laborers in the lower plains farms.

Survey results from these highland areas were confirmed by schistosomiasis prevalence data for hospital patients in the Sudan Interior Mission hospitals in Sodo (Wollamo area) and Shashemene (southern Ethiopia lakes area), the Belessa Mission Hospital in Hosanna (Kembata Hadya area), the Attat Mission Hospital near Endeber (Gurage area), the Health Center in Fiche and Government Hospital in Debre Berhan (central Shoa Province), the Dejazmach Balcha Hospital in Addis Ababa and the Health Center in Tensae Berhen (Arussi Province). Only in the Tensae Berhan Health Center were more than 1% of the in- and outpatients diagnosed as positive for schistosomiasis mansoni in 1974 and 1975.

Snail surveys. In 1976 permanent *Biomphalaria pfeifferi* populations were found in all five farms in the upper valley from which parasitological data had been obtained (Wonji, Nura Era, Golgota, Abadir and Metahara) and in one farm in the middle valley (Melka Sadi). All other irrigation farms in the middle valley and those in the lower plains lacked this species, based on our surveys. In the Middle Awash Corporation farm, near the Melka Sadi banana plantation, only one small specimen of *B. pfeifferi* was found, in spite of intensive searches during the wet and dry seasons. *B. pfeifferi* is common in the smaller supply canals, in drains of all sizes and in reservoirs in Wonji, Nura Era, Golgota, Abadir and Metahara. It still, however, is confined to certain

TABLE 4

Schistosomiasis haematobium in migrant labor populations and indigenous pastoralists in and around the Awash irrigation farms

Farm surveyed	Sample	Males		Females		Total	
		No. exam.	% pos.	No. exam.	% pos.	No. exam.	% pos.
	Upper valley						
1. Golgota	Migrant labor popul.	82	1.2	36	0	118	0.8
2. Abadir	Indigenous pastoralists	27	0	34	0	61	0
3. Metahara	Migrant labor popul.	94	0	59	0	153	0
	Indigenous pastoralists	35	0	37	0	72	0
	TOTAL	238	0.4	166	0	404	0.2
	Middle valley						
1. Melka Sadi	Migrant labor popul.	136	1.4	124	0	260	0.8
2. Amibara/Melka Worer	Migrant labor popul.	382	0	91	0	473	0
3. Middle Awash Corp.	Migrant labor popul.	90	0	31	0	121	0
4. Kessem Kabena	Migrant labor popul.	55	1.8	14	0	69	0
5. Bolhamo	Migrant labor popul.	22	0	19	0	41	0
6. Galela Dora	Migrant labor popul.	30	23.3	5	0	35	20.0
	Indigenous pastoralists	46	19.6	21	42.9	67	26.9
	TOTAL	761	2.5	305	3.0	1,066	2.5
	Lower plains						
1. Dubti	Migrant labor popul.	120	0	6	0	126	0
	School children of the migrant labor popul.	76	0	88	0	164	0
2. Dit Bahari	Migrant labor popul.	135	0	0	0	135	0
	School children of the migrant labor popul.	20	0	39	0	59	0
3. Assaita town	School children of the migrant labor popul.	42	0	7	0	49	0
	School children of the indigenous pastoralists	79	5.1	5	0	84	4.8
4. Barga	Migrant labor popul.	118	0	0	0	118	0
	TOTAL	590	0.7	145	0	735	0.5
GRAND TOTAL		1,589	1.5	616	1.5	2,205	1.5

supply canals in Melka Sadi, where live specimens of this snail were found for the first time in November 1975.

Mean monthly *B. pfeifferi* densities recorded in the various farms during the 2-year study period decrease with decreasing altitude and increasing air and water temperatures (Table 3), in spite of considerable variations in irrigation practices, length of irrigation season and size of the farms studied. Mean maximum water temperatures in supply and drainage networks were 24.2°C in Wonji (1,540 m alt.), where 1.5 snails were recorded by 1 man per 1 min of sampling, and 33.9°C in Amibara (735 m), located adjacent to the Middle Awash Corporation farm, the place of the lowest altitude record of *B. pfeifferi* in the Awash Valley. Only at the Wonji farm is there a regular snail control program in this valley.

The lowest altitude location at which *B. pfeifferi* was found in a natural water body was the Biskelo stream in the upper valley, at 1,260 m. The freshwater lakes Lyadu and Hertale, the swamps in the middle and lower parts of the Awash Valley and Kessem, Kabena and Mille rivers in their lower reaches did not support this species. In streams and rivers at higher altitudes, however,

including the Bati stream at Bati, the Borchenna, the Kessem, the Arba Dima at Tensae Berhan, the Awash and most of its tributaries above Wonji, large *B. pfeifferi* populations were found toward the end of the dry season.

Prevalence of S. haematobium *infections*

Results of the examination of urine from 2,205 people in and around 13 irrigation farms are summarized in Table 4. As shown in this table, in all but one farm *S. haematobium* infection rates were below 1%. In 9 migrant labor populations and in 2 pastoral groups in the upper valley, no infections were found. Only in Galela Dora, a small Afar settlement farm located in the large swampy area in the middle valley, were high rates (20.0%) recorded among migrant farm laborers, as well as among indigenous Afar pastoralists (26.9%). In Assaita town, four of 84 local Afar school children but none of 49 school children of migrant laborer parents were infected. None of the 183 locally born children shed *S. haematobium* eggs in the villages of Mille (55 children) and Bati (46) in Wollo Province; Chacha (34) and Debre Tsegie (19) in central Shoa Province; and Miesso (29) in the Kottu Galla area.

Snail surveys. Only tetraploid *Bulinus (Bulinus) truncatus sericinus*, with 36 chromosomes, were found in the five farms for which bulinine snails could be identified (Nura Era, Golgota, Metahara, Melka Sadi and Amibara). *Bulinus* sp. also flourished in the canals in Wonji, Abadir, Dubti and Dit Bahari farms but their genetic status could not be determined. Live *B. (Physopsis) abyssincus* were obtained from Lake Lyadu and the swamps at Gewani, Kortume and Assaita and many empty shells were taken from the latter two localities.

Susceptibility of Bulinus (B.) truncatus/sericinus *to the local strain of* S. haematobium

Ten wild *B. (B.) truncatus/sericinus* (n = 36) from Metahara and 10 from Melka Sadi were individually exposed to miracidia of *S. haematobium* obtained from a patient who had lived around Gewani. None of the snails shed cercariae 23, 28, 33 and 48 days after exposure. A water temperature of 30°C was maintained in the aquaria.

DISCUSSION

Results of this study indicate that schistosomiasis prevalence has not increased markedly during the past 10 years in most Awash farms. Lemma[1] reported *S. mansoni* infection rates of between 1% and 5.2% among migrant laborers and school children in the lower plains cotton farms and in Assaita, but *S. haematobium* infections were only found in 3.5% of 144 people of various origin in Assaita and 40% (4 of 10) among local Afar tribesmen, and none among 104 migrant laborers in Dubti and 112 migrant laborers in Barga. In the middle valley, Russell reported 48% of 189 indigenous Afar pastoralists in the swamps near Gewani infected with *S. haematobium*,[7] and Lemma[1] reported rates between 41.2% and 67.9% in four swampy areas in the area between Gewani and the Middle Awash Corporation farm, all of them located away from irrigation systems. In the upper valley, 2.1% of 95 farm laborers in Abadir, 1% of 102 laborers in Metahara and 12.9% of 426 children and laborers in Wonji were infected with *S. mansoni*. The Wonji sample included 220 newly employed laborers (with a 1.4% infection rate) and 47 irrigation and canal workers (with a 60% rate).

The continuing absence of *B. pfeifferi*, the only intermediate host of *S. mansoni* in the Awash Basin, from the middle and lower Awash farms may be largely the result of high temperatures. The absence of this species from both African coasts, 10°N and 10°S at altitudes below 300 to 600 m, has been attributed to high temperatures.[8] Berrie found that *B. pfeifferi* was absent from habitats at 200 and 350 m on Tanzania's coastal plains where water temperatures reach 30°C during more than 4 months of the year, but that this snail was present in localities at 1,050 m, where temperatures are lower.[9] Several laboratory studies show that the critical temperatures for *B. pfeifferi* survival are between 28 and 30°C.[9] Mean maximum water temperatures in the canal systems in the middle valley are around these critical levels and even higher in the lower plains (Table 3). In Amibara, located adjacent to the Middle Awash Corporation farm, *B. pfeifferi* existed only briefly in 1973, and in a permanent reservoir in Melka Worer farm only a few empty shells were found.[3]

High silt load and water chemistry of the Awash and its tributaries in the middle and lower parts of the valley may also contribute to keeping *B. pfeifferi* out of the lowlands. Mean annual silt concentrations in the Awash are more than 25

times higher at Dubti than at Wonji and Metahara, owing to settlement of silt of the upper Awash in Lake Galila. Malek attributed the seasonal decline of *B. pfeifferi* in the Gezira cotton scheme in Sudan to corresponding silt increases in the Blue Nile,[10] which also originates in the Ethiopian highlands. Brown and Lemma considered that high turbidity of the middle and lower Awash contributed to keeping *B. pfeifferi* from spreading into the lowlands north of Metahara.[11] Kloos and Lemma found that the salinity and alkalinity of Awash water gradually increase downriver from Wonji.[2]

Intestinal schistosomiasis is endemic in Wonji, Abadir and Metahara farms, where *S. mansoni*-infected *B. pfeifferi* have repeatedly been found[12] (Wonji Medical Service, unpublished data). Presence of high infection rates in the indigenous populations near these three farms, whose inhabitants are in frequent contact with canal water, further suggests that transmission cycles have been established in these irrigation systems. In Nura Era, however, many of the infections among the indigenous pastoralists may have been acquired around Tensae Berhan in the nearby foothills, where they seasonally move with their livestock. The near absence of infections in the 6–15 age group in Nura Era and Golgota, in spite of intensive water contact by children from the camps, also suggests that schistosomiasis mansoni is not endemic in these two cotton farms. However, no mouse exposure studies could be made in the canals and the small number of *B. pfeifferi* (445) that were obtained from both farms and studied for cercarial shed (all negative) do not permit us to draw more definite conclusions.

The confinement of *B. pfeifferi* to several canals in the old sections of Melka Sadi, away from the labor camps, indicates that schistosomiasis mansoni has not yet become endemic in this farm. Empty *B. pfeiferri* shells were first found in 1973, 8 months after the start of irrigation,[2] and breeding populations were established in the canals in the old section of the farm in 1975. Rapid spread of *B. pfeifferi* in this banana plantation contrasts with its continued absence in the surrounding cotton schemes of Awara Melka, Kessem Kabena and Amibara.

Endemic schistosomiasis mansoni is unlikely to become widespread in the middle valley and lower plains farms, owing to the temperature and silt barriers, but imported schistosomiasis may become more prevalent in the middle valley with the arrival of larger numbers of farm laborers from eastern and northern Ethiopia. This may follow use of the newly constructed road between Tendaho and Metahara. Settlements in the eastern and northern Ethiopian highlands where *S. mansoni* infection rates above 30% have been found among school children include the towns of Bati and Adwa[13] and another 8 of 15 communities in Wollo, Tigre and Eritrea provinces,[14] and 8 of 13 villages in the Lake Tana area in Begemder and Semien Province.[15] In the main labor source areas in the central and southern Ethiopian highlands, schistosomiasis mansoni appears to be uncommon, probably due to the high altitude and low temperatures. The large flow of migrants from the Kembata/Hadya, Wollamo, Gurage, central Shoa and Kottu Galla areas (Table 2, Fig. 1) to the upper and middle parts of the valley tends to keep infection rates fairly low, even though *S. mansoni* transmission does occur in some of the farms.

Low schistosomiasis haematobium prevalence rates among farm populations are mainly due to the absence of appropriate intermediate hosts in the canal systems. *Bulinus (Physopsis) abyssinicus* has not colonized any canals in the Awash Valley, unlike in Somalia, where it is the sole transmitter of *S. haematobium* in irrigation farms.[16] That this species appears to be the only intermediate host of *S. haematobium* in the Awash Valley is suggested by the recovery of naturally infected *B. (Ph.) abyssinicus* from a swamp near Gewani, by laboratory infection of this snail with miracidia from a local Afar boy,[17] and by failure to infect *B. (B.) truncatus/sericinus* with this parasite during the present study. Widespread occurrence of *B. (B.) truncatus/sericinus* in the canal systems and low prevalence rates in the farm populations further indicate that this snail does not transmit *S. haematobium* in the Awash Valley, contrary to the suggestion made by Wu and Burch.[18] Of the other potential intermediate hosts of *S. haematobium* identified in Ethiopia—*B. (B.) tropicus*, *B. (Isidora) hexaploidus* and *B. (I.) octoploidus*[19,20]—only *B. (B.) tropicus* has been found in the vicinity of irrigation farms in the Awash Valley.[12,21] This species is nearly always insusceptible to *S. haematobium* in its area of distribution in eastern and southern Africa,[18] and Lo found

Ethiopian *B.* (*B.*) *tropicus* completely refractory to infection with an Egyptian strain of this parasite.[22] *B.* (*B.*) *tropicus* and tetraploid forms of *Bulinus* are, however, transmitters of *S. bovis* in Ethiopia, as is *B.* (*Ph.*) *abyssinicus*.[23]

Differences in sampling techniques between the randomly selected farm populations and pastoralists and children must also be expected to have influenced the results. The *S. haematobium* infection rates reported for the randomly selected migrant labor populations might have been higher had the late afternoon and evening urine samples been collected.

An important conclusion from this study is the observation that the planned extension of existing irrigation farms into and the construction of new agricultural schemes in the swamps near Gewani and Assaita will probably result in establishment of the *S. haematobium* transmission cycle if *B.* (*Ph.*) *abyssinicus* becomes adapted to the new canals and no control programs are instituted. *B. pfeifferi* and *S. mansoni* may become endemic in the middle valley and lower plains if the temperatures, silt concentrations and chemistry of Awash water are changed sufficiently by the two proposed high dams at Awara Melka and Tendaho for the survival of this host snail in these hot lowlands.

ACKNOWLEDGMENTS

The authors are indebted to Bahta Mazengia of the Institute of Pathobiology for examining all stools and urines, to Belete Kirub, Assefa Gebre, and Dr. Giuseppe DeSole for assistance during surveys, to Dr. Pierre Bonnemaison of UNDP for help with transportation and to Prof. D. S. Brown for identifying snails. Drs. Donald Heyneman and Fred L. Dunn of the G. W. Hooper Foundation, University of California, San Francisco, kindly read the manuscript and offered many helpful suggestions. The assistance of farm managers, government officials and the farm labor populations is gratefully acknowledged. This study was supported in part by USAID Grant 252800000.

REFERENCES

1. Lemma, A., 1969. Bilharziasis in the Awash Valley. I. An epidemiological study with special emphasis on its possible future economic and public health importance. *Ethiop. Med. J., 7:* 147–176.
2. Kloos, H., and Lemma, A., 1974. Bilharziasis in the Awash Valley. II. Molluscan fauna and agricultural development. *Ethiop. Med. J., 12:* 157–173.
3. Kloos, H., Polderman, A. M., DeSole, G., and Lemma, A., 1977. Schistosomiasis haematobium among seminomadic and agricultural Afar in Ethiopia. *Trop. Geogr. Med.* (In press.)
4. Awash Valley Authority, 1974. Proposed AVA development programme during the fourth five-year plan. Unpublished document, Addis Ababa.
5. Kloos, H., 1977. Schistosomiasis and irrigation in the Awash Valley of Ethiopia. *Unpublished Ph.D. dissertation, University of California, Davis.*
6. Ritchie, L. S., 1948. An ether sedimentation technique for routine stool examination. *Bull. U.S. Army Med. Dept., 8:* 444–448.
7. Russell, H. B., 1958. Final report, Pilot Mobile Health Team, Ethiopia. *WHO document EM/PHA/62.*
8. Sturrock, R. F., 1965. The development of irrigation and its influence on the transmission of bilharziasis in Tanganyika. *Bull. W.H.O., 32:* 225–236.
9. Berrie, A. D., 1970. Snail problems in African schistosomiasis. *Adv. Parasitol., 8:* 43–96.
10. Malek, E. A., 1958. Factors conditioning the habitat of bilharziasis intermediate hosts of the family Planorbidae. *Bull. W.H.O., 18:* 785–818.
11. Brown, D. S., and Lemma, A., 1970. The molluscan fauna of the Awash River, Ethiopia, in relation to the transmission of schistosomiasis. *Ann. Trop. Med. Parasitol., 64:* 533–538.
12. Aram, R. R., 1973. Epidemiological studies of schistosomiasis at the Metahara and Tendaho estates. Unpublished report. University of Sussex, Ecology Unit, Sussex, England.
13. Buck, A. A., Spruyt, D. J., Wade, M. K., Deressa, A., and Feyessa, E., 1965. Schistosomiasis in Adwa. *Ethiop. Med. J., 3:* 93–106.
14. McConnell, E., and Armstrong, J. C., 1976. Intestinal parasitism on the Central Ethiopian Plateau. *Ethiop. Med. J.* (In press.)
15. Polderman, A. M., 1974. Intestinal schistosomiasis north and west of Lake Tana, Ethiopia. *Trop. Geogr. Med., 26:* 170–177.
16. Arfaa, F., 1975. Studies on schistosomiasis in Somalia. *Am. J. Trop. Med. Hyg., 24:* 280–283.
17. Lo, C. T., 1970. Snail intermediate host of *Schistosoma haematobium* in Ethiopia. *OAU Proc. Symposium on Schistosomiasis,* Addis Ababa, 85–88.
18. Wu, S. K., and Burch, J. B., 1974. Experimental infection of *Bulinus sericinus* with *Schistosoma haematobium. Malacol. Rev., 7:* 5.
19. Burch, J. B., 1972. Names for two polyploid species of African *Bulinus* (Basommatophora: Planorbidae) in Ethiopia. *Malacol. Rev., 5:* 7–8.
20. Burch, J. B., 1973. Some current research on the genus *Bulinus. Malacol. Rev., 6:* 43.
21. Brown, D. S., and Wright, C. A., 1972. On a

polyploid complex of freshwater snails (Planorbidae: *Bulinus*) in Ethiopia. *J. Zool., 167:* 97–132.

22. Lo, C. T., 1972. Compatibility and host-parasite relationships between species of the genus *Bulinus* (Basommatophora: Planorbidae) and an Egyptian strain of *Schistosoma haematobium* (Trematoda: Digenea). *Malacologia, 11:* 225–280.

23. Lo, C. T., and Lemma, A., 1975. Studies on *Schistosoma bovis* in Ethiopia. *Ann. Trop. Med. Parasitol., 69:* 375–382.

Kloos, H, Lemma, A. 1977. *Ethiop. med. J.* 15,

BILHARZIASIS IN THE AWASH VALLEY
III. EPIDEMIOLOGICAL STUDIES IN THE NURA ERA, ABADIR, MELKA SADI AND AMIBARA IRRIGATION SCHEMES

Helmut Kloos, PhD [1] and Aklilu Lemma, ScD.

ABSTRACT. *The prevalence of* Schistosoma mansoni *infections in farm and pastoral populations and the occurrence of host snail populations in Nura Era, Abadir, Melka Sadi and Amibara irrigation schemes were described and compared. In Abadir, 7.6 percent of migrant farm labourers and their families but 14.6 percent of local pastoralists were infected. In Nura Era the rates were 6.3 percent to 14.8 percent, in Melka Sadi 2.1 percent and 0 percent, and in Amibara 3.7 percent and 0 percent. The difference in infection rates among these populations appears to be mainly due to variations in* B. pfeifferi *occurrence but further studies are needed to identify clearly the transmission sites and to determine transmission levels in individual farms. Although infection rates among farm populations are still fairly low, endemic intestinal schistosomiasis may spread into the rapidly expanding Amibara-Bolhamo irrigated areas. The fairly small, localized* B. pfeifferi *populations in farms may be controlled by spot application of molluscicides, but other control measures, including provision of potable and acceptable water supplies for the farm populations, chemotherapy, elimination of snail habitats by engineering measures, sanitary improvements, and health education should also be considered.*

During the previous study in this series (1) it was suggested that epidemiological studies be made in Nura Era, Abadir, Melka Sadi, and Amibara irrigation schemes in the Awash Valley, owing to the likelihood of *S. mansoni* transmission in those farms. Nura Era and Abadir are cotton farms located in the upper part of that valley, whereas Melka Sadi is a banana plantation and Amibara a cotton farm in the middle valley. This paper reports the prevalence of schistosomiasis mansoni and occurrence of *Biomphalaria pfeifferi* in these four irrigation schemes. The endemicity of infection in Wonji and Metahara sugar estates and absence of *S. mansoni* transmission in the middle and lower parts of the Awash Valley have been described elsewhere (2). *S. haematobium* is unlikely to be transmitted in the Awash Valley farms, owing to the failure of *Bulinus abyssinicus* to colonise the canals and the absence of other appropriate snail hosts (1,2).

Institute of Pathobiology, Addis Ababa University

[1] Present address: 2307 N Backer, Fresno, Ca 93703, USA.

MATERIALS AND METHODS

Ten percent random surveys were made of the total farm labour populations in Nura Era, Abadir, Melka Sadi, and Amibara (Figure 1) and non-random surveys of locally born pastoralists who come into contact with these irrigation systems. A total of 1,160 stool specimens was obtained from labour populations and 183 specimens from pastoralists. One gram of each specimen was examined using the Ritchie (4) concentration method. Monthly snail surveys were made between

November 1975 and July 1976 on a time-restricted basis using a dip net in the canal systems, as previously described (1). Samples of canal water were analysed for chemical composition and silt content and temperatures were recorded. No longitudinal studies of snail infection or mouse exposure to potentially infective canal water could be made and the samples of children were not large enough to permit definite statements on the endemicity of *S. mansoni* in individual farms. However, inclusion of data on locally born pastoralists in all farms can provide a measure of endemicity as these populations live in and around certain irrigation schemes where they are exposed to canal water.

RESULTS

S. mansoni infection rates among migrant farm populations were 7.6 percent (of 264 persons) in Abadir, 6.3 percent (of 252) in Nura Era, 2.1 percent (of 192) in Melka Sadi, and 3.7 percent (of 161) in Amibara. Among indigenous groups the rates were 14.8 percent among 88 Arussi Galla pastoralists at Nura Era, 14.6 percent among 48 Kereyu Galla at Abadir, none of 28 Afar at Melka Sadi, and none of 19 Afar at Amibara.

Nura Era and Abadir: Tables 1 and 2 show the age and sex distribution of infection and the age and sex structure of the farm populations in Nura Era and Abadir. These tables show higher rates

TABLE 1: *S. mansoni* infection rates, by age and sex, in Nura Era

Age	Males		Females	
	Number examined	Percent infected	Number examined	Percent infected
1 - 5	15	0	13	0
6 - 10	10	0	11	9.1
11 - 15	5	0	6	0
16 - 20	31	3.2	18	16.7
21 - 25	34	11.8	24	8.3
26 - 30	34	5.9	11	0
31 - 35	18	16.7	3	0
36 - 40	9	0	1	0
41+	7	0	2	0
Total	163	6.1	89	6.7

TABLE 2: *S. mansoni* infection rates in Abadir, by age and sex

Age	Males		Females	
	Number examined	Percent infected	Number examined	Percent infected
1 - 5	17	0	10	10.0
6 - 10	13	30.8	10	10.0
11 - 15	15	13.7	6	0
16 - 20	26	7.7	17	3.9
21 - 25	42	7.3	11	9.1
26 - 30	35	5.7	12	0
31 - 35	20	10.0	7	0
36 - 40	12	8.3	3	0
41+	6	0	1	0
Total	186	8.6	78	5.1

in males than females in Abadir, indicating that schistosomiasis mansoni is primarily an occupational infection on that scheme, and no markedly different rates between the sexes in Nura Era. The highest age-adjusted rates in Abadir were in boys aged 1 - 15 years and in Nura Era in females 16 - 20 years and in males 21 - 35 years. This difference may be due to the more intensive use of the large main canal and drain in Abadir than the smaller secondary canals in Nura Era by children (mainly boys) for swimming and bathing, as well as to the greater exposure of labourers in the *B. pfeifferi* infested field canals in the latter. The higher rate in females than males up to 20 years of age in Nura Era appears to be due to the intense water contact young females have with canals during laundering and water fetching for the household.

Definite distribution patterns of infection were discerned within farms. In Nura Era the highest rates (16.7 and 23.1 percent) were found in the large permanent labour camps 3 and 5, and no infections in eight of the 10 predominantly seasonal labour camps that house mostly cotton pickers, who work only three to four months of the year in this farm. All camp populations except one continued to obtain all their household water from canals in 1976. In Abadir, rates were highest in camp 4 (16.3 percent) and no infections were recorded in camp 1, thought to be primarily the result of the confinement of permanent *B. pfeifferi* populations and thus transmission sites to the main drain at camp 4 and their absence from the fast-flowing main canal on which camps 1, 2 and 3 are located (Figure 2). Only a few labourers in camp 1 came in contact with the small snail-infested canals in the fruit farm section. In 1976 the management began to provide artesian water (of a highly mineralised type) by tank truck and standpipe for most farm residents, causing a reduction in the amount of laundering, bathing, and water fetching in the canals around all five camps. However, some people rejected this warm deep-well water, claiming that it was unsuited for properly washing clothes, unfit for preparing *tella* (beer), and that it tasted bad, causing them to use canal water instead. The elimination of the small dams at camp 4, which resulted in the destruction of the snail populations, and the disappearance of the drainage swamp in the northwest corner of the farm after the excavation of new canals are considered to have decreased transmission levels.

Melka Sadi and Amibara: Unlike Nura Era and Abadir, no infected indigenous pastoralists were found in Melka Sadi and Amibara. Live *B. pfeifferi* were first discovered in November 1975 in Melka Sadi, three years after commencement of irrigation, although *Bulinus forskalii*, *Physa acuta*, and *Melanoides tuberculatus* had already invaded the canals by 1973. In 1976, breeding populations of *B. pfeifferi* were found at all sites where this snail occurred. *B. pfeifferi* was confined to several slow-flowing supply canals and ponded ditches in the old section of the farm and remained absent from the drainage system, although all canals contain water throughout the year. High salinity and alkalinity values, a result of the highly saline soil in Melka Sadi, appear to be the major factors involved (Table 3). Not only *B. pfeifferi* but all other snails commonly found in the Awash Valley schemes, except *M. tuberculatus*, were absent from the drainage canals, swamps, and lake.

B. pfeifferi habitats in Melka Sadi are still located away from the labour camps and villages, banana packing centres, and main water contact points (Figure 3), minimising the amount of water contact children and women have. The management is supplying all labour camps except Quissa with artesian water (also highly mineralised) by pipe and truck but amounts are too small and, as in Abadir, this water is not well accepted by the labour population. In Quissa, many people use fresh water from the snail-free wells they dug.

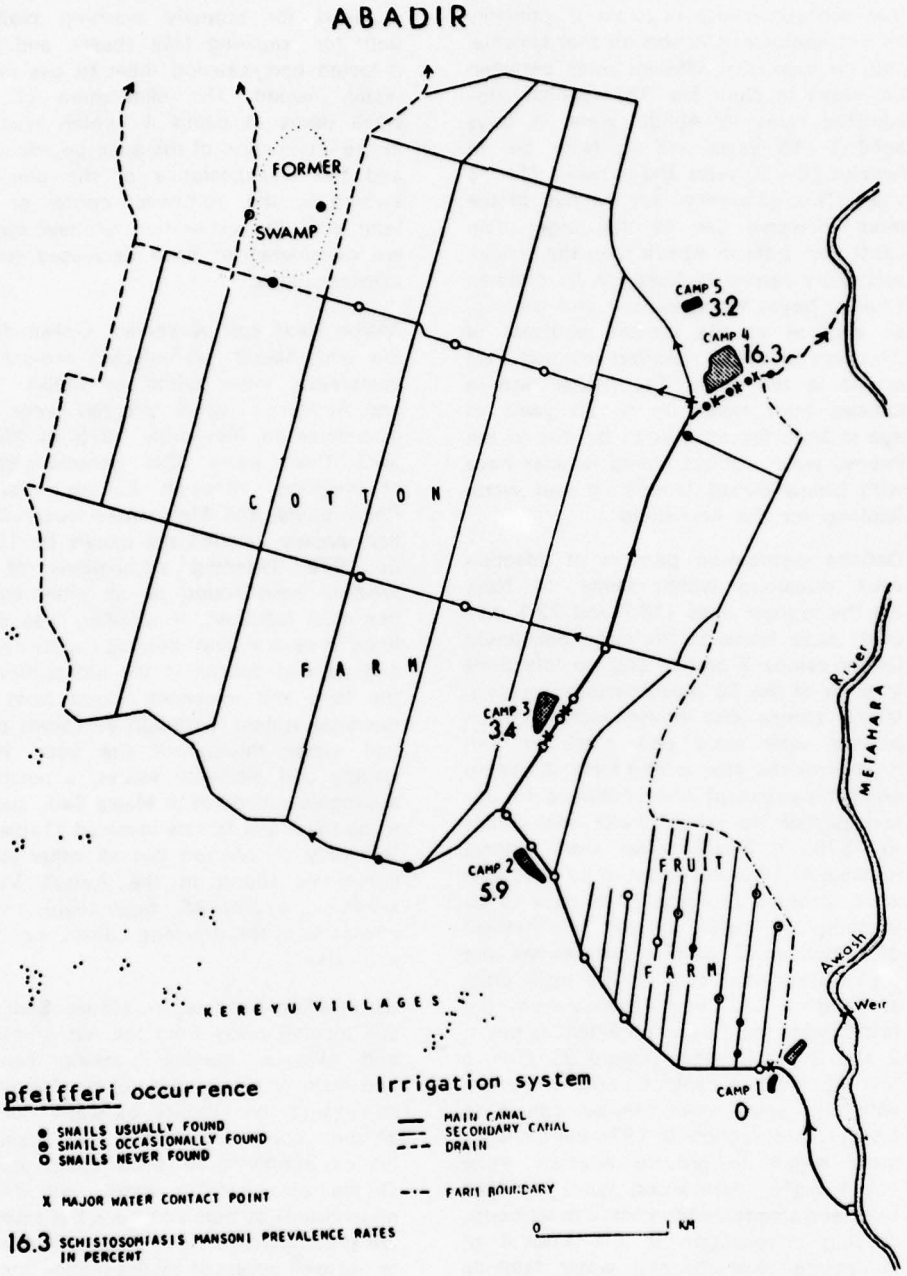


Figure 2. *S mansoni* infection rates, *B. pfeifferi* occurrence, and the major human water contact points in Abadir

TABLE 3: *B. pfeifferi*, salinity and alkalinity in supply canals, drains and drainage swamps and a lake in Melka Sadi

Type of habitat	No. of water samples taken	Salinity (conductivity in micromhos)	Alkalinity (pH)	*B. pfeifferi*
Supply canal	6	280 - 360	7.0 - 7.9	present
Drains at camps 1 and 2 and in north-east section of farm	4	364 - 1,280	7.8 - 8.6	absent
Drainage swamp outside of farm	1	940	9.1	absent
Drainage swamp inside farm	1	1,450	9.2	absent
Drainage lake	1	860	8.7	absent

Only six of the 161 (3.7 percent) migrant farm labourers and their families and none of the 19 Afar settlers studied in Amibara shed *S. mansoni* ova. *B. pfeifferi* was not found in the canal system. Most residents continue to obtain the well accepted (chlorinated) Awash water from the standpipes in the main village.

DISCUSSION

This study indicates that schistosomiasis mansoni is still relatively uncommon in the Awash Valley irrigation schemes although it is spreading into areas formerly free of it. The main depressants of *S. mansoni* transmission are probably high temperatures and silt load, seasonality of irrigation in the cotton schemes, and low infection rates in migrant labourers (4). Water chemistry in the drainage system in Melka Sadi suggests unsuitability for *B. pfeifferi*. It is not known, however, if the densely shaded banana groves in Melka Sadi prevent *B. pfeifferi* from invading the central sections of this scheme, although Brown (5) identified shade as a limiting factor in the distribution of *Biomphalaria* in forested areas of Ethiopia. The main factors contributing to the increase and spread of infection include the frequent contact farm populations (both labourers and their families) have with the canal systems, especially around the labour camps, the scarcity of potable and acceptable water supplies, poor sanitary conditions, and the high incidence of labour migrations. Few latrines exist in the labour camps and the nearby canals serve as sources of household water and places to wash laundry and to bathe. While efforts are being made in Abadir and Amibara to improve the living conditions of camp populations, much uncontrolled water contact and contamination continues in the fields, a problem that cannot be easily corrected in these hot lowlands without health education and improvements in the economic situation of labourers.

S. mansoni is currently transmitted in Wonji, Abadir, and Metahara farms, in all of which *S. mansoni* infected *B. pfeifferi* have been found repeatedly (2). The presence of high infection rates among indigenous pastoralists in Nura Era and Abadir also indicates that the parasite is being transmitted in these irrigation systems, especially since these population groups spend all their lives in the vicinity of the farms (their homelands), unlike the highland-born migrant farm labourers. The complete absence of infections in Afar (or Adal) pastoralists and of *B. pfeifferi* in the middle and lower Awash Valley irrigation schemes is evidence that lowlands below 1,000 m. or above the 26°C isotherm are still free of infection (2, 6). However, *B. pfeifferi* appears to be gradually spreading into the middle part of the valley, as reflected by its establishment in the canals in Melka Sadi (740 m.) and Ambash (730 m.), but the

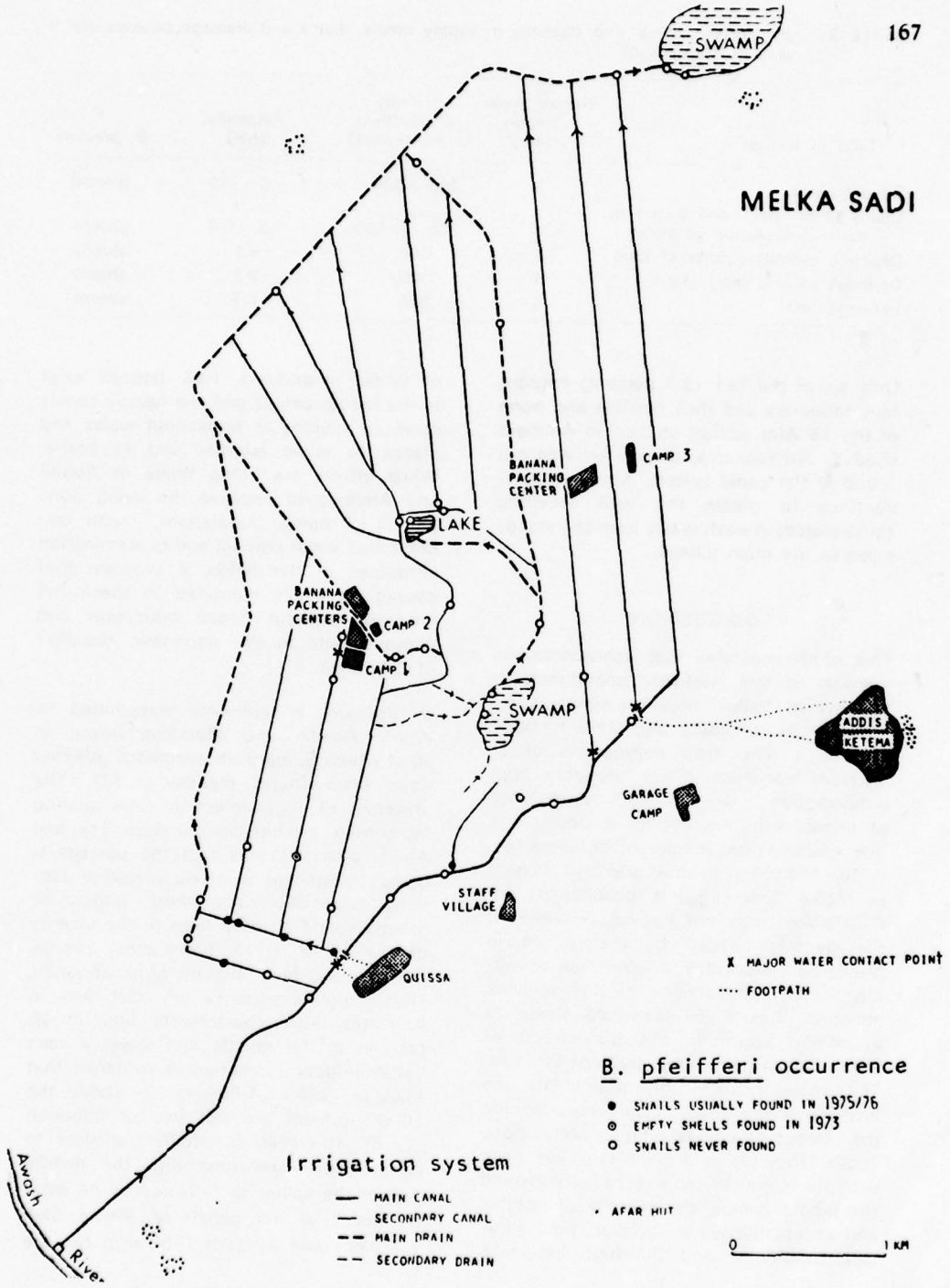


Figure 3. *B. pfeifferi* occurrence and the major human water contact points in Melka Sadi

continued absence of this snail from rivers, lakes and swamps in these lowlands. Year-round irrigation in Melka Sadi is more conducive to snail survival than the seasonal irrigations in the cotton farms, where most canals dry out during the off season (1, 2). *S. mansoni* is being introduced into the irrigation systems by farm labourers who originated in the upper valley schemes or in the endemic centres in various parts of Ethiopia. These migrations explain the presence of infected persons in Melka Sadi, Amibara and the other schemes in the Awash lowlands where no schistosome transmission occurs at present.

Longitudinal studies of snail occurrence and cercarial shed and annual surveys of prevalence and incidence of intestinal schistosomiasis at Wonji and Metahara (Dr. Bekele Tekle Haymanot, personal communication) should also be initiated in Melka Sadi, where transmission may soon commence. Snail surveillance studies should be continued in the surrounding cotton farms of Bolhamo, Kessem Kabena, Awash Melka and the Middle Awash Corporation, all of which are presently being expanded. The uneven distribution of infected people among labour camps and of *B. pfeifferi* in the canal systems suggests that there are still relatively few transmission sites in the individual farms, making local application of molluscicides a feasible control measure. Little attention has been given in the past to domestic water supply and use patterns in the farms, but this study shows that they are significant in the epidemiology of schistosomiasis mansoni. Well intended piped water supplies may be neglected if they do not meet the needs of the population, a problem identified elsewhere (7, 8) or, as in Wonji, may cause a high incidence of fluorosis (Wonji Medical Service, unpublished data).

Epidemiological studies in all 11 irrigation schemes in the middle and lower parts of the Awash Valley (Figure 1) suggest that while schistosomiasis mansoni is unlikely to spread in the near future beyond the temperature barrier in the Melka Sadi-Amibara area, vesicular schistosomiasis may become more prevalent in the swamps at Gewani and Assaita, where new irrigation schemes are to be developed (2).

The first author wants to thank the Director of the Institute of Pathobiology and other officials of Addis Ababa University for accommodation in that institute and for technical assistance. Mr. Belete Kirub, Dr. Giuseppe DeSole and Mr. Assefa Gebre generously assisted during the surveys and Mr. Bahta Mazengia examined the stools. The managements and medical personnel of Wonji, Nura Era, Metahara, Abadir, Melka Sadi and Amibara helped in many ways. Dr. Pierre Bonnemaison of UNDP helped with transportation and Mr. Firdu Zawide, Mr. Getachew Feleke and Mr. Samuel Mehari of the Awash Valley Authority made various data available. Professor Donald Hayneman of the Hooper Foundation, University of California, San Francisco, kindly read the manuscript and offered many constructive criticisms. This study was supported in part by a USAID grant.

REFERENCES

1. Kloos, H and Lemma, A. 1974. Bilharziasis in the Awash Valley. II Molluscan fauna in irrigation farms and agricultural development. *Ethiop. med. J.* 12, 157.
2. Kloos, H. and Lemma, A. 1977. Schistosomiasis in irrigation schemes in the Awash Valley, Ethiopia. *Amer. J. Trop. Med. Hyg.* 26, in press.
3. Kloos, H. 1977. Schistosomiasis and irrigation in the Awash Valley of Ethiopia. PhD dissertation, University of California. Davis.
4. Ritchie, LS. 1948. An ether sedimentation technique for routine stool examination. *Bull. US Army Med. Dept.* 8, 326.
5. Brown, DS. 1964. The distribution of intermediate hosts of Schistosoma in Ethiopia. *Ethiop. med. J.* 2, 250.
6. Brown, DS and Lemma, A. 1970. The molluscan fauna of the Awash River, Ethiopia, in relation to the transmission of schistosomiasis. *Ann. Trop. Med. Parasit.* 64, 533.
7. Husting, EL. 1970. Sociological patterns and their influence on the transmission of bilharzia. *Centr. Afr. J. Med.* 16, 5.
8. Pitchford, RJ. 1972. Aspects of bilharzia control. *Prevent,* 1 (No. 2), 37.

HAEMATOBIUM SCHISTOSOMIASIS AMONG SEMINOMADIC AND AGRICULTURAL AFAR IN ETHIOPIA

H. KLOOS, A. M. POLDERMAN, G. DESOLE AND AKLILU LEMMA
Institute of Pathobiology, University, Addis Ababa, Ethiopia

Received December 9th, 1976

Summary. Parasitological and malacological surveys were made in the Afar tribal area on the flood plains of the Awash River. *S. haematobium* – infections are most prevalent among seminomadic Afar living around the swamps and lakes in the middle part of the Awash Valley. Infection rates between 6 and 52% were found among seminomadic Afar and between 0 and 27% in agricultural groups. The highly localised distribution of vesical schistosomiasis is maintained by the distribution of the swamps, lakes and the human population and by migration patterns. On the marshy plain near Gewani significantly higher infection rates occur among Afar females than males. This is apparently due to sex differences in water contact patterns.

INTRODUCTION

A review of the schistosomiasis literature on Africa reveals a conspicuous underrepresentation of data about the occurrence of the disease among pastoral nomads and seminomads. From an epidemiological point of view this is regrettable, because high levels of transmission may occur at the scarce permanent water collections where humans, intermediate hosts and parasites are concentrated (e.g. Wright, 1970). From a health planning viewpoint, there is also a need for more information, as many national governments increasingly emphasize water development projects in subhumid areas and the settlement of pastoralists in irrigation farms.

This paper describes some aspects of the epidemiology of *S. haematobium* – infections among pastoral and agricultural Afar in the Awash Valley of Ethiopia (*Fig 1 and 2*). The main interest is on the geographical distribution of the parasite and its intermediate host and on sex differences in infection. Russell (1958) found *S. haematobium* infections of about 50% at Gewani; Lemma (1969) reported rates between 41 and 68% from Angelele, Kortume, Lake Hertale and Gewani and low rates from the Awash delta. Lo (1970) recovered *S. haematobium*-infected *B. abyssinicus* from swamps near Gewani and Brown and Lemma (1970) found specimens of *B. abyssinicus* in the swamp near Assaita. The Webi Shebelle and Genale river valleys in southern Ethiopia are the only other areas in this country from where *S. haematobium*-infections have been reported (Lemma *et al.*, 1976; Swedish Mission Clinic, Dolo, unpublished data).

MATERIALS AND METHODS

Epidemiological surveys in the irrigation farms and Afar villages between 1973 and 1976 are part of a longitudinal study of schistosomiasis in the Awash Valley (Kloos

Trop. geogr. Med., 29 (1977) 399–406

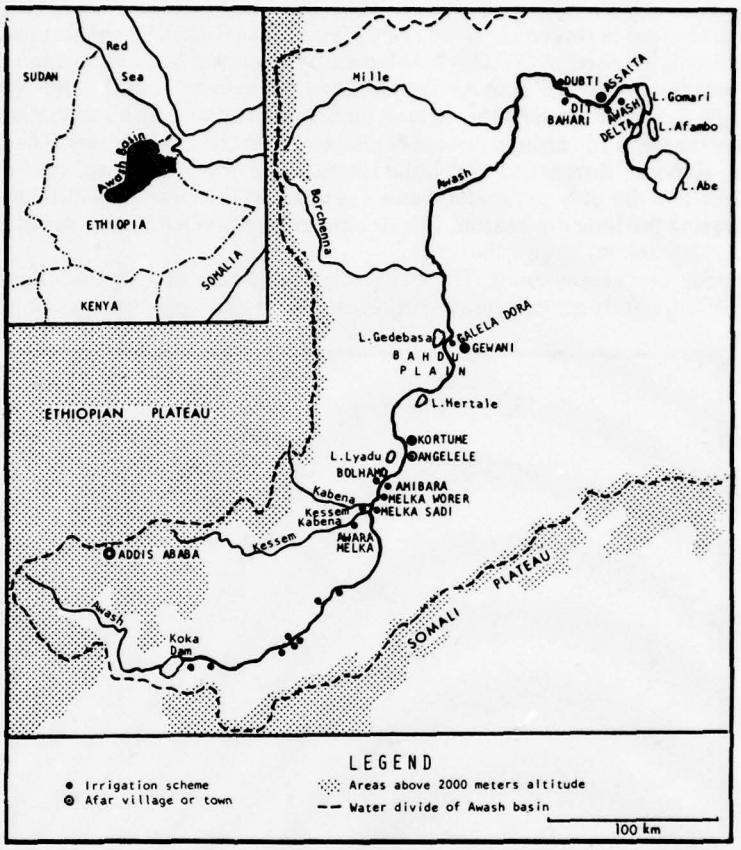


Fig. 1. The Awash Basin.

et al., in preparation). The absence of many Afar from their villages and the refusal of others to give urine specimens did not permit the use of random sampling methods. Only those people were included who could be persuaded to give specimens. In some villages locally born 1st through 4th grade students were also examined.

About 5 ml of urine was collected from each person and preserved in 7.5% formalin, centrifuged in the laboratory and examined. Information on age, sex, tribe, occupation, place of birth and duration of stay was obtained during interviews. Medical records of the Ethio-Swiss Hospital in Gewani were reviewed.

Snail surveys were made in all localities where parasitological data could be collected. A hand dip net was used. All collection sites were visited at least twice and those in the middle valley between 3 and 10 times.

The description of Afar culture and water contact patterns is based on interviews conducted with interpreters and on personal observations in the field.

STUDY AREA AND PEOPLE

The area covered in this study is the flood plains in the Awash Valley basin, the most densily populated parts of the Afar tribal territory (*Fig 1 and 2*). The elevation of these plains decreases from 760 m at Awara Melka to 300 m in the Awash delta. A hot and arid tropical climate prevails throughout these lowlands, with mean annual temperatures ranging from 26° around Awara Melka to 29° in the lower plains. The Awash, Kessem, Kabena, Borchenna and Mille rivers, all of which originate in the humid highlands, are the only perennial water courses and the swamps and lakes shrink in size during the long dry season. The swamps are maintained by the seasonal floods of the Awash and its large tributaries.

The Afar are seminomads. Until the commencement of irrigation agriculture in the 1960's, nearly all Afar moved seasonally with their livestock – camels, cattle,

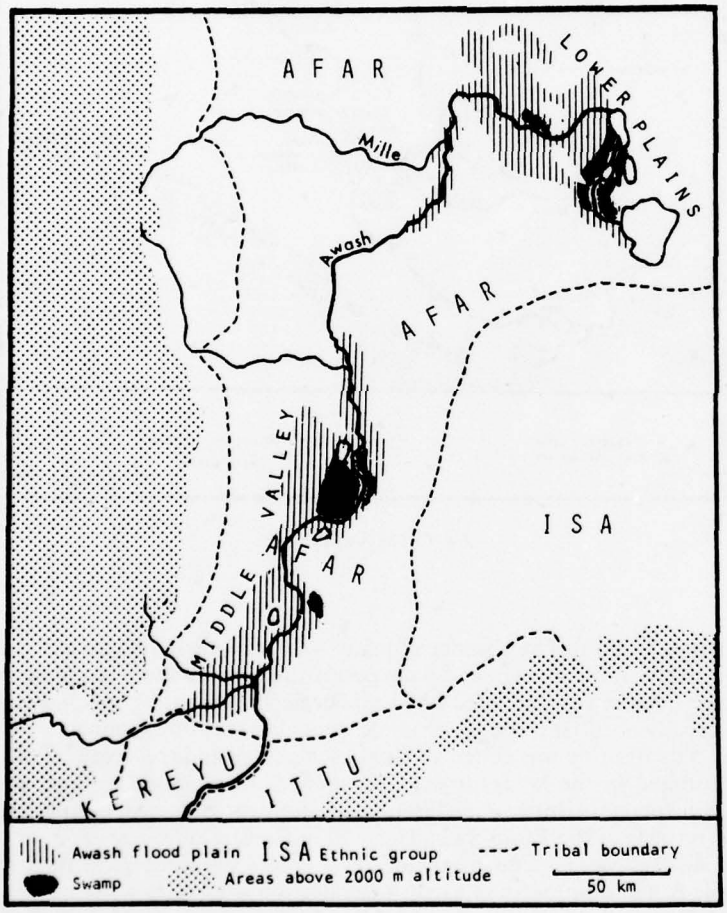


Fig. 2. The middle and lower parts of the Awash Valley: Physical features and tribal distribution.

sheep and goats – between the permanent grazing areas near the Awash and the rocky areas along the edge of the flood plains, to escape the seasonal floods and mosquitoes. Only in the Awash delta have some Afar traditionally cultivated crops (maize and cotton). The flood plains are now rapidly being developed to irrigation farming. In 1976 19 schemes covered nearly 60,000 hectares of the most fertile land in the valley (*Fig. 1*).

About 75,000 Afar live on the flood plains, concentrated mainly on the Bahdu Plain and in the Awash delta. The other pastoral groups in the Awash basin, including the Isa, Ittu and Kereyu, do not come into contact with the swamps (*Fig. 2*). Approximately 50,000 migrant labourers from the Ethiopian highlands are working in the expanding cotton and banana plantations. Pastoral Afar are gradually settling in and around these farms to work there.

RESULTS

Geographical distribution of S. haematobium infections in man

A highly uneven distribution of *S. haematobium* infections was noted in the study area (*Table I Fig 1 and 2*). Higher infection rates (14–52%) were found in the populations inhabiting the swamp and lake area of the middle valley than those in the farming areas in the middle valley and lower plains (0–16%). At the Ethio-Swiss Hospital in Gewani 177 of 445 (39.8%) Afar females but only 95 of 733 (13.0%) males were found to be infected. Our surveys on the nearby Bahdu Plain and Galela Dora farm showed 21 out of 56 (34.4%) females and 16 out of 145 (11.0%) males to be infected. Lake Lyadu is the only other area where marked sex differences in infection were observed. The only non-Afar group with high *S. haematobium* infection rates were migrant farm labourers in the Galela Dora maize farm on the Bahdu Plain.

Geographical distribution of the intermediate host

Live specimens of *Bulinus abyssinicus* were obtained from the swamps at Gewani, Kortume, Assaita and from Lake Lyadu. The lakes in the lower plains could not be surveyed for snails and searches for *B. abyssinicus* in Lake Hertale proved negative. The *Bulinus* sp. in the canals of Melka Sadi and Amibara belong to the tetraploid (n = 36) *truncatus* group. *Bulinus forskalii* exists in all large swamps and in the canals of most farms. Only *Melanoides tuberculatus* was found in the Awash and its tributaries in the lowlands and no snails at all in the saltwater lakes, the numerous hot springs and the deep wells of the Afar. The results of the malacological surveys in the irrigation farms were published earlier (Kloos and Lemma, 1974).

DISCUSSION

S. haematobium appears to be transmitted only in the swamps and freshwater lakes in the Awash basin unlike in Somalia, where the intermediate host, *B. abyssinicus*, exists also in irrigation canals (Arfaa, 1975). *B. truncatus* and *B. forskalii*, both widespread in the Awash farms, lakes and swamps, are insusceptible to infection with Ethiopian strains of *S. haematobium* (Burch, 1973; Brown and Wright, 1972; Lo, 1972). High population density, the presence of large permanent swamps and the

TABLE I

S. HAEMATOBIUM INFECTIONS IN SEMINOMADIC AND AGRICULTURAL AFAR AND SOME MIGRANT LABOURERS

Locality	*age in years distribution (in %)*			*urine examinations positive*		
	2–15	*16–30*	*>30*	*total (%)*	*males*	*females*
1. *Farming area in the middle valley*						
Bolhamo (N)	46	33	21	(6.1)	2/33	0/0
Melka Sadi (A)	15	30	55	(3.6)	3/83	0/0
Amibara (A)	32	37	31	(0)	0/7	0/10
Melka Worer (N, A)	9	65	26	(2.7)	1/31	0/6
Melka Worer (S)	92	8	0	(0)	0/26	0/0
2. *Swamp and lake area in the middle valley*						
Lake Lyadu area (N)	44	28	28	(23.0)	0/24	9/15
Angelele (N)	100	0	0	(14.3)	1/7	0/0
Kortume (N)	30	34	36	(52.0)	14/29	12/21
Lake Hertale area (N)	32	50	18	(31.8)	6/18	1/4
Gewani area:						
Ethio-Swiss Hospital (N)	not known			(23.1)	95/733	177/445
Bahdu Plain (N)	47	31	22	(14.2)	7/99	12/35
Galela Dora farm (N, A)	48	36	16	(26.9)	9/46	9/21
Galela Dora farm (H)	27	73	10	(20.0)	7/31	0/4
3. *Lower plains*						
Assaita, clinic (N)	33	31	36	(15.8)	3/19	0/0
Assaita, school (S)	89	9	2	(1.5)	1/60	0/5
Awash delta (N, F)	31	37	32	(2.4)	6/202	0/46

Legend: (N) Afar seminomads; (F) Afar subsistence farmers; (A) Afar agricultural labourers and settlers; (S) Afar students; (H) Migrant farm labourers from the highlands. Urine examinations "positive" indicate the numbers of urines containing *S. haematobium*-eggs and the numbers of urines examined, respectively and the total percent of positives.

water use patterns of the Afar are major factors in the high schistosomiasis prevalence on the Bahdu Plain. About 10,000 Afar live most of the year on this fertile plain, depending on the swamps and lakes for some of their domestic and livestock water needs and for the collection of some food plants. The Afar in the Awash delta, by contrast, use mostly deep wells, irrigation canals and the Awash.

The seasonal migrations of the Afar contribute to maintaining the clustered distribution of schistosomiasis and are unlikely to result in much spread of the parasite between the middle valley and the lower plains, because most tribesmen seasonally move in a direction nearly perpendicular to the Awash and they seldom visit areas far downstream or upstream from their permanent villages. Other factors are the desert that separates the middle valley from the lower plains and the Afar tribal division into two branches, the Adoimara in the former area and the Asaimara in the latter. The presence of six young children among the infected Afar in the lower plains and the common use of the *B. abyssinicus*-infested swamp near Assaita further indicate that most *S. haematobium* infections in this area are autochthonous. The few infections among adult labourers in the middle valley, however, were probably imported from the swamp and lake area.

Fig. 3. Afar reed huts made of *gededa* (*Typha sp.*) along the Awash River.

The observed sex differences in infection are mainly due to the Afar division of work and the use of three swamp plants. Only females collect the water lily *fura* (*Nymphea coerulea*), valued as a specialty food by Afar men, the unidentified grass *gedleboyta*, the sweet roots of which are eaten during famines, and the cattail *gededa* (*Typha* sp.), used for mats that cover the Afar huts (*Fig. 3*). The potato-like tuber of the water lily (*fura*) and the small seeds of its flower pod (*buri*) (*Fig. 4 and 5*) are collected mainly on the Bahdu Plain and to a lesser extent in the other localities near swamps and lakes. Few Afar living around the irrigation farms use *fura* and the tribesmen in Amibara, Melka Worer and Melka Sadi no longer eat this food. Only females collect the water plants. During this activity adolescent girls and adult women may stand as long as half a day in waist-deep water. Afar men have relatively little contact with the swamps and lakes. Their daily activities centre around scouting for pasture and guarding livestock and villages from enemies and wild animals.

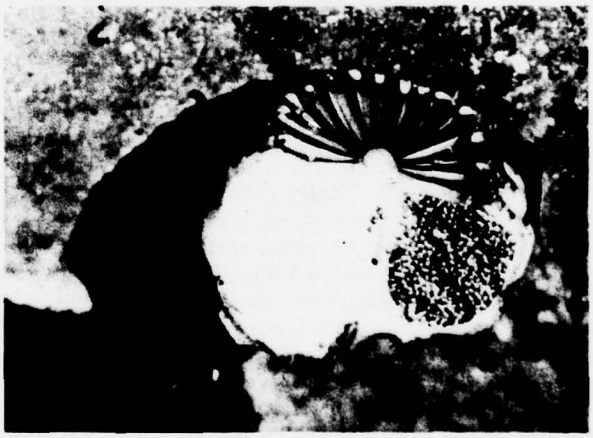


Fig. 4. Cut-open section of the flower pod *buri* (*Nymphea coerulea.*).

Fig. 5. A swamp on the Bahdu Plain containing many water lilies (*Nymphea coerulea*) ready for collection.

Whereas children above three years of age herd small animals (goats and sheep) in the vicinity of villages, men herd cattle and camels considerable distances away from the settlements, spending days and even weeks in the uplands. Fishing, another form of water contact usually resulting in high infection rates in endemic areas (Farooq *et al.*, 1966; Roundy, 1971), is not practiced by the Afar, who do not eat fish. This food habit is widespread among pastoral peoples in Africa (Simoons, 1974). The main types of water contact Afar men have with potentially infective water are religious ablution and laundering of clothes during periods when the Awash water is too silty. Laundering is done by both sexes, unlike in the Ethiopian highlands, where mainly women do this work (Polderman, 1975).

The regulation of the Awash flow through the construction of Koka Dam and irrigation schemes reduced the seasonal floods, the number and size of the swamps and apparently the incidence of *S. haematobium*-infection. Lemma (1969) found higher prevalence rates in all localities in the swamp and lake area in the middle valley than those reported here, although the same examination techniques were used, by the same technician. If the Afar continue to change their pastoral way of life to farming and if the Awash plains are further developed, according to the plans of the new Ethiopian government (Awash Valley Authority, 1974), then the incidence of *S. haematobium*-infections in the marshy areas is likely to decrease. However, the disease may become endemic in the irrigation farms if *B. abyssinicus* establishes itself in the canals. The spread of *B. pfeifferi* snails into the Afar lands at Melka Sadi and Amibara (Kloos and Lemma, 1974; Kloos *et al.*, in preparation) may set up *S. mansoni* transmission cycles in these farms, because this parasite is continuously being introduced by migrant labourers from the Ethiopian highlands, where it is common in many localities (Polderman, 1974; McConnell and Armstrong, 1976). In such an event intestinal schistosomiasis would become a new disease of the Afar, who have been free of it up till now. River basin development may affect schistosomiasis occurrence in many other pastoral groups in Africa, a subject about which little is known.

The authors are indebted to Dr. Hans Aebersold for permission to review clinical records at the Ethio-Swiss Hospital in Gewani, to Dr. Pierre Bonnemaison of UNDP for assistance with transportation

and to Dr. D. S. Brown for the identification of snails. Ato Bahta Mazengia of the Institute of Pathobiology kindly examined all urine specimens. This study was supported in part by a USAID grant.

REFERENCES

Arfaa, F., *Amer. J. trop. Med. Hyg.*, 24 (1975) 280.
Awash Valley Authority. Master Plan for the Awash Valley. Unpublished, Addis Ababa (1974).
Brown, D. S. and Wright, C. A., *J. Zool.*, 167 (1972) 197.
Burch, J. B., *Malac. Rev.*, 6 (1973) 43.
Farooq, M. *et al.*, *Bull. Wld. Hlth. Org.*, 35 (1966) 293.
Kloos, H. and Lemma, A., *Eth. med. J.*, 21 (1974) 157.
Lemma, A., *Eth. med. J.*, 7 (1969) 147.
Lemma, A. *et al.* Preliminary Report on Schistosomiasis in the Lower Webi Shebelle Valley. Institute of Pathobiology, unpublished. Addis Ababa (1976).
Lo, C. T., *Symposium on Schistosomiasis*, OAU, Addis Ababa (1970), pp. 85–88.
Lo, C. T., *Malacologia*, 11 (1972) 225.
McConnell, E. and Armstrong, J. C., *Eth. med. J.*, 23 (1976) in press.
Polderman, A. M., *Trop. geogr. Med.*, 26 (1974) 170.
Polderman, A. M. *The Transmission of Intestinal Schistosomiasis in Begemder Provincæ, Ethiopia. Acta Leidensia*, 42 (1975), 1–192.
Roundy, R. W., *Habitat, fishing and Schistosome Transmission in the Nile Basin*, Unpublished M. A. thesis, University of California, Los Angeles, 1971.
Russell, H. B., *Wld Hlth Org.*, Document EM/PHA/62 (1958).
Simoons, F., *J. Ecol. Food Nutr.*, 3 (1974) 89.
Wright, C. A. *Human Ecology in the Tropics.* edited by J. B. Garlic and R. W. J. Keay. New York (1970), pp. 67–79.

Am. J. Trop. Med. Hyg., 27(5), 1978, pp. 928-930

SCHISTOSOMIA HAEMATOBIUM IN THE WABI SHEBELLE VALLEY OF ETHIOPIA

G. DE SOLE,* A. LEMMA, AND BAHTA MAZENGIA†
Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia

Abstract. A survey of the lower Wabi Shebelle Valley of southeastern Ethiopia was made to assess the presence of schistosomiasis haematobia in the modernized plantation of Gode and adjacent areas. The disease is present in Kellafo, Mustahil, and in the Burukur flood plain 100 km downstream from Gode, and absent further north. This is related to the ecology of the different areas.

Reports of urinary schistosomiasis in the lower Wabi Shebelle Valley of southeastern Ethiopia have been unconfirmed, although the disease has been recorded from the adjacent area of Somalia.[1-3] The introduction of irrigation schemes by the Ethiopian Relief and Rehabilitation Commission stressed the importance of a survey of the area.[4-6]

ECOLOGY AND DEMOGRAPHY

The study area extended from the Somali border to the town of Imi, 400 km upstream (Fig. 1). The altitude varies from 365 m in the north to 195 m in the south. The Somali population was composed of about 26,600 nomads, 11,700 semi-nomads, and 40,200 farmers. The majority of the last-named are Rer-Barre tribesmen (Bantu) living in the plain between Kellafo and Mustahil. They are traditional farmers, digging canals during the period when the river was at its lowest level so that the flood waters would cover their fields which they sowed with sorghum, maize, and occasionally sesame after the water subsided. In Gode, modernized agriculture had only recently been introduced, whereas in Imi and Kugno agriculture was traditional and the yield rather poor. The livestock consisted of cattle, sheep, goats, and camels.

The lower Wabi Shebelle Valley receives about 300 mm of rain per year, mainly during late March, April, and May and again in October and early November. The mean annual air temperature is 28°C with a maximum of 30°C and a minimum of 18°C. There is a strong wind from the southwest from April to October (mean daily speed 4–6 m/sec), and from the northeast from November to March (mean daily speed 2–3 m/sec). The average relative humidity is about 55% without any great daily or monthly variation. Mean daily evaporation from a container is 10 mm.

The flow rate of the Wabi Shebelle varies seasonally at Gode between a minimum of 4 m^3/sec and a maximum of 600 m^3/sec. The annual average varies between 80 and 100 m^3/sec. The river overflows in the Imi-Kugno area for a period of 10–20 days per year (local information). At Gode, the river never overflows because the banks are high. In the plain of Kellafo, Mustahil and Burukur the river overflows about twice a year, and divides into many branches to form a vast flood zone.

METHODS

Urine samples were collected and examined, either immediately or after preservation in formalin. Age, sex, occupation, ethnic group, place of birth, and travel history were recorded for each person examined. The majority of the people studied were males. Collection of samples from females in Muslim areas is always difficult.

RESULTS

In Imi, all urine samples collected from 150 Somali tribe members were negative. Of these samples, five were from females between the ages of 10 and 50 years.

In Kugno, urine samples were collected from 100 Somalis. Only one infection with *Schistosoma*

Accepted 26 November 1977.

* Present address: c/o Ranger Rogers, 3011 Willamette, Little Town, Colorado 80121.
† Deceased.

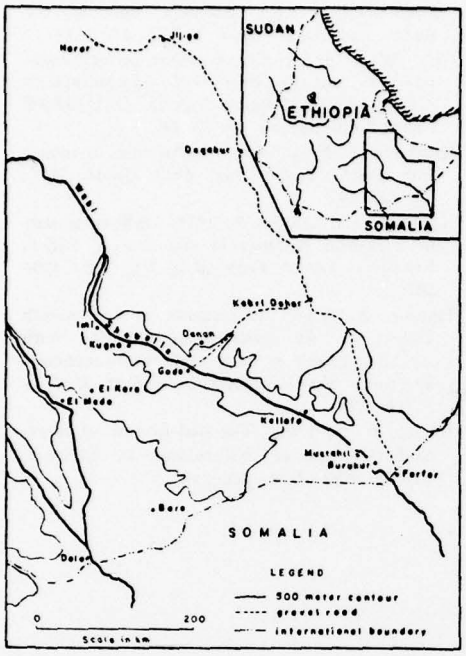


FIGURE 1. Map showing study area in the Wabi Shebelle Valley of Ethiopia.

haematobium was found. The Sherif Wornef is the predominant subtribe in Kugno. However, the only positive case found was in a Sahoule tribesman, one of the only two members of this subtribe present in the group examined. No *Bulinus* sp. snail was found in either area.[7]

In Gode, 261 urine specimens were examined, 83 from villagers, 134 from canal workers, and 44 from persons in a rehabilitation camp. Only two villagers were found infected. Both were adult males, one from Gode and one from Kellafo. The birthplace of 134 canal workers was recorded in order to estimate the likelihood that the disease will be introduced from adjacent areas. It was found that 79 (59.0%) were from Gode, 34 (25.4%) from Danan, 10 (7.5%) from Kellafo, 6 (4.5%) from Imi, and the remaining 5 (3.6%) were from different areas of the Ogaden. A few *Bulinus* shells were found in one canal only.

In Kellafo, of the 136 Rer-Barre tribesmen examined 32 (23.5%) were infected. The two adult females examined were both negative.

In Mustahil, of the 72 people examined 14 were town-dwelling Amharas (highlanders), none of whom was infected; 30 were seminomadic Ogaden tribesmen, 9 of whom (30.0%) were infected; and 28 were Rer-Barre farmers, 21 of whom (75.0%) were infected. Only six women were examined, five Amharas, all negative, and one Ogaden who was positive.

In Burukur, of the 73 people examined eight were Amharas, none of whom were infected; 60 were Ogaden, 27 of whom (45.0%) were positive. Only 11 adult females were examined. Of these, one was an Amhara woman who was negative and ten were Ogaden women, six of whom were positive. The prevalence of *S. haematobium* in Kellafo, Mustahil, and Burukur is shown in Table 1. *Bulinus* snails were found in the flood zone between Kellafo, Mustahil, and Burukur.

TABLE 1

Prevalence of S. haematobium *by age group in Kellafo, Mustahil, and Burukur, Wabi Shebelle Valley, Ethiopia*

Age group (yr)	Kellafo		Mustahil		Burukur	
	No. exam.	No. (%) pos.	No. exam.	No. (%) pos.	No. exam.	No. (%) pos.
0–5	4	0	0	–	0	–
6–10	12	2 (17)	8	0	17	4 (24)
11–15	20	7 (35)	16	9 (56)	15	11 (73)
16–20	25	6 (24)	11	6 (54)	9	6 (67)
21–25	27	5 (19)	9	6 (67)	6	2 (33)
26–30	18	5 (28)	7	3 (49)	9	3 (33)
31+	30	7 (23)	21	6 (29)	17	3 (18)
Total	136	32 (24)	72	30 (42)	73	29 (40)

DISCUSSION

Schistosomiasis haematobia appears to be well established in the flood plain of Kellafo, Mustahil, and Burukur but does not seem to be present farther north. In Imi and Kugno the flood period may be too short to allow the intermediate snail host to establish itself in the area. In Gode, the river does not overflow and only recently have canals been built which create a suitable habitat for the snail intermediate host. On the plain at Kellafo, Mustahil, and Burukur the division of the river into many branches and the occurrence of 2–3 periods of annual overflow create a suitable habitat for the snails.

Further agricultural development of the lower Wabi Shebelle Valley, which seems inevitable

under any future administration, will almost certainly modify the ecology and demography of the involved areas to create favorable conditions for the transmission of *S. haematobium*.

ACKNOWLEDGMENTS

We are grateful to the Ethiopian Relief and Rehabilitation Commission for information on the study area, to Mr. B. Assafa for the collection of snails, and to Dr. E. McConnell for editing the manuscript, and to Dr. Kloos for drawing the map.

REFERENCES

1. Arfaa, F., 1975. Studies on schistosomiasis in Somalia. *Am. J. Trop. Med. Hyg., 24:* 280–283.
2. Ayad, N., 1956. Bilharziasis survey in British Somaliland, Eritrea, Ethiopia, Somalia, the Sudan, and Yemen. *Bull. W.H.O., 14:* 1–117.
3. Nuh, M. Y. H., 1970. A report on schistosomiasis in Somalia. *Proc. OAU Symposium on Schistosomiasis, Niamey, Nigeria.* OAU/STRC Publications Bureau, pp. 35–44.
4. Bruijning, C. F. A., 1969. Bilharziasis in irrigation schemes in Ethiopia. *Trop. Geogr. Med., 21:* 280–292.
5. Kloos, H., and Lemma, A., 1977. Schistosomiasis in irrigation schemes in the Awash Valley, Ethiopia. *Am. J. Trop. Med. Hyg., 26:* 899–907.
6. Lemma, A., 1960. Bilharziasis in the Awash Valley. I. An epidemiological study with special emphasis on its possible future economic and public health importance. *Ethiop. Med. J., 7:* 147–175.
7. Brown, D. S., 1964. The distribution of intermediate hosts of Schistosoma in Ethiopia. *Ethiop. Med. J., 2:* 250–259.

Reprinted from Annals of Tropical Medicine and Parasitology, Vol. 72, No. 5 (1978)

Schistosoma mansoni distribution in Ethiopia: a study in medical geography

By HELMUT KLOOS*, AKLILU LEMMA and GIUSEPPE DESOLE

The George Williams Hooper Foundation, Medical Center, University of California, San Francisco, California 94143, U.S.A. and Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia

Received 15 July 1977

The distribution of *Schistosoma mansoni* among Ethiopian children indicates that this parasite is endemic only at altitudes between 500–1000 and 2000–2200 m in different parts of the country, but that the development of water resources may result in extension of the endemic areas into some lowlands.

Climate and Topography

Climates in Ethiopia range from hot desert (below 500 m in the Danakil Depression, Red Sea coast, lower part of the Awash Valley and the southeastern lowlands), to warm, semi-arid uplands (500–2000 m), and cool, humid highlands (above 2000 m). At low altitudes mean annual temperatures are 26–32°C and mean annual precipitation ranges from 100–300 mm (in the eastern and southern lowlands) to 400–800 mm (in the western lowlands). At intermediate altitudes temperatures are 18–25°C and precipitation 400–1200 mm, again highest in the western part of the country. On the extensive Ethiopian and Somali plateaus (altitude 2000–3000 m, topped by mountain ranges up to 4500 m) temperatures are 8–18°C and rainfall 600–2200 mm, highest in the southwest and lowest in the north. Many perennial surface waters exist on the well watered plateaus but most of the rivers, streams and lakes in the lowlands disappear during the long dry season of October–March.

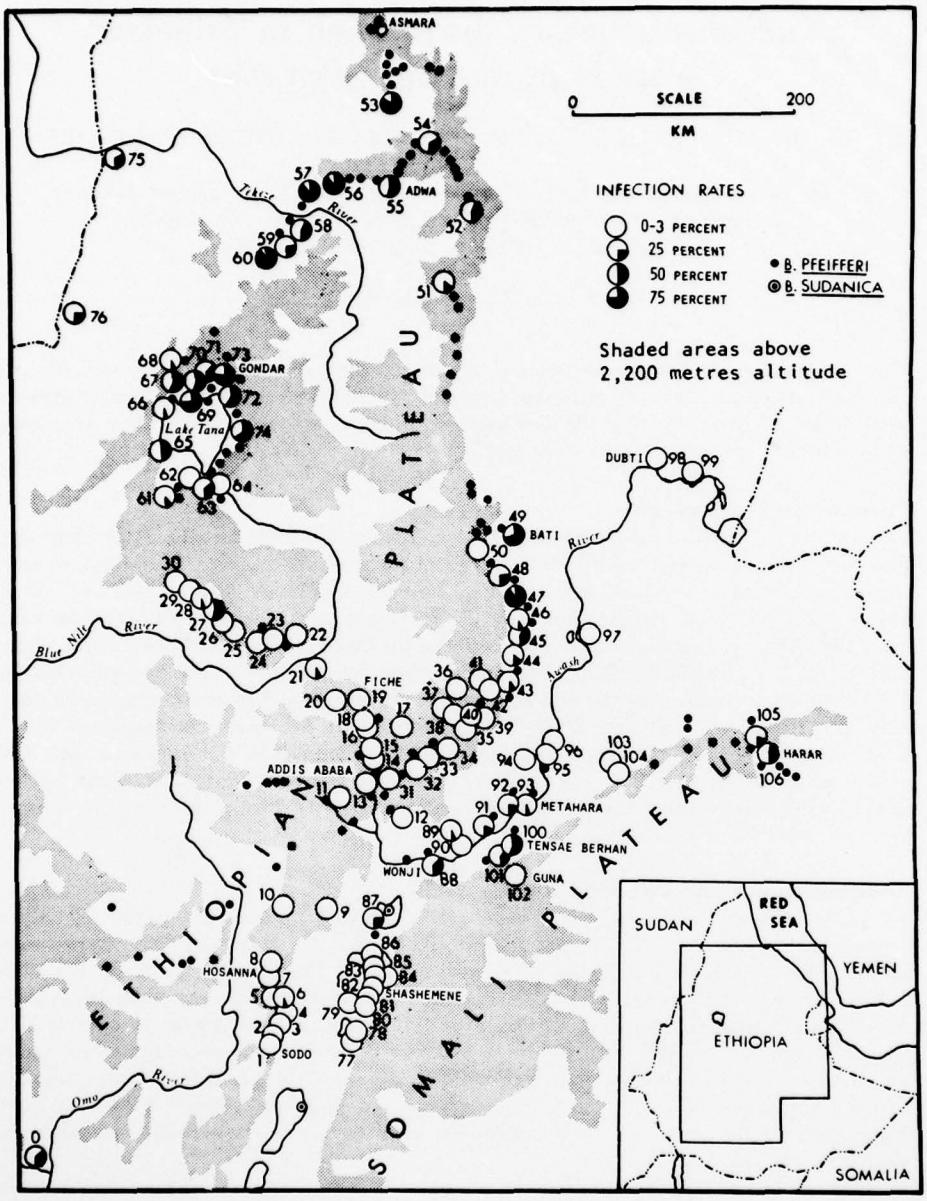
Endemic Areas and Infection Rates

The results of earlier studies on the geographic distribution of *S. mansoni* in Ethiopia (Ayad, 1956; Buck *et al.*, 1965; Chang, 1961; Kubasta, 1964; Lemma, 1969) were mapped by Schaller and Kuls (1972) and Yelizarov (1975). Their maps show schistosomiasis mansoni established in the well known endemic centres in the northern provinces of Tigre and Eritrea, the Lake Tana and Blue Nile areas, around Harar town and Wonji irrigation scheme, as well as in the lower Awash, Webi Shebelle and Omo river valleys, and in some parts of Bale and Sidamo provinces. The more recent results of the examination of stool specimens of locally born school, farm and pastoral children are shown in the Table and on the map. The relationship between *S. mansoni* prevalence and altitude is shown in the Fig.

Only one of 29 communities located above 2200 m had *S. mansoni* infection rates above 3%, whereas 42 of the 77 communities at or below 2200 m had rates above 5% (and up to 94%, at Zerima). Below 960 m infections were found only in the two villages surveyed in the northwestern lowlands and in Mui National Park. A weak inverse relationship exists between all prevalence data for locally born children and altitude ($r = -0{\cdot}1887$), but

* Present address: 2307 N. Backer, Fresno, California 93703, U.S.A.

0003–4983/78/050461+10 $01.00/0



Map. Distribution of *S. mansoni* and *Biomphalaria* spp.

TABLE

Results of schistosomiasis surveys in locally born children

Village, town, or farm	*Altitude (metres)*	*Sample**	*Number examined*	*Method†*	*Percent infected*	*Source*
SOUTHERN PART OF ETHIOPIAN PLATEAU AND FOOTHILLS						
0. Mui National Park	700–900	E†	86	R	42	Institute of Pathobiology
		P†	21	R	33	unpublished data
1. Hamus Gebaye	2000	F	14	R	0	Present survey
2. Boditti	1800	F	13	R	0	,,
3. Shone	1800	S	59	R	0	,,
4. Durame	2000	S	44	R	0	,,
5. Angecha	2300	S	50	R	6	,,
6. Serera	2700	S	26	R	0	,,
7. Hosanna	2300	S	87	R	0	,,
8. Shurmo	2350	F	46	R	2	,,
9. Butajira	2100	S	13	R	0	,,
10. Asawde Yefeterke	2400	F	20	R	0	,,
CENTRAL PART OF ETHIOPIAN PLATEAU						
11. Wollenchomi	2200	S	115	R	0	Aram (1973*b*)
12. Debre Zeit	1900	S	90	R	0	Lemma, Demisse and Mazengia (1968)
13. Addis Ababa	2400	S	266	R	0	,,
14. Chancho	2600	S	35	MIFC	0	McConnell and Armstrong (1976)
15. Gorfu	2600	S	37	MIFC	0	,,
16. Muke Turi	2600	S	35	MIFC	0	,,
17. Weberi	2575	S	35	,,	0	,,
18. Debre Tsegie	2600	S	25	R	0	Present survey
19. Fiche	2800	S	69	R	0	,,
Fiche	—	S	25	MIFC	0	McConnell and Armstrong (1976)
20. Gebre Guracha	2575	S	35	MIFC	0	McConnell and Armstrong (1976)
21. Filkilik	1900	S	35	,,	9	,,
22. Yetman	2400	S	33	,,	0	,,
23. Wegel	2475	S	35	,,	0	,,
24. Lumane	2500	S	35	,,	0	,,
25. Amanuel	2400	S	35	,,	0	,,
26. Dembecha	2100	S	32	,,	0	,,
27. Jiga	1900	S	40	,,	55	,,
28. Finote Salem	1900	S	40	,,	5	,,
29. Mankusa	2000	S	42	,,	0	,,
30. Bure	2100	S	41	,,	2	,,
31. Lege Tafo	2500	S	34	,,	0	,,
32. Sendafa	2550	S	34	,,	0	,,
33. Aliltu	2625	S	35	,,	0	,,
Aliltu	—	S	42	R	0	Present survey
34. Sheno	2825	S	35	MIFC	0	McConnell and Armstrong (1976)
35. Chacha	2750	S	35	,,	0	,,
36. Jihar	2650	S	34	,,	0	,,
37. Inewari	2650	S	34	,,	0	,,
38. Mendita	2800	S	35	,,	0	,,
39. Ankober	2900	S	35	,,	3	,,
40. Debre Berhan	2750	S	35	,,	0	,,
Debre Berhan	—	S	71	R	0	Present survey

TABLE (*continued*)

Village, town, or farm	*Altitude (metres)*	*Sample**	*Number examined*	*Method†*	*Percent infected*	*Source*
41. Sela Dingay	2850	S	39	MIFC	0	McConnell and Armstrong (1976)
42. Debre Sina	2650	S	37	,,	0	,,
EASTERN FOOTHILLS OF ETHIOPIAN PLATEAU						
43. Robi	1400	S	31	,,	10	,,
44. Yewaha	1250	S	31	,,	16	,,
45. Efeson	1500	S	46	,,	41	,,
46. Karakore	1850	S	43	,,	7	,,
47. Kemse	1500	S	36	,,	92	,,
48. Harbu	1600	S	40	,,	25	,,
49. Bati	1650	S‡	51	,,	51	,,
Bati	—	S	50	R	72	Present survey
50. Kembolcha	1900	S	48	MIFC	2	McConnell and Armstrong (1976)
NORTHERN PART OF ETHIOPIAN PLATEAU						
51. Mekele	2200	S	53	,,	17	,,
52. Wikro	2050	S	64	,,	47	,,
53. Adi Kwala	2000	S	35	,,	83	,,
54. Inticho	2000	S	34	R	35	Lemma *et al.* (1975)
55. Adwa	1800	S	509	,,	56	,,
56. Seleklaka	1950	S	29	MIFC	90	McConnell and Armstrong (1976)
57. Inda Baguna	1900	S	29	MIFC	90	McConnell and Armstrong (1976)
58. Maitseberi	1500	S	48	D	40	,,
59. Adi Arkai	1600	S	25	D	24	,,
60. Zerima	1300	S	34	MIFC	94	,,
LAKE TANA BASIN						
61. Dangla	2150	S	42	,,	12	,,
62. Wetet Abay	1950	S	42	,,	0	,,
63. Merawi	2000	S	45	,,	33	,,
64. Meshenti	1950	S	42	,,	0	,,
65. Kunzila	1850	S	24	S	46	Polderman (1974)
66. Delghi	1850	S	28	R	7	,,
67. Gella Dubba	2000	S	30	,,	60	,,
68. Aykel	2200	S	32	,,	8	,,
69. Gorgora	1850	S	57	,,	77	,,
70. Kolla Dubba	2000	S	56	,,	55	,,
71. Sakalt	2000	S	36	,,	39	,,
72. Tadda	1800	S	41	S	59	,,
73. Addis Alem	2000	S	63	S	79	,,
74. Emfraz	1900	S	35	R	63	,,
NORTHWESTERN LOWLANDS						
75. Settit Humera	550	S	20	R	30	,,
76. Metemma	700	F	8	,,	25	,,
SOUTHERN PART OF RIFT VALLEY						
Lake Awasa area:						
77. Dalati	1550	F	14	R	0	Institute of Pathobiology unpublished data (1968)

AD-A080 030 CALIFORNIA UNIV SAN FRANCISCO F/G 6/5
STUDIES ON MOLLUSCICIDAL AND OTHER PROPERTIES OF THE ENDOD PLAN--ETC(U)
JUN 79 A LEMMA, D HEYNEMAN, H KLOOS N00014-76-C-0218

UNCLASSIFIED NL

6 OF 6
AD
A080030

END
DATE
FILMED
2-80
DDC

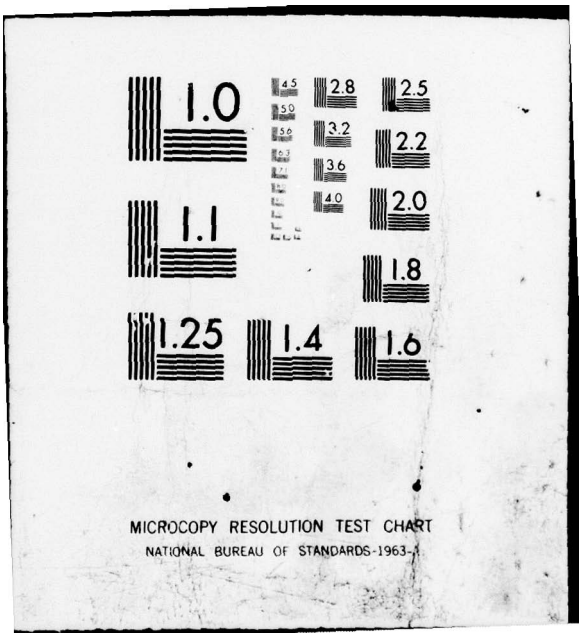

1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

TABLE (*continued*)

Village, town, or farm	*Altitude (metres)*	*Sample**	*Number examined*	*Method†*	*Percent infected*	*Source*
78. Loke	1550	F	15	,,	0	,,
79. Adarie	1550	F	25	,,	0	,,
80. Malkadoret	1550	F	35	,,	0	,,
81. Sama	1550	F	40	,,	0	,,
82. Birbow	1550	F	45	,,	0	,,
Lake Langano area:						
83. Bosuma	1600	F	18	,,	0	,,
84. Kello	1600	F	15	,,	0	,,
85. Chancho	1600	F	10	,,	0	,,
86. Ashelamo	1600	F	23	,,	0	,,
Lake Ziway area:						
87. West shore and islands	1625	F	52	S	25	Central Laboratory, Addis Ababa, unpublished data
NORTHERN PART OF RIFT VALLEY (AWASH VALLEY)						
88. Wonji	1540	S	82	R	21	Present survey
Wonji	—	F	20	,,	40	,,
89. Wollenchiti	1400	S	44	,,	6	,,
90. Bofa	1200	S	16	,,	0	,,
91. Nura Era	1100	P	31	,,	19	,,
92. Abadir	960	P	41	,,	20	,,
93. Metahara	960	P	47	,,	4	,,
Metahara	—	F	45	,,	4	,,
94. Awara Melka	750	P, F	38	,,	0	,,
95. Melka Sadi	740	P	15	,,	0	,,
96. Amibara-Melka Worer	740	P	33‡	,,	0	Present survey
97. Galela Dora	620	P	26	S, D	0	Ethio-Swiss Hospital, unpublished data
98. Dubti	380	P	51	D	0	Dubti Clinic, unpublished data
99. Assaita	350	P	35	R	0	Present survey
SOMALI PLATEAU AND FOOTHILLS						
100. Tensae Berhan	1550	S, F	143	,,	59	Polderman (1976)
101. Dolchia	1800	S, F	31	,,	45	Present survey
102. Guna	2800	S	35	,,	0	,,
103. Miesso	1300	S	44	,,	0	,,
104. Asbe Teferi	1750	S	24	,,	4	,,
105. Alemaya	2100	S	53	,,	19	Lo, Flemings and Lemma (1973)
106. Harar	1950	S§	53	,,	39	,,
Harar	—	S§	158	,,	30	Polderman (1976)

* S = school children; F = farmers' children (non-school); P = pastoralists' children (non-school); E = dependents of park employees.

† R = Ritchie concentration method, MIFC = merthiolate-iodine-formaldehyde concentration method, S = simple sedimentation method, D = direct smear method. The use of various methods of stool examination renders the results in the table not strictly comparable. Nevertheless, earlier studies on their relative efficacy (Duncan, Lemma and Mazengia, 1970) show that the Ritchie concentration method is only moderately more efficient than the other three.

‡ Some adults included.

§ Some children born elsewhere included.

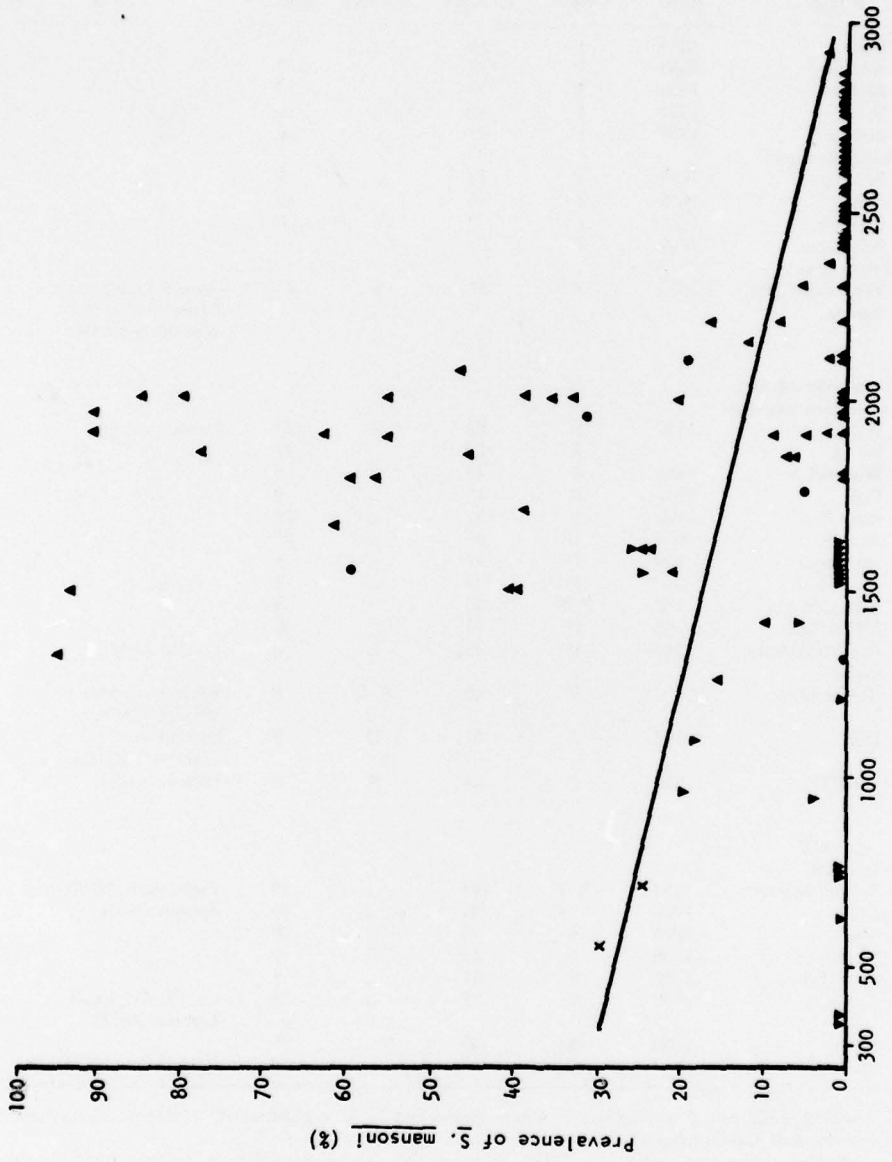


Fig. Relationship between *S. mansoni* prevalence and altitude in Ethiopia. (▲) Ethiopian Plateau, (▼) Rift Valley, (●) Somali Plateau, (×) northwestern lowlands. $r = -0{\cdot}1187$, $P < 0{\cdot}05$, $b = -0{\cdot}008$.

a stronger relationship emerges when considering the two plateaus alone ($r = -0{\cdot}4834$). The sharp increase in infection on the plateaus at around 1300 m and the abrupt fall in rates between 2000 and 2200 m also indicate that the lower and upper altitudinal boundaries of *S. mansoni* transmission in large parts of Ethiopia are located near these contours, respectively.

The 2000 and 2200 contours lie between the 16 and 18°C isotherms in Ethiopia (Kloos, 1977). Brown (1964) considered that low temperatures prevent the completion of the *S. mansoni* cycle at higher altitudes. A somewhat lower upper altitudinal transmission ceiling (1800–2000 m) was found in East Africa by McCullough (1972) and Diesfeld (1969). *Biomphalaria pfeifferi* is widely distributed on the Ethiopian and Somali plateaus (Aram, 1973*a*; Brown, 1965; Yasuraoka, 1973). It occurs seasonally between the two rainy periods (Aram, 1973*b*) and some of the brief malacological surveys made by various investigators have apparently failed to recover it from habitats where it is established. Polderman (1975) found *B. pfeifferi* naturally infected with *S. mansoni* and successfully infected mice in ponds in the Lake Tana Basin (1900 m), the highest location where such studies have been made in Ethiopia.

Vertical mobility is characteristic of Ethiopian populations along the escarpments of the two high plateaus, suggesting that some of the infections found at altitudes above 2000 m may have been acquired at lower levels. Many people commonly travel to markets, fields and community water sources, and make social visits that may require them to cross several hundred metres of altitude (Roundy, 1976). In some areas, however, the upper altitudinal transmission boundary is quite sharply defined, as shown by the high prevalence of infection in Tensae Berhan (1550 m) and Dolchia (1800 m) and its absence from nearby Guna (2800 m), as well as by similar differences between two villages in the Lake Tana basin (Polderman, 1974).

The lower altitudinal boundary of *S. mansoni* transmission is less well defined than its upper limit but some lowlands remain free of intestinal schistosomiasis. No endemic centres are known in the Awash Valley below Metahara (960 m) (Kloos and Lemma, 1977) or in the southern part of the Rift Valley, and *Biomphalaria* was not found in the Genale (Ayad, 1956; Brown, 1964), Webi Shebelle (DeSole *et al.*, 1978) or lower Omo (by Dr. DeSole in 1976) river valleys. High temperatures, and to a lesser extent high river silt load, apparently prevent *B. pfeifferi* from colonising all natural waters and 11 of the 12 irrigation systems in the middle and lower parts of the Awash Valley. Only in the new Melka Sadi scheme has this species of snail become established. Transmission had apparently not begun in 1976, but the influx of many migrant farm labourers from the endemic areas in northern and southern Ethiopia to Melka Sadi must presumably result eventually in the introduction of *S. mansoni* into the canal system (Kloos and Lemma, 1974, 1977). Labour migrations also have been associated with high *S. mansoni* rates in agricultural schemes in the Settit-Humera area (Polderman, 1974). The absence of schistosomiasis from the southern Rift Valley appears mainly due to the absence of snail hosts from the saltwater lakes Abyata, Langano, Shala and Awasa (Brown, 1965; Aram, 1973*a*), also indicated by the confinement of high infection rates to the freshwater lake Ziway, the only *Biomphalaria sudanica*-infested lake in this part of the rift. The apparent absence of infection in children in several foothill villages of the southern Ethiopian and Somali plateaus may be due to the unsuitability of village ponds, springs and rocky streams for *Biomphalaria* snails, but this problem needs further study. In the western lowlands, however, the parasite appears to be transmitted. Ayad (1956) cited reports of autochthonous cases and Polderman (1974) found infected locally born children in Metemma and Settit-Humera. The recent discovery of high infection rates in many localities in the foothills of the Ethiopian and Somali plateaus suggests that many endemic foci in Ethiopia remain unknown.

DISCUSSION

Most communities included in this study are located along major roads, but the focal distribution of high *S. mansoni* infection rates, especially in isolated areas such as the Blue Nile Gorge (Fuller Torrey, 1966), Lake Tana area and Mui Valley indicates that this parasite is of some antiquity in Ethiopia, contrary to the reports by Ayad (1956) and Lemma (1969). Similarly, the general decrease in the prevalence of schistosomiasis mansoni from northern to central Ethiopia appears more closely related to increase in altitude and decrease in temperature on the Ethiopian Plateau along the north/south axis than to population movements, contrary to the suggestion by McConnell and Armstrong (1976). There have been many extensive movements of armies, merchants and whole tribes during the past 3000 years in Ethiopia (Trimingham, 1952; Pankhurst, 1968). The assertion that there is little human contact with *B. pfeifferi*-infested waters in the highlands (Ayad, 1956) must also be discounted as an explanation for the rarity of infection in certain portions of the plateaus. The highest population densities in Ethiopia, i.e. 25–300 persons/km^2, occur in the highlands (Kuls, 1968; Imperial Ethiopian Government, 1974) and the general lack of piped water supplies necessitates the use of most streams, lakes and swamps, many of which support *B. pfeifferi*. This suggests that the infection is less likely to increase with further population increase and economic development in the cool, well watered highlands than in certain lowlands, particularly those of western Ethiopia and at intermediate altitudes, where *S. mansoni* and its snail host already occur and where irrigation development is planned.

At altitudes below 750 m or beyond the 26 °C isotherm in eastern and apparently southern Ethiopia, high temperatures may represent a strong barrier to the spread of *B. pfeifferi* (Brown and Lemma, 1970; Kloos and Lemma, 1977), as already noted for the coast of East Africa (Berrie, 1970; Sturrock, 1966). Sturrock (1965) considered that high temperatures prevent the establishment of *B. pfeifferi* along both African coasts between 10°S and 10°W, but these temperature effects may extend up to 16°N in the eastern and southern Ethiopian lowlands, where the rainshadow produced by the Ethiopian highlands results in particularly dry and hot climates. The confinement to areas above 500 m of the few known localities for *B. pfeifferi* in Somalia (Ayad, 1956) is in agreement with the broad distribution pattern of climate and endemic schistosomiasis mansoni in Northeast Africa. Nevertheless, longitudinal studies on incidence and prevalence of infection and on snail occurrence are needed for the eight river basins in Ethiopia (where irrigation development is scheduled) before more definite predictions can be made about further schistosomiasis spread in the lowlands (Kloos, 1977).

SUMMARY

Schistosoma mansoni occurs locally at altitudes below 2200 m in Ethiopia, but is absent below about 1000 m from the lower Awash, Webi Shebelle, Genale and Omo river valleys in the eastern and southern parts of the country. The results of faecal and snail surveys by several investigators are reported and mapped.

The recent discovery of endemic foci of *S. mansoni* in widely separated and remote areas of Ethiopia indicates that schistosomiasis mansoni is an older disease of Ethiopians than was thought previously. Altitude, rather than population movements and water use patterns, account for much of the spatial distribution of *S. mansoni* and its intermediate hosts, *Biomphalaria pfeifferi* and *B. sudanica*. Low water temperatures prohibit schistosome survival on the high plateaus, but high temperatures prevent *Biomphalaria* snails from

colonising the hot eastern and southern lowlands. In the more humid and cooler western lowlands, conditions for snail survival are more favourable. Development of irrigation agriculture may break down the temperature barrier in some areas, as indicated by the spread of *B. pfeifferi* and the influx of infected migrant farm labourers into the lower parts of the Awash Valley.

ACKNOWLEDGEMENTS. The senior author wishes to thank the Director of the Institute of Pathobiology and other officials of Addis Ababa University for accommodation in that Institute and for generous technical assistance. We are also indebted to Mr. Belete Kirub, Dr. A. M. Polderman and Mr. Assefa Gebre for assistance during field work, to Mr. Bahta Mazengia for examining stools and Professor Donald Heyneman for critically reading the manuscript. The study was supported in part by a USAID grant. Professor Harry J. Costis generously helped with computer analysis at California State University Fresno.

REFERENCES

ARAM, R. H. (1973*a*). *Report of studies on schistosomiasis in Ethiopia by the University of Sussex Ecology Unit, 1970–1973.* 15 pp. Unpublished report.

ARAM, R. H. (1973*b*). *Studies on* Biomphalaria pfeifferi *in three Ethiopian highland streams.* University of Sussex. 32 pp. Unpublished report.

AYAD, N. (1956). Bilharziasis survey in British Somaliland, Eritrea, Ethiopia, Somalia, the Sudan and Yemen. *Bulletin of the World Health Organisation*, **14,** 1–117.

BERRIE, A. D. (1970). Snail problems in African schistosomiasis. *Advances in Parasitology*, **8,** 43–96.

BROWN, D. S. (1964). The distribution of intermediate hosts of *Schistosoma* in Ethiopia. *Ethiopian Medical Journal*, **2,** 250–259.

BROWN, D. S. (1965). Freshwater gastropod mollusca from Ethiopia. *Bulletin of the British Museum of Natural History* (*Zoology*), **12,** 39–94.

BROWN, D. S. & LEMMA, A. (1970). The molluscan fauna of the Awash River, Ethiopia. *Annals of Tropical Medicine and Parasitology*, **64,** 533–538.

BUCK, A. A., SPRUYT, D. J., WADE, M. K., DERESSA, A. & FEYESSA, E. (1965). Schistosomiasis in Adwa: a report of an epidemiological pilot study. *Ethiopian Medical Journal*, **3,** 93–106.

CHANG, W. P. (1961). Report on epidemiological study on bilharziasis in Gorgora, north shore of Lake Tana. *Gondar Health Series*, **1,** 4–7.

DESOLE, G., LEMMA, A. & MAZENGIA, B. (1978). Preliminary report on schistosomiasis in the Webi Shebelle Valley. *American Journal of Tropical Medicine and Hygiene* (in press).

DIESFELD, H. J. (1969). Die Befundhäufigkeit von Helminthen beim Menschen in Kenia in Beziehung zu Umweltfaktoren. *Tropenmedizin und Parasitologie*, **20,** 311–333.

DUNCAN, J., LEMMA, A. & MAZANGIA, B. (1970). A comparison of certain stool examination techniques with special reference to their efficacy in surveys for schistosomiasis in the Awash Valley. *Ethiopian Medical Journal*, **8,** 17–25.

FULLER TORREY, E. (1966). A medical survey of the SaySay people in the Blue Nile Gorge. *Ethiopian Medical Journal*, **4,** 155–165.

IMPERIAL ETHIOPIAN GOVERNMENT (1974). *The Demography of Ethiopia. Statistical Bulletin* (Central Statistical Office), No. 10, 57 pp.

KLOOS, H. (1977). *Schistosomiasis and irrigation in the Awash Valley of Ethiopia.* 284 pp. Ph.D. dissertation, University of California, Davis.

KLOOS, H. & LEMMA, A. (1974). Bilharziasis in the Awash Valley. II. Molluscan fauna in irrigation farms and agricultural development. *Ethiopian Medical Journal*, **12,** 157–173.

KLOOS, H. & LEMMA, A. (1977). Schistosomiasis in irrigation schemes in the Awash Valley, Ethiopia. *American Journal of Tropical Medicine and Hygiene*, **26,** 899–908.

KUBASTA, M. (1964). Schistosomiasis mansoni in the Harar Province. *Ethiopian Medical Journal*, **2,** 260–271.

KULS, W. (1968). Jüngere Wandlungen in den Enseteanbaugebieten Südäthiopiens. *Acta Geographica*, **20,** 185–199.

LEMMA, A. (1969). Bilharziasis in the Awash Valley. I. Epidemiological study with special emphasis on its possible future economic and public health importance. *Ethiopian Medical Journal*, **7,** 147–175.

LEMMA, A., DEMISSE, M. & MAZENGIA, B. (1968). Parasitological surveys of Addis Ababa and Debre Zeit school children with special reference to bilharziasis. *Ethiopian Medical Journal*, **6,** 61–71.

LEMMA, A., GOLL, P., DUNCAN, J. & MAZENGIA, B. (1978). *Control of schistosomiasis with use of endod in Adwa, Ethiopia: Results of a 5-year study*. 31 pp. *Proceedings of the International Conference on Schistosomiasis*, (Cairo), Vol. 1, 415–436.

LO, C. T., FLEMINGS, M. B. & LEMMA, A. (1973). Schistosomiasis in Harar, Alemaya and the Damota Valley, Ethiopia. *Ethiopian Medical Journal*, **11,** 271–278.

MCCONNELL, E. & ARMSTRONG, J. C. (1976). Intestinal parasitism in fifty communities on the central plateau of Ethiopia. *Ethiopian Medical Journal*, **14,** 159–168.

MCCULLOUGH, F. S. (1972). The distribution of *Schistosoma mansoni* and *S. haematobium* in East Africa. *Tropical and Geographical Medicine*, **24,** 199–207.

PANKHURST, R. (1968). *Economic history of Ethiopia: 1800–1935*. 772 pp. Addis Ababa: Haile Sellassie I University Press.

POLDERMAN, A. M. (1974). Intestinal schistosomiasis north and west of Lake Tana, Ethiopia. *Tropical and Geographical Medicine*, **26,** 170–177.

POLDERMAN, A. M. (1975). The transmission of intestinal schistosomiasis in Begemder Province, Ethiopia. Ph.D. dissertation, Rijksuniversiteit Leiden (Netherlands), published in *Acta Leidensia*, **42,** 1–193.

POLDERMAN, A. M. (1976). Schistosomiasis in Ethiopia. *World Health Organization Assignment Report*, EM/SCHIS./64 EM/ETH/MPD/003/RB.

ROUNDY, R. W. (1976). *Studies of communicable disease transmission resulting from cultural behavior in Ethiopian rural highland-dwelling populations: A cultural medical-geographical study*. 422 pp. Ph.D. dissertation, University of California, Los Angeles.

SCHALLER, K. F. & KULS, W. (1972). *Äthiopian-Ethiopia*. Geomedical Monograph Series, Vol. 3. 180 pp. Heidelberg: Springer-Verlag.

STURROCK, R. F. (1965). The development of irrigation and its influence on the transmission of bilharziasis in Tanganyika. *Bulletin of the World Health Organization*, **32,** 225–236.

STURROCK, R. F. (1966). The influence of temperature on the biology of *Biomphalaria pfeifferi* (Krauss), an intermediate host of *Schistosoma mansoni*. *Annals of Tropical Medicine and Parasitology*, **16,** 100–105.

TRIMINGHAM, J. S. (1952). *Islam in Ethiopia*. 299 pp. London: Oxford University Press.

YASURAOKA, K. (1973). A survey of the snail vectors of schistosomiasis in Ethiopia. *World Health Organization Assignment Report*, EM/SCHIS/54, ETHIOPIA 2101/R.

YELIZAROV, V. A. (1975). Data on the medical geography of Ethiopia. *Soviet Geography*, **16,** 321–329.

Printed in Great Britain by Henry Ling Ltd., at the Dorset Press, Dorchester, Dorset

Teklehaimanot, R and Goll, PH. 1978. *Ethiop. med. J.* 16, 115

INVESTIGATIONS INTO THE CONTROL OF SCHISTOSOMIASIS AT THE HVA WONJI-SHOA SUGAR ESTATES IN ETHIOPIA

2. Interim evaluation of the project

Redda Teklehaimanot, MD[1] and Peter H Goll, MSc.[2]

ABSTRACT: *An account is given of the attempt to control transmission of* Schistosoma mansoni *by the use of molluscicides on a sugar estate in Ethiopia. Annual fluctuations of populations of snails* (Biomphalaria pfeifferi) *and their distribution in various habitat types are discussed in relation to application of the molluscicides, N-tritylmorpholine and niclosamide. The use of these products in their respective situations was successful in controlling snail populations, but results of a 10 percent random stool survey of 14 plantation villages indicated that a low level of transmission persists. The most likely modes of transmission are considered and improvements in the application of the control methods are suggested. Clinical aspects of the infection are discussed briefly but there is little evidence of severe symptoms in this locality.*

The background to the project has been described by Duncan and Lemma (1). This paper constitutes an interim evaluation of the control measures adopted during the period until June, 1973, when the HVA Medical Services Department maintained continuous monthly snail surveys on the basis of which molluscicide applications were made covering the entire estate. Routine examination of *Biomphalaria pfeifferi* for infection with *Schistosoma mansoni* was introduced in order to locate possible transmission sites and to discover any seasonal fluctuations of infected snails.

Results of stool surveys will be presented together with some observations of the water contact behaviour of estate residents. Comments will be made on the extent of disability in clinical and social terms caused by the disease.

1 Wonji Hospital, HVA, Ethiopia

Present address: Dept. of Neurology, McGill Hospital, Montreal, Canada

2 Institute of Pathology, Addis Ababa University

Present address: Medical Research Council Laboratories, Fajara (Banjul) The Gambia.

MATERIALS AND METHODS

Intermediate snail host surveys: Representative examples of the types of water body to be found on the estates, in main and secondary supply canals, tertiary or field drains, main drains and wadooks (night storage reservoirs) were surveyed monthly and all molluscs recorded according to the methods of Duncan and Lemma (1). Rainfall and temperature data were provided by the Agricultural Services Department of HVA Wonji/Shoa. Water temperature was not recorded until 1973 but for discussion of the influence of this factor before 1973 air temperature was taken as a suitable indication.

Molluscicide applications: During the period under review the timing of the molluscicide treatments was determined by increased abundance of *B. pfeifferi* populations. N-tritylmorpholine (Frescon) as a 16.5 percent weight/volume emulsion concentrate was used at a concentration

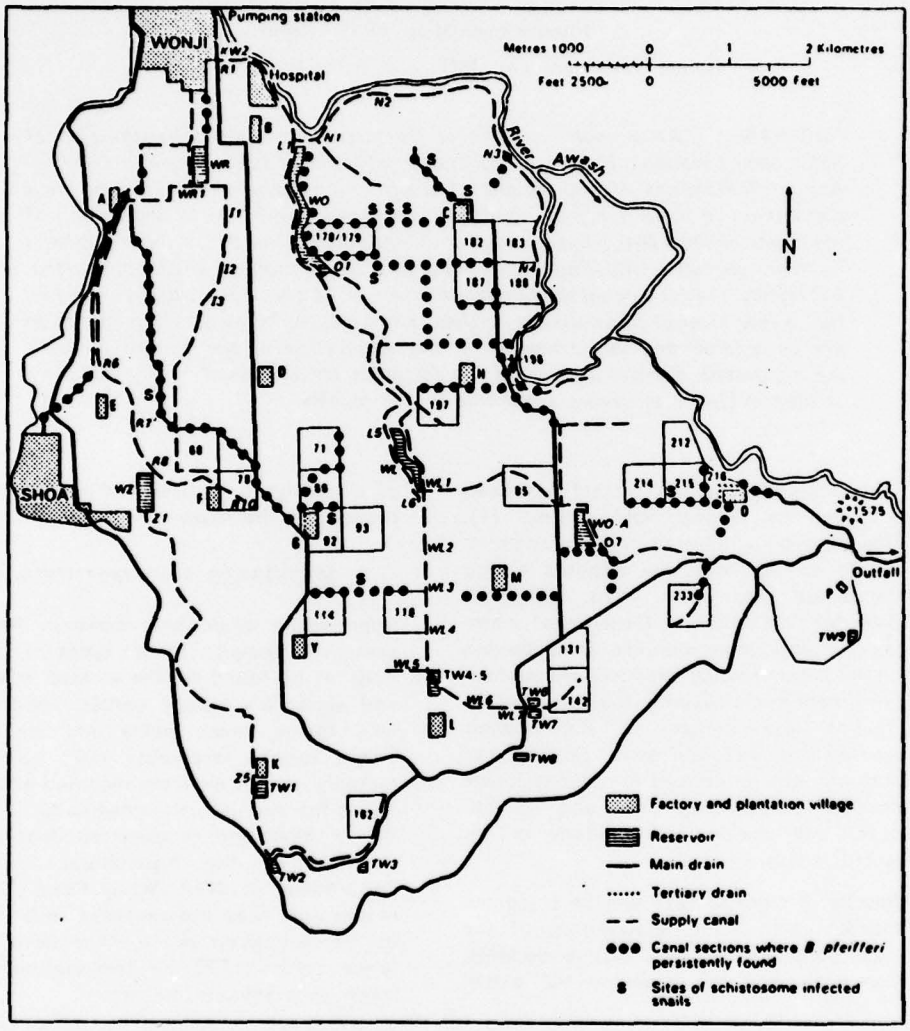


Figure 1. Sketchmap of Wonji-Shoa Sugar Estates indicating water distribution and drainage network; factory and plantation villages; and distribution of *B. pfeifferi* populations.

of 0.025 ppm for 72 hours on two occasions, 20 days apart, while niclosamide (Bayluscide) as a 70 percent wettable powder was used at 0.25 ppm for eight hours.

The use of these molluscicides was initially integrated, the former being used in the main supply and the latter in smaller secondary supplies and the tertiary drainage system. Applications of N-tritylmorpholine were protracted and cumbersome, and often rendered impracticable by irrigation practice, so that by 1972 it was partially replaced by niclosamide applications.

One branch continued to be treated with N-tritylmorpholine from KW2 (figure 1) with a booster dispenser at WR, but on the other branch niclosamide was dispensed from L1 and N1 to avoid dissipation of chemical into wadook WO. Discharges from wadooks, WO, WL, WZ and WR (figure 1) continued to be treated with niclosamide.

Distribution of the chemicals was monitored by analysis of water samples according to the methods of Beynon and Thomas (2) and Strufe (3) for N-tritylmorpholine and niclosamide respectively. Bioassay, using previously collected *B. pfeifferi*, was also carried out.

Cercarial examination: The infection rate of *B. pfeifferi* with *S. mansoni* was assessed from samples collected mostly from field drains at 100 m intervals using a drag scoop. From each drain 200 - 300 specimens were collected. The method of shedding was essentially that of Webbe (4). If schistosome-type cercariae were shed, then snails from the positive samples were exposed individually on the following day in order to determine percent infection rate. This routine was considered to be less time-consuming and less subject to error than the crushing method, although only patent infections were detected.

Population under survey: There are about 3000 permanent and 2000 seasonal labourers who together with dependents comprise a population of about 20,000. They are housed in two large factory villages, one attached to each of the two mills, and 14 smaller plantation villages distributed throughout the estate, designated alphabetically in figure 1.

Stool examination: Until 1970, all stool samples at Wonji Hospital were examined by the direct (unpreserved) film method, where two faecal smears were made on the same slide. One smear was stained with D'Antoni's iodine solution and the other with 2 percent Eosin and both were read by the same technician. The routine diagnostic procedure adopted in 1970 for all stool examinations was Ritchie's formol ether concentration method which had been recommended as the method of choice by Duncan *et al.* (5) and which is more efficient in detecting light infections (6).

The direct smear method was repeated in 1973 in order to allow some comparison with pre-1970 data but the definitive prevalence was the estimate obtained by the concentration method. This 10 percent random survey for *S. mansoni* infection among the plantation villages was considered suitable as an interim evaluation of the efficacy of the first three years of control measures.

RESULTS

Snail populations: The main and secondary supply canals and field drains, which were surveyed regularly are indicated in figure 1. The density fluctuations of *B. pfeifferi* are shown in figure 2, where the monthly data for the supply canals and for the drains have been aggregated in each case and a mean number of snails per sample plotted. Throughout the three years of this report the pattern for field drains was roughly parallel to that for the affluent system. Wadooks and the proximal sectors of their discharge systems were surveyed initially on a regular basis

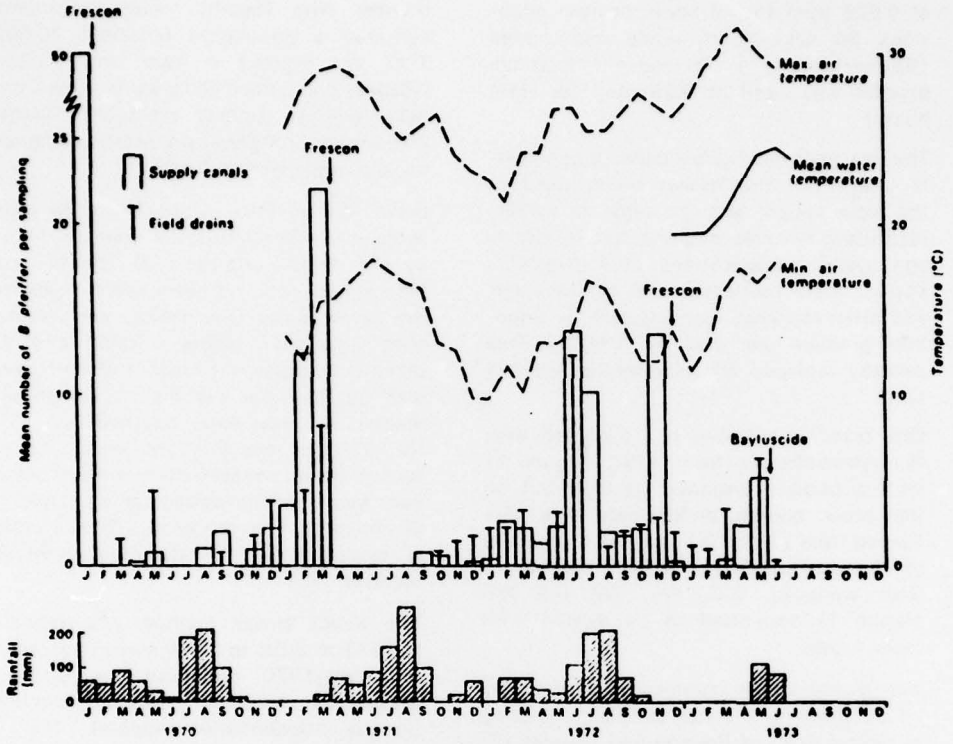


Figure 2. Density fluctuations of *B. pfeifferi* in supply canals and field drains in relation to rainfall and temperature. Molluscicide applications to the supply system are indicated.

but only isolated specimens were found occasionally so sampling was abandoned.

Molluscicide applications: Figure 2 shows that, apart from the increase observed in January 1971 when molluscicide application was unavoidably delayed until March, there was a marked reduction in density of *B. pfeifferi* after each chemical treatment. In general field drains remained free of snails for many months after treatment.

Infection of B. pfeifferi with S. mansoni: Table 1 shows a distinct seasonal fluctuation in the recovery of infected snails. In 1972 no infected snails were found until June, after which time the occurrence and percentage infected increased from 0.5 percent to a peak of about 2 percent for individual drains in mid-September. In 1973, although one isolated infected population was found in January (but none of four mice which were exposed to cercariae derived from it became infected), no infections were found until May, increasing to a peak in November after the period under review. The infection rate per sample varied between 0 - 3 percent and 0.5 - 2 percent for individual drains sampled, these being measures of the intensity and distribution of infection in the irrigation system respectively. All these data were from field drains but infected *B. pfeifferi* were occasionally found in the secondary supply system, R6 — R8, and in the main drain near Camp C, adjacent to field 176.

TABLE 1: Examination of *B. pfeifferi* for infection with *S. mansoni* in field drains, where the total number of snails is the pooled collection from a variable number of drains sampled over the course of one month.

Date	Total number of snails examined	Number infected	Percentage infection rates for individual drains
1972			
January	Not recorded	0	0
February	"	0	0
March	"	0	0
April	1342	0	0
May	2732	0	0
June	3072	1	0.5
July	2740	13	0.5-1.9
August	—	—	—
September	1704	5	0.5-1.1
October	3030	15	0.4-1.0
November	2154	0	0
December	1575	0	0
1973			
January	703	3	0.4
February	429	0	0
March	552	0	0
April	1988	0	0
May	2740	2	0.4
June	772	2	0.5
July	891	0	0
August	1710	12	0.4-1.9
September	1908	3	0.5-1.0

Sampling of field drains was erratic so that no drain could be sampled continuously or for the same months each year, but the combined results from all the drains indicate widespread, intermittent occurrence of infected snails throughout the estates, although they are probably absent at the southern end.

Stool survey of plantation villages:

Sample structure: In order to ensure that the 10 percent random sample of the plantation village survey was representative of the population, and in the absence of a complete official census, a subsidiary survey of three villages was carried out. The age structure of the combined data for males and females is compared with that of the sample in figure 3. It can be seen that, with the exception

TABLE 2: Prevalence of *S. mansoni* infection in a 10 percent random survey of plantation villages in 1973

Plantation village	Total number examined	Percentage positive	
		Direct smear	Concentration
A	66	13.6	19.7
B	67	0	6.0
C	79	7.6	12.7
D	76	2.6	13.2
E	105	6.7	17.1
F	93	6.5	32.3
G	77	5.2	16.9
H	70	2.9	15.7
K	85	2.4	10.6
L	112	0.9	7.1
M	127	3.1	15.7
O	69	2.9	5.8
P	18	0	16.7
Y	147	2.0	10.9
MEAN PREVALENCE	—	3.9	14.3

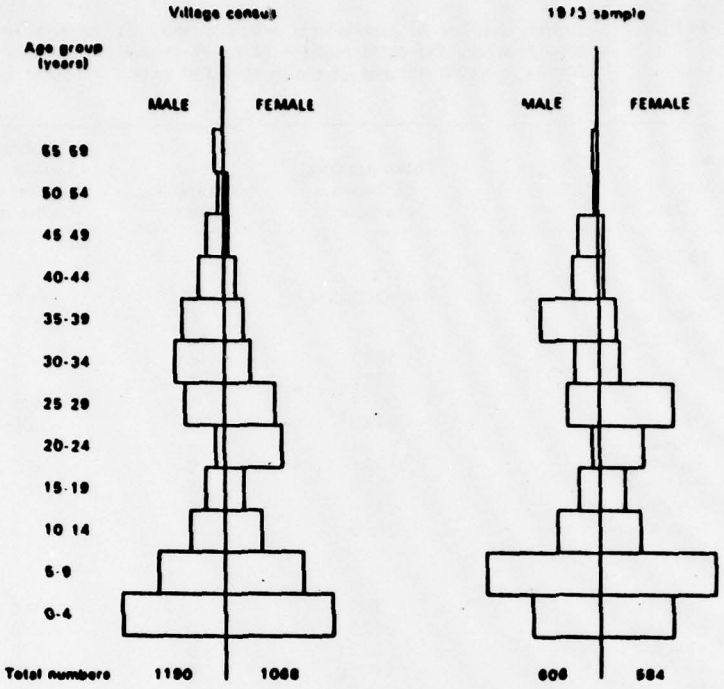


Figure 3. Age frequency pyramids for plantation population and stool survey sample.

of children aged less than four years, there is a very close correspondence between the two.

Stool survey: As shown in Table 2, in 1973 the concentration method gave an overall estimate of prevalence three times that obtained by the direct smear. However, there is no clear indication that the value of 14.3 percent represents a significant increase during those five years.

Table 3 also shows the variation in prevalence between villages, from 5.8 percent in village 0 to 32.3 percent in village F.

The distribution of *S. mansoni* infection in relation to sex was examined. The mean infection rate was 24.5 percent for males and 5.1 percent for females. When viewed according to age (figure 4) there is an apparent bimodal distribution for infected males about the ages of 12 and 29 years, while females show a much smaller single peak at age 12 years.

TABLE 3: Prevalence of *S. mansoni* amongst schoolchildren from both plantation and factory villages

Year	Number examined	Number positive	Percentage prevalence
1971	238	22	9.24
1973	238	31	13.00

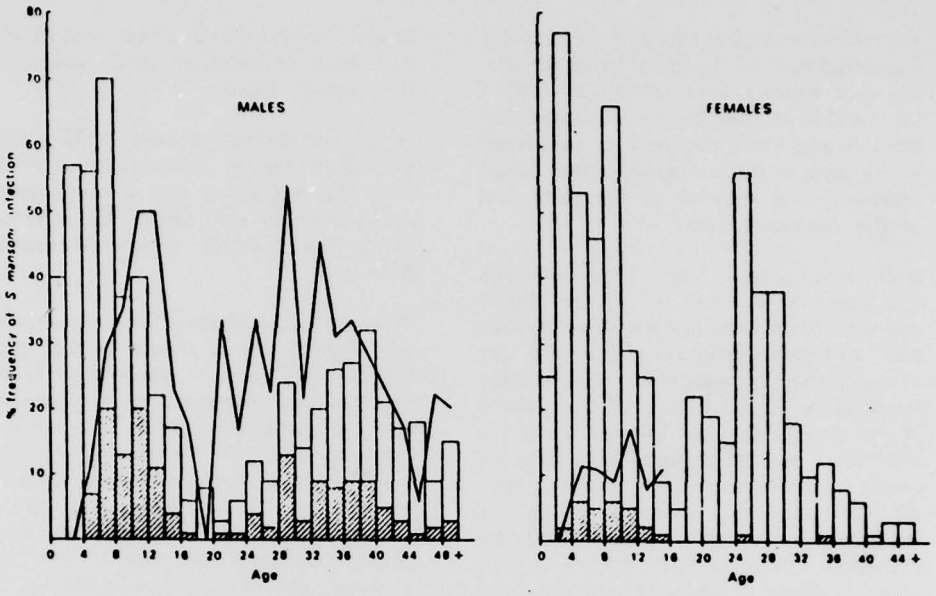


Figure 4. Age distribution and percentage frequency of *S. mansoni* infection in males and females at Wonji-Shoa Sugar Estates in 1973. Open histogram is total number in age group examined; hatched histogram is number positive; solid line is percent infected.

Survey of schoolchildren: In 1971, 238 children (13 - 15 years old) born on the estates and drawn from factory and plantation villages were examined for *S. mansoni* infection. Another group of 238 was examined in 1973 in the same age group, and from Table 3 it can be seen that there was an increase in prevalence of 3.5 percent, although the difference is not statistically significant. In addition, 189 of the 238 examined in 1971 were re-examined in 1973 and it can be seen from Table 4 that there was an increase of infection rate of 6.0 percent.

DISCUSSION

The programme of snail control initiated by Duncan and Lemma (1), and its continuation reviewed here, is essentially a demonstration of the integrated use of well-tested molluscicides in a novel irrigation system in Ethiopia, the results of which, it is hoped, will be of use in future irrigation schemes in the Awash River basin and elsewhere in the country. That population of *B. pfeifferi* in the various ecosystems found on these estates can be controlled by molluscicides is without doubt but the evidence of continued transmission of the infection as shown by the survey of 1973 suggests that a reappraisal of the measures is required.

Snail populations and control by molluscicides: Populations of *B. pfeifferi* vary in abundance with respect to time and habitat type. In Wonji Shoa, temporal fluctuations are the product of both climatic factors and water management practice, while the nature of the irrigation system itself, by provision of a variety of habitats, influences spatial distribution

and abundance. Duncan and Lemma (1) suggested that the dominant factor governing this species was rainfall in that it increased in number during the dry season months and then declined at the onset of the rains, when low levels of population prevailed until the rise in the early part of the following year.

Data accumulated since then indicates that the situation may not be as simple, and that temperature and irrigation practice may also have influence. Bruijning (7) believed that it was most unlikely that temperature would limit snail populations at any time of the year in this part of the Rift Valley. But the population fluctuations observed can be more adequately explained if temperature is considered to modify the effect of rainfall. The time at which *B. pfeifferi* shows a rapid decline is also when irrigation is stopped and the character of habitats is liable to change.

Thus, in the dry season of October, 1970, to February, 1971, there was a steady rise in populations of *B. pfeifferi* in the supply system which accorded with expectation. Unseasonal rain fell in November and December, 1971, and levels remained very low until an abrupt rise in June, 1972, just at the onset of the rains. They declined in July and by August were down to pre-rain levels. During the following season there was a dramatic rise within one month of the end of the rains, which had not been seen before.

Onset of the rains is usually accompanied by a fall in mean temperature followed by a steady rise through the dry season, December to May, and this was the case in 1970 - 1971 when *B. pfeifferi* populations followed the expected pattern. In 1971 - 1972, a continuous fall in temperatures accompanied the unseasonal rain and they remained low until February and *B. pfeifferi* was apparently suppressed until May. Measurements of daily water temperatures from November to April suggest that they were less than optimal for breeding of this species, which elsewhere has been shown to be close to 25°C, with little or none at 18°C, which was the average here.

In the following season, 1972 - 1973, the slight fall in temperature after the rains did not occur and it continued to rise, remaining well above the optimum; hence the dramatic expansion seen in November.

In an irrigation system, it is not obvious why rainfall *per se* should control populations. The stress of water flow is ever present, but minimal during the rains when the intake is closed. The converse may be true for the drainage system. Field drains in particular represent a series of intermittent habitats which are at different stages of an ecological succession at any one time according to the stage of cane growth of adjacent blocks. The average density of *B. pfeifferi* in seven such drains which were known always to contain the species unless treated revealed a similar pattern to that of the affluent system. Peaks were seen in March, 1971, June, 1972, and May, 1973, corresponding with major peaks in the supply canals. This similarity contrasts with the situation described by Dazo *et al.* (8) in an irrigation system in Egypt, where there was a distinct seasonal difference in the pattern of density fluctuations of *B. truncatus* living in supply canals and those living in drains. The former showed a peak in June and the latter in December. The synchrony seen at Wonji-Shoa emphasises the dominant effect of climatic factors which may be modified by water management practice.

The parallel fluctuations of populations in different parts of the system also suggests that the sampling programme was reasonably efficient. However, since the object was to detect the point at which populations were entering a phase of rapid expansion and so to apply molluscicides, the fact that such sudden increases took place in June and November, 1972, with-

out forewarning suggests that the frequency of sampling should be increased to fortnightly.

On each occasion when molluscicides were applied, massive reductions in snail numbers were recorded. Distribution of the chemicals throughout those parts of the system concerned was generally good. The subsequent length of time that populations remained depressed was determined by climatic factors. Thus, after the N-tritylmorpholine application in March, 1971, there was no significant population of *B. pfeifferi* present in the supply system until June, 1972, whereas after the application in December, 1972, repopulation occurred within six months, prompting a treatment in June, 1973. It would appear that one treatment per annum of the affluent system is insufficient to maintain control.

In this study temperature and irrigation practice have been observed to cause fluctuations in the populations of snails. Could these fluctuations observed during three years be due to natural variations in response to these factors alone or has the use of chemical induced or contributed to the changes? In 1971 there was no peak observed at the end of the dry season following the use of N-tritylmorpholine in March. In 1972, there was a massive increase in November soon after the resumption of irrigation.

What is the situation in the main drains and wadooks? The former do support *B. pfeifferi*, especially in the upper reaches, but except for the one close to Camp C (figure 1) they do not present an obvious hazard owing to their inaccessibility. Water volume and velocity increase down the course of the drain and daily fluctuations of level discourage colonisation by snails, unless the drain is overgrown. Treatment of these would be problematic but it should probably be restricted to focal application in places where habitual water contact is known to occur.

Wadooks support only very sparse populations of *B. pfeifferi* or, more often, none at all, which is in contrast to the situation described by Shiff (9) in an irrigation system in Rhodesia, where night storage dams seemed to be the preferred habitat. Nevertheless, work elsewhere in Ethiopia (Aram; personal communication) suggests that this species does not settle and flourish in large, open water bodies and on the rare occasions when it is found in such situations it is probably only for a very short period. Jobin and Michelson (10) showed experimentally that *B. glabrata* was stranded at vertical drawdowns ranging from 23 cm/h on a 5:1 slope and 0.1 cm/h on a 100:1 slope in daytime and, although no measurements have been made, it does seem reasonable to conclude that the daily drawdown experienced here, where up to 50 percent of the total volume is discharged, would not encourage *B. pfeifferi*. The steep sided margins deter people from swimming in most wadooks. This indicates that such wadooks are not important as transmission sites.

S. mansoni infections in B. pfeifferi: Until 1971, the need for the application of molluscicide had been determined on the basis of snail density, but now that there is evidence from the human infection survey that measures applied so far have not been entirely successful in interrupting transmission it seems reappraisal is necessary. One important parameter not utilized previously was the rate of *S. mansoni* infections in *B. pfeifferi*. Data accumulated to date suggest that infected snails are only found towards the end of the dry season and until a month or so after the rains. The reason for seasonal cercarial production is not obvious. The length of the prepatent period for *S. mansoni* in *B. pfeifferi* was shown by Foster (11) to vary with temperature and perhaps with the relatively low water temperatures, frequently below 18°C. Low temperatures are recorded between November and February and they cause delays in the maturation of infections acquired just before and during this period, giving rise to a bunching of

Chronic liver disease was seen occasionally (eg in 11 of 1993 hospital admissions in 1971) but *S. mansoni* was not suspected. In a country where cirrhosis and other chronic liver diseases are common (14, 15) it is difficult to incriminate *S. mansoni* without confirmatory liver biopsies or rectal snips.

Mass chemotherapy has never been attempted on the estates for economic reasons, but all infected cases found during laboratory investigations and field surveys (except for the group of schoolchildren involved in the incidence studies) have been treated with niridazole (Ciba 23 644 BA (Ambilhar)) 25 mg/kg, in two or three divided doses for seven days as inpatients in Wonji Hospital. This drug has been reasonably well tolerated but nausea and/or vomiting in about 15 percent, abdominal pain in 10 percent, and headache in 15 percent of cases were seen as side effects. Psychosis, hallucination, acute confusional state or suicidal depression, which occurred in 2 percent, stopped without sequelae when the drug was discontinued. This latter complication occurred in three patients (between 1970 and 1973) who were on concurrent administration of isoniazid for pulmonary tuberculosis. The drug was not well tolerated in cases of advanced liver disease or severe anaemia.

Sanitation and health education: The HVA Ethiopia Company is intent upon raising sanitary standards in all villages through its medical services. The original latrines overhanging canals, as described by Bruijning (7), have all been abolished. Pit latrines have also gradually been replaced by flush toilets connected to septic tanks or cesspools. There is a piped water supply in all villages, and villagers are encouraged to keep their villages clean, often by disciplinary action. But in spite of all these facilities contamination of the environment continues. The health education programme has not been comprehensive enough, since it is limited to schools and the few community centres available.

CONCLUSIONS

Since, on balance, it seems most probable that transmission of *S. mansoni* is still occurring at Wonji-Shoa, several recommendations are made for the future of the programme:

1. The frequency of the snail survey should be increased to fortnightly in order to improve the efficancy of molluscicide applications.

2. To substantiate the apparent seasonal fluctuation of infected snails a limited mouse exposure programme would be helpful in determining whether low density cercarial populations are present at times other than those already indicated. It would also be helpful to determine whether transmission is possible at all times when infected snails are found; and whether transmission is possible in those areas where snail densities are low, or even apparently absent. Maximum mouse infectivity does not necessarily correspond with maximum snail density (16) so that data obtained from mouse exposure experiments must be interpreted with caution. Incidence of infection is partly a product of water velocity so that stationary sentinel animals may not truly reflect the epidemiological situation obtaining in the human infection cycle. However, if combined with a modified snail sampling procedure, mouse exposure experiments could help to elucidate transmission potential on the estates and possibly explain some of the apparent contradictions.

3. If the seasonal fluctuation of infected snails proves to be a regular pattern then application of molluscicides could be adapted accordingly. Attention should be focussed on eliminating the snail population present immediately before the rains but before irrigation is stopped in order to minimise the post-rains potential; and again immediately upon resumption of irrigation to eliminate those survivors which most likely show high infection rates. These treatments would most efficiently be done with niclosamide, which obviates

the need for long term availability of irrigation water at a time when plantation requirements tend to be erratic whereas it would be necessary if N-tritylmorpholine was used. This procedure should be carried out annually without reference to snail densities and combined with at least one interim treatment, so raising the annual number of treatments to a minimum of three.

4. Attempts should also be made to define those sites responsible for the elevated transmission amongst males, particularly in the period August - November when infection in snails is thought to be high, but also at a time when irrigation is restarted causing changes in distribution and character of water bodies. Careful observation of sites favoured by children for swimming and further water contact studies in the villages with high prevalence rates, such as A and F, might be profitable as outlined by Farooq (17). A survey of the two factory villages is also essential; the pattern of infection in populations of these two villages could provide further information on the location of transmission sites so far overlooked.

The authors gratefully acknowledge the continued interest and cooperation of the Management of HV Ethiopia and of Dr Aklilu Lemma, Director of Institute of Pathobiology, Addis Ababa University, and also the technical support of Ato Makonnen Yimam in the laboratory and Ato Birhanu Sileshi in the field.

REFERENCES

1. Duncan, L and Lemma, A. 1976. Investigations into the control of schistosomiasis at the HVA Wonji-Shoa sugar estates in Ethiopia. 1. Initiation of the project *Ethiop. med. J.* 14, 3.
2. Beynon, KI and Thomas, GE. 1967. Analytical methods for determination of the molluscicide N-tritylmorpholine in water. *Bull. Wld Hlth. Org.* 37, (1) 47.
3. Strufe, R. 1963. Modified methods for determining Bayluscide in field trials. *Pflanzenschutz-Nachrichten 'Bayer'*, 16 (5), 221.
4. Webbe, G. 1962. The transmission of *S. haematobium* in an area of Lake Province, Tanganyika. *Bull. Wld Hlth Org.* 27, 59.
5. Duncan, J, Lemma, A and Mazengia, B. 1970. A comparison of certain stool examination techniques with special reference to the efficacy for schistosomiasis surveys in the Awash Valley. *Ethiop. med. J.* 8, 17.
6. Ritchie, LS. 1948. An ether sedimentation technique for routine stool examinations. *Bull. US Army Med. Dept.* 8, 326.
7. Bruijning, CFA. 1969. Bilharziasis in irrigation schemes in Ethiopia. *Trop. geog. Med.* 21, 280.
8. Dazo, BC, Hairston, NG and Dawood, IK. 1966. The ecology of *Bilunus truncatus* and *Biomphalaria alexandrina* and its implications for the control of bilharziasis in Egypt — 49 project area. *Bull Wld Hlth Org.* 35, 339.
9. Shiff, CJ, Clarke, V de V, Evans, AC and Barnish, G. 1973. Molluscicide for the control of schistosomiasis in irrigation schemes. A study in Southern Rhodesia. *Bull. Wld Hlth Org.* 48, 299.
10. Jobin, WR and Michelson, EH. 1969. Operation of irrigation reservoirs for the control of snails. *Am. J. trop. Med. Hyg.* 18, 297.
11. Foster, R. 1964. The effect of temperature on the development of Schistosoma massoni Sambin 1907 in the intermediate host. *J. trop. Med. Hyg.* 67 (12), 289.
12. Francis, TI and Wright, SG. 1971. *Schistosoma mansoni* infestation of the lower bowel in Nigerians. *Trop. & Geog. Med.* 23, 84.
13. Cowper, SG. 1965. Schistosomiasis in Nigeria. *Ann. trop. Med. Parasit.* 57, 307.
14. Coady, A. 1965. The possibility of factors of plant (particularly fungal) origin in Ethiopian liver disease 1. *Ethiop. Med. J.* 3, 173.
15. Teferra, A and Abdulkadir, J. 1968. The interim report of the Medical Registration Committee. *Ethiop. Med. J.* 6, 91.
16. Webbe, G. 1965. Transmission of bilharziasis: 2. Production of cercariae. *Bull. Wld. Hlth Org.* 33 (2), 155.
17. Farooq, M. 1966. Importance of determining transmission sites in planning bilharziasis control. Field observations from the Egypt — 49 project area. *Am. J. Epidem.* 83, 603.

Am. J. Trop. Med. Hyg., 28(3), 1979, pp. 526–530

SCHISTOSOMIASIS IN OMO NATIONAL PARK OF SOUTHWEST ETHIOPIA

GEORGE K. FULLER,* AKLILU LEMMA, AND TRINIDAD HAILE
Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia

Abstract. Schistosomiasis mansoni infection was found in more than 50 tourists who had visited Omo National Park, Ethiopia, and bathed and swum in the Mui River. A survey revealed *Schistosoma mansoni* infection in 41% of Park residents and in 33% of the neighboring Suri people. Eggs were found in stools and adult worms at autopsy of wild *Papio anubis* and *Cercopithecus aethiops*. Trematode larvae were found in 27% of *Biomphalaria pfeifferi* snails found in the Mui River. The source of the disease and the implications of its spread with the future development of the Omo Valley are discussed.

Since 1974 more than 50 tourists have developed schistosomiasis (*Schistosoma mansoni*) after visiting the Omo National Park in southwest Ethiopia, with the latest patients being diagnosed in July 1978. Cases have been diagnosed at the Institute of Pathobiology, Addis Ababa University, Addis Ababa, as well as in institutions in Europe and the United States. These cases involve people who have utilized campgrounds located along a small stretch of the Mui River where clear pools were used for swimming and bathing about 30 km from the river's confluence with the Omo River (Fig. 1). This has led to a growing concern over the source of the disease and its transmission in southwest Ethiopia; thus, the Institute of Pathobiology undertook a study to determine the sources and extent of the disease.

The Omo National Park, a savannah rich in plainsland game, stretches along the western side of the Omo River for over 100 km. The Park extends westward into the foothills of the Maji Mountains (Fig. 2). A number of different ethnic groups live near the Park including the Suri (Surma, Tid) people living at the Park's western border and extending south and west of the Park to the Sudan border, and the Deze (Maji) people living north and west of the Park and forming the major portion of the Park's labor force. To date, there are no major irrigation sites in the lower Omo Basin, though small plots are irrigated by the Dassanetch near the Omo Delta on an experimental basis.

Accepted 14 October 1978.

* Present address: 4091 Lincoln Avenue, Oakland, California 94602.

MATERIALS AND METHODS

Collection and examination of the snail intermediate host

Snails were collected with gloves and dip nets from rocky pools along the course of the Mui River (Fig. 3) and at other water sources in the area. Collections were concentrated along a 2-km stretch of the Mui River around the National Park Headquarters (sites A–C) and upriver 22 km near a Suri (Surma) village (site D). Snails were crushed and examined on location under a dissecting microscope for larval forms of trematodes, particularly *Schistosoma mansoni*; *S. haematobium* is apparently absent from this area (Dr. Guiseppe DeSole, manuscript in preparation).

Stool collections and examinations

Stool specimens were collected from the Suri (Surma) people living on the western border of the Park, preserved in 5% formalin, and examined at the Institute of Pathobiology (by Ato Bahta Mezengia), using the Ritchie concentration method.[1] Stool specimens of primates from defecation sites and of animals at autopsy were also collected and preserved for later examination.

Nonhuman primates

Primates of three different species, *Papio anubis*, *Cercopithecus aethiops*, and *Colobus abyssinicus*, were shot by Park authorities and examined at autopsy for adult schistosome worms.

RESULTS

Snails found in the Mui River included *Biomphalaria pfeifferi*, *Lymnaea* spp., and *Bulinus* spp.

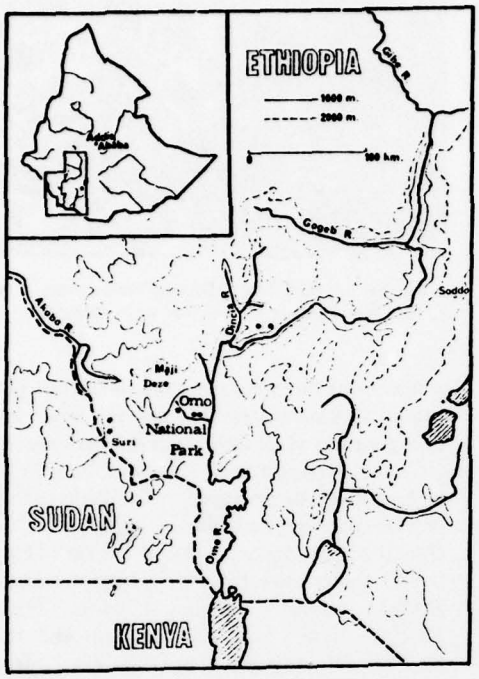


FIGURE 1. Map of southwest Ethiopia showing Omo National Park and location of *Biomphalaria* snail collection sites (dots).

FIGURE 2. Facing the Omo National Park from the Maji Mountain foothills.

Lymnaea are common all year round. *Biomphalaria* are abundant from December through March, but difficult to find during and after the rains of April and May. We have found this host of *Schistosoma mansoni* in many small streams in the Omo and Sobat basins (Fig. 1). *Bulinus* spp. appear not to occur in large numbers even in the dry season. *B. pfeifferi* occurred in large numbers in the Mui River around Park headquarters and campgrounds, as well as at various points examined for 22 km above the headquarters, though it was much less abundant 1 km below Park headquarters where the river enters the flat savannah that extends to the Omo.

On examination, the *B. pfeifferi* and *Lymnaea* spp. collected in the vicinity of the Park headquarters (sites A, B, and C) were both found to be infected with trematode larvae. Overall, 49 (27%) of 184 *B. pfeifferi* collected at various sites along a 2-km stretch at and above the Park headquarters were infected (Table 1). Thirty-seven (about 75%) of *B. pfeifferi* infections could be identified as *S. mansoni*. Of 35 *Lymnaea* examined, two were infected with trematode larvae.

From site D, 22 km upstream, no infected snail was found among 100 *B. pfeifferi* and 22 *Lymnaea* examined. This is a point where people from

FIGURE 3. Mui River in Omo National Park near tourist camp site.

TABLE 1

Trematode infection rates in Biomphalaria pfeifferi *and* Lymnaea *spp. collected from different sites along the Mui River*

Site*	Snail species	No. examined	No. positive	% positive
"A"†	*B. pfeifferi*	83	25‡	30
	Lymnaea	35	2	6
"B"	*B. pfeifferi*	49	12‡	24
	Lymnaea	–	–	–
"C"	*B. pfeifferi*	52	12‡	23
	Lymnaea	–	–	–
"D"	*B. pfeifferi*	100	0	0
	Lymnaea	22	0	0

* See text.
† One kilometer below site "A." *B. pfeifferi* were scarce but *Lymnaea* were plentiful. Some of *Lymnaea* were infected.
‡ Approximately ¾ of the trematode larvae seen were definitely identified as those of schistosomes.

the Suri villages collect water and bathe during the dry season, as here the water flows directly out of the ground (Figs. 4, 5).

One-time stool examinations (Table 2) revealed

FIGURE 4. Suri woman washing in Mui River where *B. pfeifferi* were collected.

FIGURE 5. Suri boy collecting water from a pool in Mui River where *B. pfeifferi* were collected.

a number of intestinal parasites in the test population. In general, the Suri had far fewer intestinal parasites of all kinds than did the Park labor force. With respect to *S. mansoni* (Table 3) 41% of 86 individuals employed in the Park and their families were infected. Of this group, most came from the Maji area northwest of the Park (Fig. 6) but a number came from other highland areas of Ethiopia, where *S. mansoni* is known to occur. Fourteen were young children born and raised in Mui. Of these children, 29% were infected, showing active current transmission in the immediate area. Among the Suri (Surma) living 22 km upstream from the Park headquarters, 7 of 21 examined were infected with *S. mansoni*.

Stool specimens collected from baboon defecation sites were *S. mansoni*-positive in 2 of 4 specimens. One grivet monkey stool specimen

TABLE 2

Number and percentage of individuals with intestinal parasites found in single-stool specimens, using the Ritchie concentration method for a population survey in Omo National Park

Parasite*	No. positive	% positive
Schistosoma mansoni	43	40.2
Fasciola gigantica	2	1.9
Taenia spp.	3	2.8
Ascaris lumbricoides	21	19.6
Trichuris trichiura	41	38.3
Ancylostoma duodenale	19	17.8
Entamoeba histolytica	6	5.6
Giardia lamblia	6	5.6
Total no. examined:	107	

* Identified by Bahta Mazengia of the Institute of Pathobiology.

TABLE 3

Number and percentage of individuals with S. mansoni *eggs in stool specimens by age and by sex*

Age group in years	No. examined	No. positive	% positive
0–5	16	3	18.8
6–10	14	6	42.9
11–20	21	11	52.4
21–30	40	17	42.5
31–40	13	5	38.5
>40	3	1	33.3
Total:	107	43	40.2
Male	65	30	46.2
Female	42	13	31.0

collected was also positive, but none of four colobus monkey stools was positive. Stool specimens collected from animals at autopsy revealed *S. mansoni* eggs in 1 of 2 grivet monkeys (*C. aethiops*) and 1 of 2 baboons (*P. anubis*), but none was found in either of two colobus monkeys (*C. abyssinicus*).

At autopsy, adult *S. mansoni* worms were found in one baboon and one grivet monkey. The baboon had less than 30 worms in its mesenteric and portal veins. The grivet had well over 100 adult worms. The negative grivet was shot several kilometers from the river.

DISCUSSION AND SUMMARY

Though schistosomiasis has been thoroughly described from many areas of Ethiopia,[2-6] until now transmission has not been documented in the Omo River Basin. Yet this basin comprises about 217,000 km^2, or nearly one-fifth of the Ethiopian land mass.[7] Kloos and Lemma point with particular interest to the very low infection rates found among farm laborers in the Awash valley who came from the Kembata/Hadya, Wollamo, and Gurage areas, areas which border the Omo River in the central highlands.[6] The present study demonstrates that the Omo Basin, with its many small tributaries, is not only a potentially favorable area for transmission to occur, but that active *S. mansoni* transmission is well-established among at least some populations in the region.

It is difficult to determine whether schistosomiasis was recently introduced to southwest Ethiopia or whether it has been present and unrecognized for a long period of time. Finding

FIGURE 6. Maji Mountains countryside where Deze Park employees originate.

S. mansoni among children born in the Omo National Park, as well as in baboons and grivet monkeys there, indicates active transmission in the Park. Even though most of the workers come from the Maji area, this does not rule out its recent introduction by workers originating in other regions of Ethiopia. However, with infection common among the indigenous Suri, schistosomiasis is not likely to be a recent introduction. The Suri have little contact with the Park village and throughout their territory within Ethiopia *Biomphalaria* snails are readily found.

Kubasta has suggested for Harar in eastern Ethiopia that Egyptians were the source of the disease in the late 19th century when they occupied part of the country.[2] With *S. mansoni* occurring in Uganda, Kenya, and the Sudan, as well as Ethiopia,[8,9] there is no reason to attribute occurrence of schistosomiasis in the southwestern part of Ethiopia to recent introduction. In light of its wide distribution, schistosomiasis may be no less ancient in Ethiopia than it is in Egypt.

It is noteworthy that transmission of schistosomiasis in the Omo National Park is occurring at around 650 m altitude with *B. pfeifferi* as the vector. In the Awash Valley, *B. pfeifferi* has been found only as low as 740 m (at the Melka Sadi plantation) and the lowest altitude at which *B. pfeifferi* has been found in a natural water body in the Awash Basin is 1,260 m.[6] This would suggest that *S. mansoni* might present a greater health problem in the lower Omo Basin than it has in the Middle and Lower Awash, and that the range of *B. pfeifferi* in Ethiopia, especially in the southern part, may be even greater than was originally suggested by Brown and Lemma.[10]

FIGURE 7. Dassanetch women building a home in the lower Omo Basin near the Omo Delta showing the striking difference in terrain from the Suri area and Omo National Park.

The range of hills and low-lying mountains that extend to the Sudan border and form a natural divide between the Sobat Basin and the Omo Basin appears to form a favorable habitat for *B. pfeifferi* (Fig. 1). The flatter, low-lying plains of the lower Omo presently are much less likely to sustain a significant *B. pfeifferi* population, as few water sources other than the silt-laden Omo exist (Fig. 7). With the introduction of irrigation, this situation could rapidly change.

Determining the distribution of the disease is of importance and needs further study, owing to its potential impact on the resident population and on the tourist industry in the Omo National Park. Schistosomiasis should be a major consideration in any future plans to develop the Omo Valley.

REFERENCES

1. Ritchie, L. S., 1948. An ether sedimentation technique for routine stool examination. *Bull. U.S. Army Med. Dept., 8:* 326.
2. Kubasta, M., 1964. Schistosomiasis mansoni in Harar Province. *Ethiopian Med. J., 2:* 260–267.
3. Lemma, A., 1969. Bilharziasis in the Awash Valley. 1. An epidemiological study with special emphasis on its possible future economic and public health importance. *Ethiopian Med. J., 7:* 147–176.
4. Schaller, K. F., 1972. *Athiopiea: Eine geographisch-medizinische Landskunde; Ethiopia: A Geomedical Monograph.* Springer-Verlag, Berlin, Heidelburg, New York, 188 pp.
5. Polderman, A. M., 1974. Intestinal schistosomiasis north and west of Lake Tana, Ethiopia. *Trop. Geogr. Med., 26:* 170–177.
6. Kloos, H., and Lemma, A., 1977. Schistosomiasis in irrigation schemes in the Awash Valley, Ethiopia. *Am. J. Trop.-Med. Hyg., 26:* 899–907.
7. Butzer, K. W., 1971. *Recent History of an Ethiopian Delta.* Department of Geography, University of Chicago, Chicago, 183 pp.
8. Ayad, N., 1956. Bilharziasis survey in British Somaliland, Eritrea, Ethiopia, Somalia, The Sudan, and Yemen. *Bull. W.H.O., 14:* 1–117.
9. Nelson, G. S., 1958. *Schistosoma mansoni* infection in the West Nile District of Uganda. Part II. The distribution of *S. mansoni* with a note on the probable vectors. *East Afr. Med. J., 35:* 335–344.
10. Brown, D. S., and Lemma, A., 1970. The molluscan fauna of the Awash River, Ethiopia, in relation to the transmission of schistosomiasis. *Ann. Trop. Med. Parasitol., 64:* 533–538.

THE EPIDEMIOLOGY OF SCHISTOSOMA MANSONI INFECTION IN TENSAE BERHAN, ETHIOPIA. II. HUMAN WATER CONTACT PATTERNS

Helmut Kloos (Ph.D.) and Aklilu Lemma (Sc.D.), Institute of Pathobiology
1979

Human exposure to infective water is the most critical link in the transmission of schistosomiasis. Although information on type and role of human behavior relevant to schistosome transmission and on underlying sociocultural variables are prerequisites for successful control programs, few such studies have been made. This paper is based on water contact observations and household surveys in the town of Tensae Berhan in central Ethiopia. Possible methods for the reduction of exposure to infective water are discussed within the context of the local schistosomiasis control program.

Few systematic studies on human behavioral patterns in schistosomiasis epidemiology to date, including those by Dalton (2) and Jordan (9) in St. Lucia, Dalton and Pole (3) in Ghana, Jobin and Ruiz-Tiben (7) and Lipes and Hiatt (13) in Puerto Rico, Husting (6) in Rhodesia, Farooq and Mallah (4) in Egypt, and Lemma and coworkers, and Polderman (16) in Ethiopia, have analyzed duration and frequency of water contact in relation to infection. The objective of control programs based upon this approach is to identify, measure and influence the relevant sociocultural variables to reduce or eliminate exposure to potentially dangerous water (2).

During the first schistosomiasis control project at the village level in Ethiopia, in Adwa, attempts to modify human water use patterns failed (12). In the second project of this type, begun in 1974 in Tensae Berhan, central Ethiopia (13), we attempted to quantitatively determine who engaged in what type of water contact and to identify sociocultural factors influencing these activities. By comparing the results of this baseline study with those of another study planned at the end of the 5-year project (1982), it is hoped to assess the epidemiological significance of human contact with the local rivers and to evaluate the effects of the proposed schistosomiasis control program in this community.

PROJECT AREA

Tensae Berhan is situated on a small plateau about 40 meters above the deeply incised Ferekasa, Homba and Arba Dima rivers, which pass by the town. The hot and semiarid climate prevents the formation of any other permanent surface waters and thick, extensive basalt strata prohibit the digging of wells by the local population. Tensae Berhan offers many opportunities for socioecological studies of schistosome transmission, because human water contact is highly localized at the town's 4 major river fording points. They are located at 2 of the 3 local rivers, the only water source for the town, except for small amounts of rain water. About 90% of all water contacts the town's population has with the rivers and an estimated 5% of all defecations take place at the 4 main contact and fording sites along the Ferekasa and Arba Dima rivers. Few persons frequent the rivers between the fording sites, owing to the steep slopes and cliffs extending to the water's edge (Fig. 1) and the general inaccessibility of intervening wooded areas.

The people and community leaders of Tensae Berhan are aware of their poor water supply and continuously try to improve the water that is

increasingly polluted because of population growth in the river basin. Several years ago residents built a pipeline parallel to the Ferekasa River, leading to a reservoir in town to which raw river water is pumped. Unfortunately, the reservoir pipeline becomes choked with silt every year during the big rains and remains closed until put into operation again after the rains. All residents must obtain most of their domestic water from the rivers during the seasonal dysfunction of the reservoir.

MATERIALS AND METHODS

Parasitological Survey

In a parasitological survey carried out by the Institute of Pathobiology in November 1975, all occupied houses in Tensae Berhan were numbered and mapped. Ten percent of all households, representing 796 persons, were randomly selected for study and examined for intestinal parasites, especially S. mansoni (13).

Water Contact Observations

A water contact survey was carried out in November 1975 and a second one in July 1976. Four stations were selected along the Ferekasa and Arba Dima rivers (Fig. 1) to observe water contact and contaminative activities. They are located at the 4 major contact sites along these rivers in Tensae Berhan, at all of which B. pfeifferi occurs seasonally (13). Eight local students, working in shifts from 6:45 A.M. to 6:30 P.M., were positioned at those stations for 7 consecutive days in November (during the dry period) and 2 students made observations for the same length of time at stations 1 and 2 in July (during the rainy period). They recorded all human water contact and contaminative activities within their field of vision, by time of arrival of individuals at the water. Activities were recorded under 13 categories (tables 1 and 2), by sex, estimated age and, at site 1, religion. The observers knew nearly all people at the rivers, and checking the estimated age data with information obtained from 42 water users themselves established that more than 90% of the entries were correct. Duration of individual water contact and proportion (percentage) of body surface wetted under the various categories were recorded for 566 persons at the 4 contact sites, providing a representative picture of the community's water use patterns at the 3 rivers. Contacts of nonresidents, mostly visitors, were recorded separately. Employment of local observers minimized behavioral changes by persons coming to the rivers, so no attempts were made to disguise the observers' presence.

Epidemiologically relevant activities were studied under 3 categories: water contact (potential exposure of humans to schistosome cercariae), contamination (defecation, leading to possible release of schistosome ova by infected individuals) and preventive behavior (activities, customs and beliefs that could lead to avoidance of exposure or contamination), criteria suggested by Dunn (5). Whereas water contact and fecal contamination favor transmission, preventive behavior and noncontamination tend to reduce transmission. Following the terminology developed by Farooq and Mallah (4), swimming, bathing, laundering and ablution were classified as "mixed activities" due to the possible involvement of both water contact and contamination.

Interview Surveys

Interviews were made with householders and persons encountered at the rivers to determine the numbers of persons with river contact away from the observation sites and at the reservoir, the number using rain water, and the number of visitors or transients using the rivers. Information was also obtained on the average number of times per day or week water cas carried and clothes laundered and children swam or played in the rivers.

RESULTS

Water Contact

During the 492 hours of observation at the 4 study sites along the rivers, 30,925 occurrences of water contact and contamination (excluding the 2,130 contacts by nonresidents) were recorded. They consisted of 28,023 exposure activities, 2,788 mixed activities and 114 contaminative activities (Table 3). Each person coming to the rivers engaged on the average in 2.3 contact activities. Marked differences in type of water contact were observed among the different age groups and between males and females (tables 1 and 2).

Swimming, playing in water, ablution (ritual washing of Moslem men before prayer) and fetching water with the ensera (clay water jar) were confined to specific age groups or to one of the sexes; fording the rivers, laundering, bathing, drinking water, and defecation were performed by the whole population. Males of all ages had more frequent and intense (greater body area exposed) water contact than females, but duration of contact was greater for females. Females ages 21-30 had more frequent contact than did other female groups (table 1-3).

Fetching water with the ensera, grouds, etc.: Fetching and carrying household water with the earthen ensera is the water contact activity most closely associated with female duties in Tensae Berhan, as in most other communities in central Ethiopia. Most females above 5 or 6 years carried domestic water on their backs up the steep slopes, but by far the most contacts (53.4%) were reported for the 11-20 age group. Water-fetching and carrying with the ensera represented 37.4% of all female contacts. Only 14 male contacts involving water fetching with tne ensera were observed and 822 water fetching activities were associated with gourds, coffee pots, bottles, tin cans, or with donkeys. Males usually obtained drinking water in this manner on their way to the fields and pastures.

During fetching and carrying water with the ensera, which involves rinsing, filling and carrying it over a distance of from 400 meters to 2 kilometers (Fig. 1), approximately 2% of the body surface is normally wetted, mostly on the hands, and mean exposure time is 117 seconds. Persons engaging in this activity seldom stepped into the water, in order not to stir up mud. However, some females did get their enseras wet while filling them, or spilled some water down their backs on their way home, in spite of using cans, gourds or large leafed twigs as oscillation stoppers. Most females arrived in groups of 2-6 to fetch water, and nearly all returned between 1 and 6 times daily, usually in the same group. Mean exposure time when fetching water with gourds and other small containers was only 25 seconds, also with a 2% body surface exposure.

Fetching water with donkeys: Mostly professional water vendors, nearly all young men, and servants fetch water with the use of donkeys. This activity exposes larger parts of the body for longer periods than does the use of the ensera. These persons commonly walk into the rivers to knee depth and hold submerged the jerry can or oil drum until filled. Both hands usually are exposed for this period, while spillage may wet other areas, exposing at least 6% of the body surface. Mean duration of individual contacts was 2 minutes, 35 seconds. Many persons worked in groups, assisting one another in loading the donkeys with the heavy cnas, commonly leading to conversations and increasing the number of people exposed.

Washing hands: Washing of hands was seldom an isolated activity. In more than 90% of all cases it was associated with river crossing, laundering, drinking water, washing arms, legs and face, ablution and bathing. Mostly males returning from the fields or from visits to other communities washed their hands at the rivers. Females tended to wash their hands at home. Washing hands, by itself, took an average of 7 seconds, exposed 2% of the skin surface and represented less than 1% of all contacts for both sexes.

Washing extremeties: The second most common activity engaged in by men was washing arms, face and legs. This represented 15.2% of all male contacts and was the third most common activity of females. Males and females between the ages of 6 and 45 commonly washed themselves at the rivers, the chief cleansing and refreshing activity engaged in by the local population between baths. During this activity, which lasted on the average nearly 12 minutes, people usually sat on rocks in the rivers or squatted on the shore. Mean exposure was 15%.

River fording: River fording was the most common type of contact activity for both sexes, 54.1% of the male and 34.1% of the female contacts. Upon close inspection, it was seen that only about 55% of those persons fording, mostly children and older persons, got their feet wet. Most persons washed their hands, face and feet or drank water after fording or while talking to friends, neighbors and other townspersons at these stops, thus prolonging exposure.

Drinking water: Drinking water was done by using the hands or cups or with gourds. This activity was most often observed in association with river crossings. Exposure and duration of contact was minimal (2% and 10 seconds), especially when a gourd was used.

Children playing: Few children were seen playing in the rivers, mainly due to their distance from the twon. It was often difficult to distinguish between playing and domestic activities. Duration of playing ranged from a few seconds to more than an hour and exposures varied similarly.

Bathing: Although fewer males and females had "mixed" contacts than water contacts, the greater extent and duration of exposure involved in the 4 mixed activities (Table 1) made them far more important in schistosome transmission than those in the water contact category. In spite of the hot climate in Tensae Berhan, there is little bathing and only hands are washed before and after meals in water brought from the rivers, chiefly because of a general modesty toward body exposure. Bathing represented only 2.5% of all observed male and 0.4% of the female

contacts. Nearly all females and children below 4 bathe at home. Furthermore, only males usually washed their whole body at the rivers (an activity that usually took about 10 minutes).

Laundering: Washing clothes, although now mostly done in Ethiopian towns with soap or detergent instead of crushed berries of the endod (Phytolacca dodecandra) bush, still retains most other traditional elements. Clothes are kneaded and stomped on slightly concave rocks, then rinsed and dried and bleached in the sun, a process that may result in exposure lasting more than half a day. On the average, washing clothes took 30 minutes, longer than any other activity, but resulted in less exposure than the other mixed activities, because only 15% of the body surface was normally exposed. Few persons walked into the rivers during laundering but many drank water, washed their faces and even bathed while their clothes dried nearby. Washing clothes is not exclusively a female activity in Tensae Berhan. Nearly 40% of the persons observed laundering were males. About 40% of the males and females were in the 6-20 age group. Young married women had the largest amounts of laundery and washed longest, and housewives and servants of large families were said to come to the rivers up to 4 times weekly.

Ablution: Ritual washing before prayer is performed only by adult Arussi males in the rivers in Tensae Berhan. About of the persons undergoing ablution during the present study took a complete bath; others only washed parts of the body, especially pubic and anal areas. Mean exposure was 50% and duration of contact 10 minutes.

Swimming: Swimming was observed in several deep pools in the Ferekasa River between sites 2 and 3, involving almost exclusively boys ages 6-18. The 4 girls ages 9-11 seen swimming were in a group, when no boys were present. Swimming is an important pasttime and social activity of boys, who usually swam and played in the river in groups of 4-15. although difficult to measure, actual time spent swimming was about 15 minutes. Most of the 55 boys interviewed at the rivers said that they went swimming whenever possible, at least once and as often as 4 times a day during the dry season. The same individuals usually were seen at the pools and inquiries established that many boys in Tensae Berhan do not know how to swim.

Diurnal Variations in Water Contact

The socioeconomic and climatic cycles determine diurnal, weekly seasonal variations in human activities in Tensae Berhan, and most types of water contact showed some degree of regularity. The daily socio-economic cycle begins around sunrise (about 6 A.M.) and ends shortly after sunset (6 P.M.). Nearly all exposure type activities, especially river fording and fetching water with the ensera, took place primarily in the mornings and evenings. Most housewives were well aware that the river water is cleanest during morning hours and tried to obtain most household water before noon. Most mixed activities were observed during the hottest part of the day. More than 48% of all persons fetching water and fording came to the rivers between 6:45 A.M. and 9 A.M. and between 5 P.M. and 6:30 P.M. and more than 50% of all persons laundering, bathing, drinking, swimming, playing and abluting had water contact between 9 A.M. and 3 P.M. (Fig. 2).

Weekly Variations

Market days and holidays introduced variations into the weekly cycle of water contacts in Tensae Berhan, which normally attracts many residents and several hundred nonresident farmers from surrounding rural areas to its Wednesday and Saturday markets. Fewer women carried water and laundered during those 2 days and more persons bathed and laundered on Sundays. The epidemiological significance of these variations is difficult to estimate as it is not known whether there are weekly changes in the biological cycle in the local rivers. The doubling of contacts by visitors on market days, however, may result in intensified transmission as some of them bathe and ablute when fording and defecate behind nearby bushes.

Seasonal Variations

The seasonality of water use in Tensae Berhan tends to favor S. mansoni transmission among farmers and other poor segments of the population and to protect the higher socioeconomic groups, including the users of the reservoir. Biomphalaria pfeifferi snails are most numerous during the 2 dry periods, before they are flushed out of their habitats during the big rains in August (13). No great differences in frequency of water contact was observed between the 2 surveys (Table 4), which were mainly due to the delay of the big rains in 1976. Normally a major change in water source occurs when the reservoir seasonally becomes inoperative. All households using the reservoir during the dry season must obtain nearly all their water from the rivers as rain water collected from the corrugated metal roofs is the only other available source until the reservoir is repaired.

Site Variations in Water Contact Activities

Marked differences in the number and type of water contact activities were observed among the 4 contact points. Site 1 was the major laundering place but only a minor river fording point. Site 2 was the primary water contact point of the Tensae Berhan population, where more than 50% of all contacts took place, followed by sites 3 (32.5%), 4 (7.4%) and 1 (6.6%). Site 2 is the most important place for river fording and associated activities, the main water-fetching point and a popular swimming area. Site 3 is a heavily frequented water-fetching point and swimming area. Site 4 is a minor contact point, owing to its location near the low population density section of the town.

Within each of the 4 contact sites water contact was place specific, reflecting efforts by the local population to obtain the cleanest water possible. The place for water fetching usually was furthest upstream, above the fording points, which in turn were located above the laundering, bathing, swimming and ablution places. People crossing the rivers commonly drank just above the fording points, to avoid water contaminated and silted up by humans and livestock.

Contaminative Activites

Defecation was seldom observed at the rivers. Most persons defecated in nearby bushes, behind large boulders in the river beds, often out of view of the observer. More males (95) than females (19) were observed defecating at the observation sites but no marked age differences were noted. About half of the 527 latrines existing in Tensae Berhan in 1975, built after encouragement by the local sanitarian, were either not

usable or not used. The primary reason given by residents for their neglact was that bad smells in latrines can cause disease.

Preventive Behavior

Forms of preventive human behavior observed at the rivers included abstention of females and many adult males from swimming and bathing in the rivers; restriction of most water fetching activities to early morning and evening hours and a desire to obtain household water in the early morning; use of reservoir and rain water; tendency to carry small children across the rivers; use of stepping stones while fording; and localization of different types of activities at specific points within fording sites. Although it is not clear to what extent these behavioral traits result from conscious effort or habit, their relevance to schistosome transmission will be explained to the resident population within the context of the local schistosomiasis control project (13) in order to further reduce their exposure to potentially dangerous water.

A drastic decline in river water contacts between the November and July surveys was observed in swimming, with 333 boys engaging in this activity in November at site 2, and 4 boys in July. Interviews showed that swimmers considered the ongoing schistosomiasis control project to have increased the level of awareness of this disease to a point where nearly all of them abstained from swimming. This change was not caused by seasonal change in climate and is all the more remarkable as the emphasis in the project was on snail control, and no systematic effort in health education had been made.

Relationship Between Water Contact and S. mansoni Infection

Although the parasitological data (13) are not strictly comparable with the water contact data, due to the differences in sample selection, values for all exposure variables (number, duration and extent of contacts) and for schistosomiasis prevalence were higher in males than females ages 1 to 30. The highest exposure and infection levels in males were recorded in the 11 to 20 group and in females in the 11 to 30 group. After their decrease to a low point in the 41 to 50 group of both sexes, infection rates increased again toward the older ages, whereas exposure values continued to decrease (tables 1-3).

Cercariometric studies were not made in Tensae Berhan but Polderman (16) found in northern Ethiopia that most S. mansoni cercariae are released by B. pfeifferi between 11 A.M. and 3 P.M. Thus the early morning and evening activities were relatively safe and the midday activities more dangerous. The concentration of mixed activities during noon and afternoon hours may be of greatest epidemiological significance, due to the common release of S. mansoni eggs during laundering or from washing the perianal area by swimmers (1,6) and persons bathing or abluting.

Although the epidemiological significance of site variation in contact activities is not known, due to lack of cercariometric data, the concentration of mixed activities below the heavily frequented water fetching points probably protects females somewhat, even though infective cercariae may be carried by the water current to each contact site.

Infection rates were higher in persons belonging to the high-risk occupations and among persons using river water exclusively than among those using the reservoir during the dry season (13). The high pressure

and velocity generated by pumping river water uphill to the reservoir over the 1-km pipeline may kill the cercariae, the absence of B. pfeifferi from the reservoir also indicates that its water is schistosome-free.

DISCUSSION

This study shows that while the age and sex distribution of water contact activities in Tensae Berhan are in general agreement with the usual distribution of S. mansoni infection in this town and elsewhere, the epidemiological and social significance of the various activities vary considerably, necessitating innovative control measures designed for the local situation.

Risk of infection for the various water contact activities, based on frequency, duration and intensity (proportion of budy surface wetted) and temporal distribution of contacts, is highest for swimming, bathing, laundering, washing extremeties, children playing, fetching water with donkeys and fetching water with the ensera, in that order. The brevity of exposure associated with river crossings, washing hands, and drinking water renders these activities by themselves relatively safe. However, the interrelatedness of contact, mixed and contaminative activities makes river crossings the most important contributor to transmission among adult males. More precise measurement of risk of exposure may be possible after development of an exposure index based on frequency, duration and intensity of exposure, and cercarial density at water contact sites, as suggested by Farooq and Mallah (4) and Kloos and co-workers (unpublished data) in Egypt.

The more frequent contact males had with the rivers than females is in contrast with studies in Rhodesia (6) and Egypt (4) and is probably due to the importance of the reservoir in Tensae Berhan as a source of domestic water.

The common practice of Tensae Berhan residents to engage in several types of water contact each time they go to the rivers and the absence of alternate water sources, aside from the reservoir, make it unlikely that cessation of any one of those activities would result in reduction of exposure below the threshold as defined by MacDonald (15). The interrelatedness of most activities implies that by providing safe, piped domestic water supplies within the town, many of these activities could then take place around the home instead, as shown also in St. Lucia (3). However, provision of proper community water supplies cannot eliminate all human water contact with the rivers, due to the high frequency of river crossings by farmers. Similarly, building of bridges at the 4 fording points without snail control, health education and provision of an alternate water supply system would probably reduce exposure by only about 10%, due to the custom of farmers cleaning themselves at the rivers, the social importance of most water use activities and the uncontrolled use of the rivers by infected visitors to Tensae Berhan. High infection rates were found in the nearby control village of Dolchia (13) and probably exist also in other communities in the vicinity of Tensae Berhan. This points out the need for snail control and supplementary health education if reductions in contamination, exposure and transmission are to be achieved.

Application of endod (Phytolacca dodecandra), a molluscicidal and larvicidal agent, in the 3 rivers in Tensae Berhan as part of a

community self-help project may reduce transmission significantly, as suggested by our earlier studies in Adwa (13). Planned systematic teaching of health-enhancing behavior may also lead to reduction of transmission, suggested by the marked decrease in swimming by boys after the completion of the schistosomiasis baseline survey in 1975. By controlling swimming, laundering, bathing, and ablution and defecation in and near the rivers, the incidence of schistosomiasis may be reduced, although systematic data on the role of contaminative behavior, similar to those obtained for hookworm by Kochar (11), have yet to be collected for schistosomiasis. The observed avoidance of exposure by some population segments and the unexpected reduction in swimming point out the need to study such desirable traits and changes in water contact in other schistosomiasis control areas, to identify and encourage various forms of preventive behavior. The enlistment of community leaders in these efforts as part of community self-help projects (7), perhaps the most crucial measure in their success, forms an integral part of the control program in Tensae Berhan.

ACKNOWLEDGEMENTS

The authors wish to thank the National Campaign students (Zematcha) Zinach Habte Mariam, Martha Habte, Etsegenet Taye, Assia Musa, Tewabich Nigatu, Gebre Hailu, Negusse Argaw, Samuel Ayele and Beyene Assefa for assistance with water contact observations and Mr. Belete Kirub, Mr. Assrat Kassie and the Zematcha Fekadu Wahabe and Solomon Habte Gebriel for help with the census and socioeconomic survey. We also wish to express our appreciation to the staff of the Institute of Pathobiology, especially Mr. Bahta Mazengia for examining all stool specimens, Dr. Anton M. Polderman and Dr. Giuseppe DeSole for assistance with research design, and Mr. Belete Kirub and Mr. Abraham Redda for help with the interview surveys. We thank the Ministry of Health and the personnel of the Tensae Berhan Health Center for their enthusiastic support and for providing accomodation for our team. Dr. Frederick L. Dunn and Dr. Donald Heyneman of the University of California, San Francisco, kindly read the manuscript and made valuable suggestions.

REFERENCES

1. Chernin, E. and Antolics, V.M. 1973. Laboratory transmission of Schistosoma mansoni to snails via fomites. J. Parasit. 59, 598.

2. Dalton, P.R. 1976. A socioecological approach to the control of Schistosoma mansoni in St. Lucia. Bull. Wld. Hlth. Org. 54, 587.

3. Dalton, P.R. and Pole, D. 1978. Water-contact patterns in relation to Schistosoma haematobium infection. Bull. Wld. Hlth. Org. 56, 417.

4. Farooq, M. and Mallah, M.B. 1966. The behavioural pattern of social and religious water-contact activities in the Egypt-49 bilharziasis project area. Bull. Wld. Hlth. Org. 35, 377.

5. Hubendick, B. (editor). 1973. A plan for a transdisciplinary study of events occurring when a tropical water development scheme is implemented, with special regard to its bearing on schistosomiasis. Report from the Nobel Workshop, Stockholm. The International Federation of Institutes for Advanced Study. 80 pp.

6. Husting, L. 1970. Sociological patterns and their influence on the transmission of bilharzia. Centr. Afr. J. Med. 16, 5.

7. Isely, R.B. and Martin, J.F. 1977. The village health committee: Starting point for rural development. Chron. Wld. Hlth. Org. 31, 307.

8. Jobin, W.R. and Ruiz-Tiben, E. 1968. Bilharzia and patterns of human contact with water in Puerto Rico. Bol. Asoc. Med. P. Rico 60, 279.

9. Jordan, P. 1972. Epidemiology and Control of Schistosomiasis. Brit. Med. J. 28, 55.

10. Jordan, P., Woodstock, L., Unrau, G.O., and Cook, J.A. 1975. Control of Schistosoma mansoni transmission by provision of domestic water supplies. Bull. Wld. Hlth. Org. 52, 9.

11. Kochar, V.K. 1974. Human factors in the ecology and epidemiology of hookworm infection in West Bengal. Ph.D. dissertation, Johns Hopkins Univ. 256 pp.

12. Lemma, A., Goll, P., Duncan, J., and Mazengia, B. 1978. Control of schistosomiasis with use of endod in Adwa, Ethiopia: Results of a 5-year study. Proc. Int. Conf. Schisto. (Cairo), 415-436.

13. Lemma, A., DeSole, G., Polderman, A.M., Mazengia, B., Redda, A., and Kloos, H. 1979. The epidemiology of Schistosoma mansoni infection in Tensae Berhan, Ethiopia. I. Prevalence of schistosomiasis. Eth. Med. J.

14. Lipes, J.K. and Hiatt, R.A. 1975. Determinants of human water contact patterns in urban Puerto Rico with special reference to schistosomiasis. Bol. Asoc. Med. P. Rico. 69 (no. 2) 35.

15. MacDonald, G. 1965. The dynamics of helminthis infections with special reference to schistosomiasis. Trans. Roy. Soc. Trop. Med. Hyg. 59, 489.

16. Polderman, A.M. 1975. The transmission of intestinal schistosomiasis in Begemder Province, Ethiopia. Acta Leidensia, 42, 1.

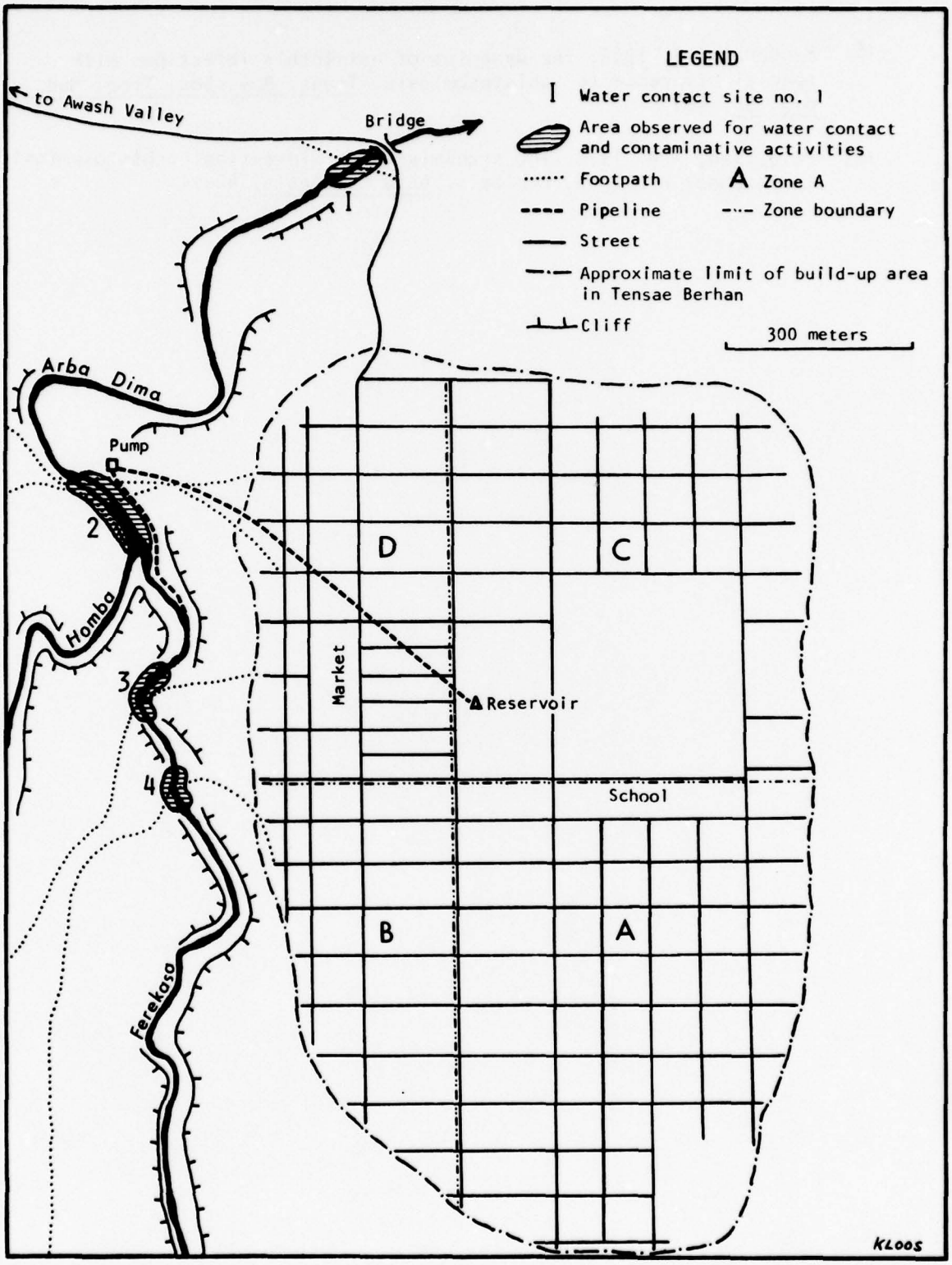


Fig.1. Map showing the four water contact sites in relation to the reservoir, roads and zones

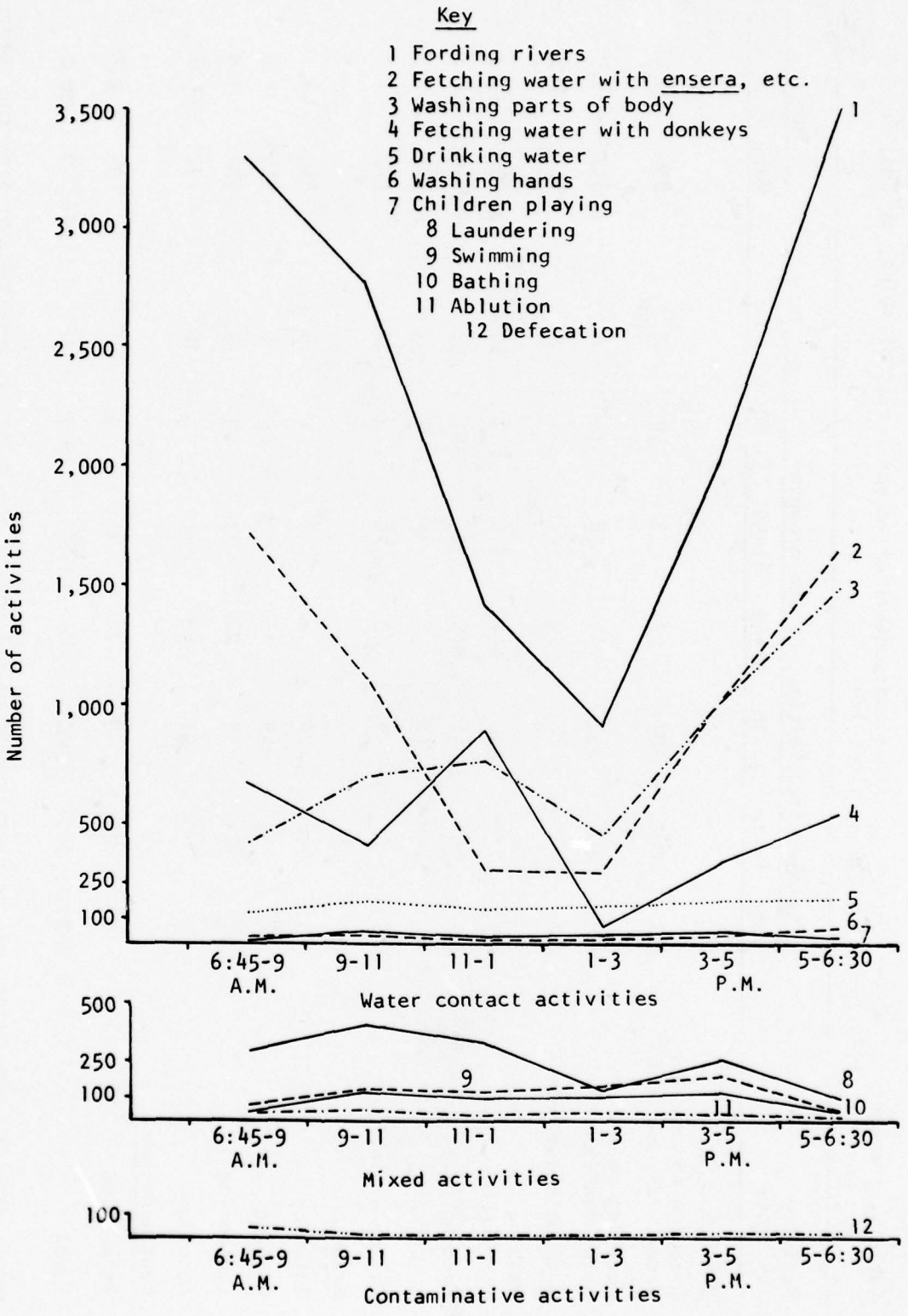


Fig. 2. Daily variations in water contact, mixed, and contaminative activities (Nov. 1975 and July 1976).

Table 1: Frequency of water contact and mixed and contaminative activities of males, by age, in November 1975 and July 1976 at sites 1-4

Activity (by type)	Number of activities, by age group(a)							Total	
	1-10	11-20	21-30	31-40	41-50	51-60	60	No.	%
Water contact									
Fetching and carrying water with gourds (b)	187	282	187	47	72	59	2	836	5.0
Fetching water using donkeys	48	864	432	210	58	14	6	1,632	9.7
Washing hands only	2	25	27	36	22	41	0	153	0.9
Washing extremities	299	738	690	444	214	108	69	2,562	15.3
Fording river	989	1,965	2,091	1,815	1,325	651	233	9,069	54.1
Drinking water	96	150	119	59	32	8	3	467	2.8
Children playing	115	62	0	0	0	0	0	177	1.1
Mixed									
Swimming	143	516	20	4	2	1	0	686	4.1
Bathing	21	99	149	83	47	12	8	419	2.5
Laundering	35	183	168	72	32	13	5	508	3.0
Ablution	0	0	31	69	45	17	10	172	1.0
Contamination									
Defecation	14	26	18	21	12	3	1	95	0.5
Total	1,949	1,910	3,932	2,860	1,861	927	337	16,776	100.0

(a)More detailed tabulations of age distribution of activities are available upon request from the authors.

(b)Includes 14 adult males using the ensera.

Table 2: Frequency of water contact and mixed and contaminative activities of females, by age, in November 1975 and July 1976 at sites 1-4

Activity (by type)	Number of activities, by age group(a)							Total	
	1-10	11-20	21-30	31-40	41-50	51-60	60	No.	%
Water contact									
Fetching and carrying water with the ensera	515	1,671	1,431	1,072	435	117	42	5,283	37.3
Fetching water using donkeys	39	188	108	78	20	8	0	441	3.1
Washing hands only	5	8	9	9	5	9	1	46	0.3
Washing extremities	248	595	645	477	153	17	3	2,138	15.1
Fording river	414	949	1,531	1,336	469	114	16	4,829	34.1
Drinking water	57	82	77	66	20	8	8	318	2.2
Children playing	50	22	-	-	-	-	-	72	0.5
Mixed									
Swimming	3	1	0	0	0	0	0	4	0.02
Bathing	11	21	16	5	0	0	0	53	0.4
Laundering	98	300	313	161	67	6	1	946	6.7
Contaminative									
Defecation	7	2	5	5	0	0	0	19	0.1
Total	1,447	3,839	4,135	3,209	1,169	279	71	14,149	100.0

(a)More detailed tabulations of age distribution of activities are available upon request from the authors.

Table 3. Mean duration of water contact, proportion of body surface exposed and number of contacts

Type of activity	Mean duration of water contact at the rivers (in seconds)	Mean percentage of body surface exposed	Number of contacts	
			Males	females
EXPOSURE ACTIVITIES:				
Fetching and carrying water with ensera	117	2	14	5,283
Fetching and carrying water with gourds, etc.	25	2	822	0
Fetching and carrying water with donkeys	155	6	1,632	441
Washing hands	7	2	153	46
Washing extremities	120	15	2,562	2,138
Drinking water	10	2	467	318
Fording rivers	15	1	9,069	4,829
Children playing	300	5	177	72
Total			14,896	13,127
MIXED ACTIVITIES:				
Bathing	600	95	419	53
Laundering	1,800	15	508	946
Swimming	900	100	686	4
Ablution	600	50	172	0
Total			1,785	1,003
CONTAMINATIVE ACTIVITIES:				
Defecation	-	-	95	19

Table 4. Number of water contacts in November and July at sites 1 and 2

Month of observation	Length of observation (hours)	Total no. of contacts	Mean no. of contacts per hour of observation	Percentage of all contacts
November (dry season)	164	10,179	62.1	52.9
July (rainy season)	164	9.072	55.4	47.1
Total	328	19.251	58.6	100.0

THE EPIDEMIOLOGY OF SCHISTOSOMA MANSONI INFECTION IN TENSAE BERHAN, ETHIOPIA. I. PREVALENCE OF SCHISTOSOMIASIS

Aklilu Lemma (Sc.D.), Giuseppe DeSole (M.D.), A.M. Polderman (Ph.D.), Bahta Mazengia, Abraham Redda and Helmut Kloos (Ph.D.)

Institute of Pathobiology, 1979

Parasitological, clinical, human water contact and malacological studies were undertaken between 1974 and 1976 in Tensae Berhan, Arba Gugu Awraja, Arsi (Arussi) Administrative Region in central Ethiopia as part of a long-range schistosomiasis control project. The primary objective of these studies was to provide baseline data in order to evaluate effects of subsequent schistosomiasis control programs. A long-range goal was to develop a sustained control project on a community self-help basis to serve as a model in other schistosomiasis affected areas. During the first control program of this type, in Adwa, Schistosoma mansoni infection rates decreased from 50% to 7% in the critical 1-6 years age group over a period of 5 years, chiefly due to the application of the molluscicide endod (powdered berries of the plant Phytolacca dodecandra) in local streams (8). Tensae Berhan was selected for the second project after it had become known from the Tensae Berhan Health Center that a large proportion of the town's population had schistosomiasis mansoni, and due to the relatively small size and proximity of this town to Addis Ababa, where the Institute of Pathobiology is located.

The potential value of the Tensae Berhan studies is also indicated by the highly localized distribution of S. mansoni infections in Ethiopia (6,14,15), favorable to small, local control programs at the community level.

This report, the first of a series, covers the parasitological, clinical and malacological findings. Results of the water contact studies are being reported in a companion paper (7).

The Town and its Setting

Tensae Berhan is located about 200 km southeast of Addis Ababa (Fig. 1), just below the northern escarpment of the southern Ethiopian Plateau, at an elevation of 1,550 meters and at the confluence of the Ferkasa and Homba rivers, where they form the Arba Dima River (Fig. 2). These 3 rivers supply nearly all of the town's water. A reservoir filled with river water during the dry season is located in the center of town. There are no wells in this rocky landscape and the smaller rivers and streams run only during the 2 rainy periods. This foothill area is well drained, preventing formation of swamps, lakes and other surface waters. The climate is of the hot semiarid savanna type. The few climatic data show that annual precipitation is around 750 mm and mean annual temperature is 23°C. Tensae Berhan is accessible by foot and donkey from all directions but by automobile only from the Metahara irrigation scheme and other Awash Valley localities in the north, and from Teferi Berhan on the Southern Plateau.

People and Economy

Tensae Berhan is a relatively new town, established by the central government as a retirement settlement for soldiers who fought in the Ethiopian-Italian War. According to information obtained from some elderly inhabitants in the town and some government records, in 1946

about 1,000 war veterans and their families who had to be rewarded for their bravery in defending their country from the Italian invaders were settled by the government in this agriculturally rich and climatically agreeable location. This area, which was earlier known as Abomsa and was used primarily for cattle grazing by the nomadic Arsi (Arussi) Galla people, was renamed Tensae Berhan by the government. The "1001" retired soldiers and their families who were said to have been settled in this town, originated from different parts of the country, including the administrative regions of Tigre (also Adwa), Begemdir (Lake Tana area), and Harar, all of which are known to be endemic regions for S. mansoni infection. This appears to be the reason why the town and immediate neighborhood of Tensae Berhan alone in this part of Arsi have endemic schistosomiasis, probably introduced by the new settlers. Over 40% of the residents were born outside of Tensae Berhan. The population of the town in 1976 was nearly 8,000. Approximately 90% of these residents were Christian Amhara, Tigre, and Shoa Galla, and 10% Moslem Arussi Galla and Gurage. The census survey revealed disproportionately large numbers of old people and relatively few young adults, especially males. The town lies in an overpopulated area characterized by outmigration from which many young males have left to work in the nearby Awash irrigation farms.

The majority of Tensae Berhan's population depends on subsistence farming, grain milling, and trading of agricultural products, although a substantial number of people are employed as government officials, shopkeepers and artisans. The main crops raised are maize, teff, sorghum and barley. Animal husbandry involves cattle, sheep and goats. Tensae Berhan attracts many farmers from the surrounding rural areas to its administrative offices, shops and semiweekly markets. The town thus has developed into an interregional market and service center between the hot lowlands (kollo) and cool highlands (dega).

MATERIALS AND METHODS

1. Parasitological Studies

The aim of the parasitological survey in 1975 was to determine the prevalence and intensity of S. mansoni infection by age group and sex in Tensae Berhan. A random sample of 10 percent of the households in Tensea Berhan was selected and stool specimens from all members of these households were examined. Two parasitological methods, the modified Kato technique (13) and Ritchie concentration method (18) were used in the field for quantitative and qualitative measurements of infection, respectively. The town was divided into four zones and all households were visited, enumerated, and mapped during the census.

Plastic 200-ml containers were passed out in the afternoon and collected the following morning. Whenever possible, three slides were prepared and examined for each specimen, using the Kato method. Approximately 225 mg of stool were used, ±75 mg per slide, measured with a decapitated syringe. One gm of formalin-preserved stool was examined using the Ritchie method. During collection of stool specimens, information was obtained on health and socioeconomic status of families and their water use behavior.

2. Clinical Observations

Six hundred and twenty of the 796 persons included in the parasitological survey were given a physical examination by a physician

(G.D.). Also, 587 thin and thick blood smears were made for study of blood parasites and 370 heparinized capillary tubes were centrifuged in a haematocrit hand centrifuge (±5 to 10 minutes at 1000 to 2000 rpm). Due to the low number of high egg excreters found during the 10% random parasitological survey, another 20 high excreters were subsequently included in the study.

3. Malacological Studies

Weekly snail collections were made between 1974 and 1976 in the Ferekasa, Homba and Arba Dima rivers (Fig. 6) at marked collection sites on a rotational basis, to determine the seasonal fluctuations of snails and to study their specific location and ecology. Biomphalaria pfeifferi, intermediate host of S. mansoni in the largest part of Ethiopia, and various species and subspecies of Bulinus, transmitters of S. bovis in this country (12), have been known to occur in these rivers. Collection sites were alternatively marked with 3 different colors on rocks, spaced about 100 meters apart and covering 2-km stretches of the 3 rivers. Sampling was systematic, but an attempt was made to gather the maximum number of B. pfeifferi, by selecting sites favorable for its breeding and survival. Collections were made with a dip net in pools and by hand-picking snails in shallow water with a gravel substratum. Collectors wore rubber boots and gloves during these activities.

Prevalence of snail infection was checked by cercarial shed from collected snails, which were then measured and uninfected specimens returned to their habitats. Occasionally, surveys were made along the Ferekasa and Homba rivers as far as 5 km upstream and along the Arba Dima River 5 km downstream of Tensae Berhan.

4. Community Involvement

The Awraja Governor, health and other officials and administrative personnel, elderly people of the town, students and teachers were briefed through demonstrations and film shows about the schistosomiasis problem in Tensae Berhan and the goal of the control project, with the aim of obtaining their cooperation and getting them involved in this local effort.

Reference Village

The village of Dolchia, located about 7 km southwest of Tensae Berhan, was chosen as a reference village, to serve as a control for future comparison. It was therefore also surveyed for S. mansoni infection.

RESULTS

1. Parasitological findings

Of the 863 persons initially included in the survey, 796 gave stool specimens, 32.7% of which were found to contain S. mansoni ova. Highest prevalence rates (70%) were found in the 11 to 15 years age group and somewhat lower rates in the 6 to 10 (50.7%) and 16 to 20 (48.2%) groups. The 46 to 55 age group gave the lowest infection levels. Rates increased again in males over 56 and women 56 to 65. Of 374 males examined, 38.8% were found to pass eggs; of the 422 females, 24.9% were egg-positive. More males than females in all age groups were egg passers (Table 1, Fig. 3).

Mean egg output by age and sex follows in a general way the age and sex adjusted prevalence rates (Table 2). Males passed on the average 85 eggs per gram of stool and females passed 65. Only 32 of the 620 (5.2%) persons examined during this and a repeat survey were found to shed more than 200 eggs per gram of stool. All but 2 of the high excreters were children between 6 and 18 years.

Few clear-cut patterns in spatial distribution of infection by zone were noted. Rates varied slightly from 39.0% in Zone C, 33% (Zone D), 31% (Zone B), and 29.1% (Zone A), but within each neighborhood, households with infected persons were quite evenly distributed (Fig. 2). However, cases did tend to be clustered in individual households. More than half of the members of 13 of the 50 hoursholds (26%) in Zone D were affected by schistosomiasis; 6 of 30 households (20%) in Zone A, 3 of 16 (19%) in Zone B, and 3 of 26 (12%) in Zone C. Although these variations may be sampling errors, it does appear that age distribution is a major factor involved, which bears upon occupation and division of work within households and water use patterns. The largest percentage of children (persons under 21 years) were found to live in Zone D (63.7% of all people in that zone); a very similar 61.3% in Zone C, and 56.0% in Zone B, but 30.8% in Zone A. Prevalence rates in children in the 2 zones located nearest the rivers (B and D) were slightly lower (36.8%) than in children in zones A and C, located farther away (43.6%) ($P>0.1$). Significantly more males than females passed eggs in zones A ($P<0.05$), C ($P<0.01$), and D ($P<0.01$).

Distribution of infection by occupation shows that rates were significantly higher among household helpers than housewives ($P<0.1$) chiefly due to the age factor. Rates were also higher in farmers and daily laborers than traders, shopowners and artisans ($P<0.05$). Both tailors examined were positive. Whereas government officials, traders and artisans have relatively little contact with the rivers, farmers and daily laborers frequently cross the rivers, and servants carry all the water for some households in clay jars, and they also wash laundry, activities mostly done by housewives and children in poorer families. Talla makers and their children frequently come into contact with the rivers, since most of them cannot afford to employ water carriers. All 20 children above 4 years of age of 9 of the 29 talla makers were positive. The low-risk occupation groups, representing mostly wealthier, middle-aged people, are concentrated in Zone D around the market area. Nearly all farmers, most of them retired soldiers, live in the peripheral, less densely populated parts of town, in Zones A, B and C. The presence of most household helpers and a large child population in Zone D (63.7% of all residents of that zone) help to counterbalance effects of the large low-risk occupational groups in that zone.

Type of water source was associated with prevalence, rates being higher among persons using river water only than among those using both river and reservoir water in all zones. This was significantly so for zones A ($P<0.01$) and D ($P<0.05$) (Table 3). The reservoir is filled only during the dry season, the pipe from the river usually becoming choked with silt after onset of the rains. The pressure and velocity of water pumped uphill to the reservoir over the 1-km pipeline may kill S. mansoni cercariae. The absence of B. pfeifferi snails from the reservoir also suggests that its water is safe. Closure of the reservoir during the season of the major rains probably has little effect on schistosome transmission, owing to sharp seasonality of B. pfeifferi occurrence and S. mansoni transmission in the rivers.

Most poor families, including talla makers, farmers, daily laborers and old people, use the rivers exclusively, where water is free. Wealthier families can afford either to employ servants, purchase water from professional water carriers, or pay the fee for use of the less turbid reservoir water. Some people, however, especially those in Zones B and D, use the rivers chiefly because they are closer to their homes than is the reservoir (Fig. 2).

Survey of S. mansoni infection among volunteers in the reference village of Dolchia showed 14 of the 31 (45.2%) examined to be positive. Sex distribution of the positive cases was 9 of 21 males and 5 of 10 females (Table 4).

2. Clinical Observations

Fifty-eight percent of the 620 persons examined parasitologically and clinically were in good health, 34% in fair and 7% in poor health, as determined by physical appearance, age, weight and height data. The nutritional status of the population was satisfactory at the time of the survey, one month after the harvest. No marasmus or kwashiorkor were found. One 2 cases of malaria were found, both believed to have originated in the Awash Valley irrigated farms. Splenomegaly was noted in 0.5% and hepatomegaly in 2.5% of the 620 persons examined, and no relationship with S. mansoni infection could be discerned. Anemia was not a problem. The haematocrit was 45 to 50% in 95% of the 620 persons studied and approximately 40% in the remaining 5%. Chronic eye lesions, mainly due to trachoma, were noted in 31% and acute lesions in 10%, the prevalence of acute lesions decreasing with age. Data from the Tensae Berhan Health Center and presence of a small middle-age group in Tensae Berhan indicate that other venereal diseases are not an important problem.

Figure 4 shows the relationship between the commonest symptoms of S. mansoni infection and egg output, determined by the Kato technique. Other intestinal parasites, especially Entamoeba histolytica, Trichuris trichiuria and Ascaris lumbricoides, are less prevalent than is S. mansoni, affecting about 12%, 20% and 23% of the population, respectively, as determined by the Ritchie method.

3. Malacological Data

Seasonal fluctuations of B. pfeifferi and proportion of S. mansoni-infected snails in the portions of the 3 rivers under intensive study are shown in Figure 5. The highest B. pfeifferi densities were noted in the dry season (February-March), when an average of more than 300 snails were collected at each of the various sites. During the following "small rains" (March-April), B. pfeifferi numbers declined again but seldom disappeared completely from these sites, and few of them were recovered in April during the "small rains." Buildup of the snail population during the following 2 dry months (May and June) was interrupted again by the "big rains" (June-September). No snails of any species were found between September and December, the period during and after the "big rains" (Fig. 5). Along the escarpment and in the foothill area below the escarpment the rivers flow rapidly, owing to their high gradient. Rapid flow during the rains, and the absence of macrovegetation result in periodic flushing out of all aquatic snails from their habitats in the 3 rivers under study. The small intermittent streams in the foothills do not support B. pfeifferi but, like the larger rivers, provide stable habitats near their source in the more humid highlands above 2000 meters

elevation (Fig. 6). Prevalence of S. mansoni infections in snails was correlated with the seasonal distribution of Biomphalaria pfeifferi. Unidentified cercariae emerging from Bulinus sp. also followed a similar seasonal pattern. B. pfeifferi was more numerous than Bulinus sp. and Lymnaea natalensis and L. truncatula, host snails of Fasciola hepatica and F. gigantica in Ethiopia. Seasonal snail occurrence, speciation, and infection was similar in all 3 streams.

DISCUSSION

1. Epidemiological Observations

Although schistosomiasis prevalence in Tensae Berhan reveals a typical age distribution of infection, with high rates in the 5 to 16 years age groups and declining rates among the middle aged, the increase in older males is unusual. Either declining immunity levels in old age or the age and occupational distribution of the population, with relatively more artisans, government officials, merchants, and other low-risk occupational groups in the middle-age category may be responsible for the relative increase in schistosomiasis among older persons. The small sample size may also have influenced the results.

Absence of definite spatial patterns in the distribution of schistosomiasis in Tensae Berhan results from the fairly even dispersal of cases throughout the town and absence of clustering in neighborhoods nearest the rivers. This chiefly reflects the reliance of large proportions of people in each zone on river water throughout the year. Age and occupational distribution of the population are additional factors involved in these findings.

Transmission may occur not only during direct contact with river water but also in the homes. This is suggested by the presence of many infections in people claiming to use only reservoir water during the dry season, including the 2 tailors and other people at low risk. Polderman (16) in the Lake Tana area of northern Ethiopia showed that cercariae in river water frequently retain their infectivity for some time while water is stored in homes for later use. He also noted high rates of infection among tailors, merchants, and other groups who seldom frequent the natural transmission sites. Unfortunately, no mouse exposure studies could be made of stored water during the present study to confirm this mode of transmission in Tensae Berhan. Nevertheless, high prevalence rates in the children and housewife populations and the low rates among people infrequently contacting river water suggest that most infections are acquired at the rivers, as is also supported by the water contact studies of Kloos and Lemma (7).

The high prevalence rates in children of talla makers points out the importance of parental occupation in evaluation of schistosomiasis among child populations. Children of talla makers commonly carry considerable amounts of water from the rivers for their mothers. Polderman (16) also noted that talla makers are in the high risk class of occupations.

No definite relationships between religion, education and schistosomiasis prevalence were noted, unlike the studies by Lemma and coworkers (8) and Polderman (16) in northern Ethiopia, and Farooq and coworkers (4) in Egypt, presumably because in Tensae Berhan other factors tend to obscure the effects, and owing to the small size of the study population.

2. Clinical Observations

The small number of high egg excreters, chiefly among children, and the apparent absence of endemic malaria are probably important factors in the low prevalence of splenomegaly and hepatomegaly. Correlation between symptoms and egg output probably is causally related, as indicated by other clinical studies (3.5). However, it is not possible to make any definite conclusions on the basis of these results alone because of the small number of high egg passers and the possible influence of other intestinal parasites.

3. Malacological Studies

The marked seasonality of B. pfeifferi in the Ferekasa, Homba and Arba Dima rivers in Tensae Berhan results primarily from the varied water velocity between dry and wet seasons. Aram (1) and Polderman (15, 16) found a strong seasonal component in the distribution of B. pfeifferi in several other Ethiopian streams. They usually found large, permanent snail populations only near stream headwaters on the high plateaus, where favorable habitats are more stable than at lower elevations. Brown and Lemma (2) recorded a richer snail fauna from streams in the plateau regions than in the lowlands of the Awash Valley.

The seasonal distribution of B. pfeifferi in Tensae Berhan may require several treatments anually in order to control this snail. Endod, like most other molluscicides, is not ovicidal, probably necessating 3 applications 6-8 weeks apart, between January and August, to kill newly hatched snails that survived the previous application as eggs. The first treatment was made in May 1977 and it is hoped that additional ones can be made in the near future. The effectiveness of endod for control of schistosomiasis was demonstrated during the Adwa control project, where B. pfeifferi was controlled and schistosomisis prevalence significantly reduced, mainly as a result of periodic endod application in the local streams (8). The development of a fermentation water extraction process that increased the molluscicidal potency of endod from 10 ppm for crude endod to 2 ppm (10) represents a major step forward to snail control technology. Also developed was a chemical assay for more effective and economical use of endod (10).

The possibility of S. bovis transmission by Bulinus spp. and of Fasciola transmission by Lymnaea spp. in the Tensae Berhan area should be considered in future studies. Lo and Lemma (12) summarized the literature on the occurrence of this parasite in Ethiopia, and found it to be transmitted by diploid (n=18) and tetraploid (n=36) forms of Bulinus. Results of longitudinal studies on Fasciola spp. and the control of Lymnaea spp. show that the parasite is widely distributed in Ethiopia and that the intermediate host can be killed at concentrations similar to those required to kill Biomphalaria pfeifferi and Bulinus spp. (9).

Acknowledgements

The writers would like to acknowledge with thanks the assistance of Mr. Belete Kirub, Mr. Asrat Kassie, Dr. Tesfamichael Tesfa Yohannes, Mr. Ian McChessney, Mr. Anton Dalhuysen, Dr. Legesse W. Yohannes and other staff of the Institute of Pathobiology, and of the Zemacha students Fekadu Wahabe and Soloman Habte Gebriel, stationed in Tensae Berhan during the surveys. Thanks is also due to Mr. Hailu Hagos and other staff of the Tensae Berhan Health Center for their help, including provision of accomodation. We are also endebted to Professor Donald Heyneman of the George Williams Hooper Foundation, University of California, San Francisco, for making valuable suggestions during the presentation and editing of this manuscript.

References

1. Aram, R.H. (1973). Studies on Biomphalaria pfeifferi in three Ethiopian highland streams. Unpublished report, Ecology Unit, University of Sussex. 32 pp.

2. Brown, D.S. and Lemma, A. (1970). The molluscan fauna of the Awash River, Ethiopia, in relation to the transmission of schistosomiasis. Ann. Trop. Med. Paras. 64, 533.

3. Cheever, A.W. (1968). A quantitative post-mortem study of schistosomiasis mansoni in man. Am. J. Trop. Med. Hyg. 11, 472.

4. Farooq, M., Nielsen, J., Samaan, S.A., Mallah, M.B., and Allam, A.A. (1966). The epidemiology of Schistosoma haematobium infections in the Egypt-49 Project Area. Bull. Wld. Hlth. Org. 35, 293.

5. Hiatt, R.H. (1976). Morbidity from Schistosoma mansoni infections: An epidemiologic study based on quantitative analysis of egg excretion in two highland Ethiopian villages. Am. J. Trop. Med. Hyg. 25, 808.

6. Kloos, H. and Lemma, A. (1978). Schistosoma mansoni distribution in Ethiopia: a study in medical geography. Ann. Trop. Med. Paras. 72, 461.

7. Kloos. H. and Lemma, A. (1979). The epidemiology of Schistosoma mansoni infection in Tensae Berhan, Ethiopia. II. Human water contact patterns. Unpublished report. Fresno.

8. Lemma, A., Goll. P., Duncan, J. and Mazengia, B. (1978). Control of schistosomiasis by the use of endod in Adwa, Ethiopia: results of a 5-year study. Proc. Int. Conf. Schisto. (Cairo), 415.

9. Lemma, A. and Yau, P. Studies on the molluscicidal properties of endod (Phytolacca dodecandra). III. Stability and potency under different environmental conditions. Eth. Med. J. 13, 115.

10. Lemma, T. and Lemma, A. (1977a). New approaches to endod (Phytolacca dodecandra) extraction--a comparitive study. Unpublished report, Institute of Pathobiology. 13 pp.

11. Lemma, T. and Lemma, A. (1977b). Colorimetric determination of endod. Unpublished report, Institute of Pathobiology, 17 pp.

12. Lo, T.S. and Lemma, A. (1975). Studies on Schistosoma bovis in Ethiopia. Ann. Trop. Med. Paras. 69, 170.

13. Martin, L.K. and Beaver, P.C. (1968). Evaluation of Kato thick-smear technique for quantitative diagnosis of helminthic infections. Am. J. Trop. Med. Hyg. 17, 382.

14. McConnell, E. and Armstrong, J.C. (1976). Intestinal parasitism in fifty communities on the Central Plateau in Ethiopia. Eth. Med. J. 14, 159.

15. Polderman, A.M. (1974). Intestinal schistosomiasis north and west of Lake Tana, Ethiopia. Trop. Geogr. Med. 26, 170.

16. Polderman, A.M. (1975). The transmission of intestinal schistosomiasis in Begemder Province, Ethiopia. Acta Leidensia. 42, 1-193.

17. Polderman, A.M. (1976). Assignment Report. Schistosomiasis in Ethiopia. WHO Document EM/SCHIS. 64 EM/ETH/MRD/003/RB.

18. Ritchie, L.S. (1948). An ether sedimentation technique for routine stool examination. Bull. U.S. Army Med. Dept. 8, 326.

Table 1: Number and percentage of persons examined and of those found infected, by age, sex, and section of Tensae Berhan, and total town population

	Zone	Age 1-5	6-10	11-15	16-20	21-25	26-35	36-45	46-55	56-65	65+	Total	%
Males	A	2/17	8/16	12/16	5/7	1/3	5/9	0/2	0/5	1/16	3/12	37/103	35.9
	B	0/12	5/8	4/6	2/3	3/8	0/2	1/4	0/3	1/6	3/4	19/56	33.9
	C	1/12	8/10	10/11	10/13	0/0	2/4	0/1	1/4	3/6	2/7	37/74	50.0
	D	4/30	11/21	16/22	16/33	3/8	5/11	4/13	1/5	0/5	2/3	62/141	44.0
	Total no.	7/71	32/61	42/55	33/46	7/19	12/26	5/20	2/17	5/33	10/26	155/374	41.4
	% infected	9.9	52.5	76.4	71.7	36.8	46.2	25.0	11.8	15.2	38.5	41.4	
	Total town population	556	499	468	402	148	313	326	176	199	146	3233	
Females	A	1/11	6/17	6/9	5/11	0/3	1/7	0/10	0/11	1/9	0/6	20/94	21.3
	B	0/12	6/12	6/6	0/2	1/7	1/4	1/7	0/5	1/1	0/0	16/56	28.6
	C	0/11	8/12	5/8	1/4	2/4	1/7	1/10	0/6	0/4	0/1	18/67	26.9
	D	4/39	18/36	14/26	3/24	2/14	4/20	5/25	0/14	1/7	0/0	51/205	24.9
	Total no.	5/73	38/77	31/49	9/41	5/28	7/38	7/52	0/36	3/21	0/7	105/422	24.9
	% infected	8.3	50.1	70.2	48.3	25.5	29.7	16.7	3.8	14.8	30.3	24.9	
	Total town population	525	560	397	301	222	447	449	218	120	45	3284	
Grand total:	No.	12/144	70/138	73/104	42/87	12/47	19/64	12/72	2/53	8/54	10/33	260/796	32.7
	% infected	8.3	50.1	70.2	48.3	25.5	29.7	16.7	3.8	4.8	30.3	32.7	
	Total town population	1081	1059	865	703	370	760	775	394	319	191	6517*	

* This excludes nearly 900 persons absent at the time of the census

Table 2: Mean (arithmetic) egg output in Tensae Berhan, by age and sex

Age	Males		Females	
	No. of positives	Mean egg output*	No. of positives	Mean egg output*
1-5	7	53	5	24
6-10	32	120	38	135
11-15	42	180	31	97
16-20	33	96	9	66
21-35	19	44	12	33
36-65	12	21	10	33
> 65	10	80	0	-

* No. of eggs per gramme of stool.

Table 3: Schistosomiasis prevalence and type of water source used*

Zone	River water only			River and reservoir water		
	No. exam.	% infect.	% of popul. using river water	No. exam.	% infect.	% of popul. using river and reservoir water
A	74	39.2	37.6	123	23.6	62.4
B	83	33.7	74.1	29	24.2	25.9
C	49	42.9	34.8	92	37.0	65.2
D	214	36.9	61.8	132	25.8	38.2
Total	420	37.1	52.8	376	27.7	47.2

* Based on results from household interviews and surveys

Table 4. Results of the parasitological survey in Dolchia (Ritchie concentration method)

Age	Males	Females	Total	% infected
6-10	2/5	3/8	5/13	38.5
11-15	3/5	1/11	4/16	66.7
16-20	1/7	1/1	2/8	25.0
21-35	3/4	-	3/4	75.0
Total	9/21	5/10	14/31	45.2

2/5: two infected persons out of five examined

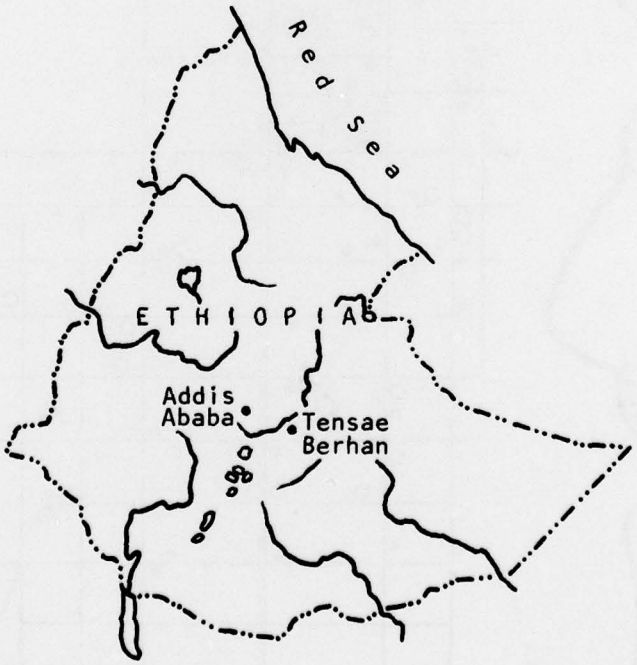
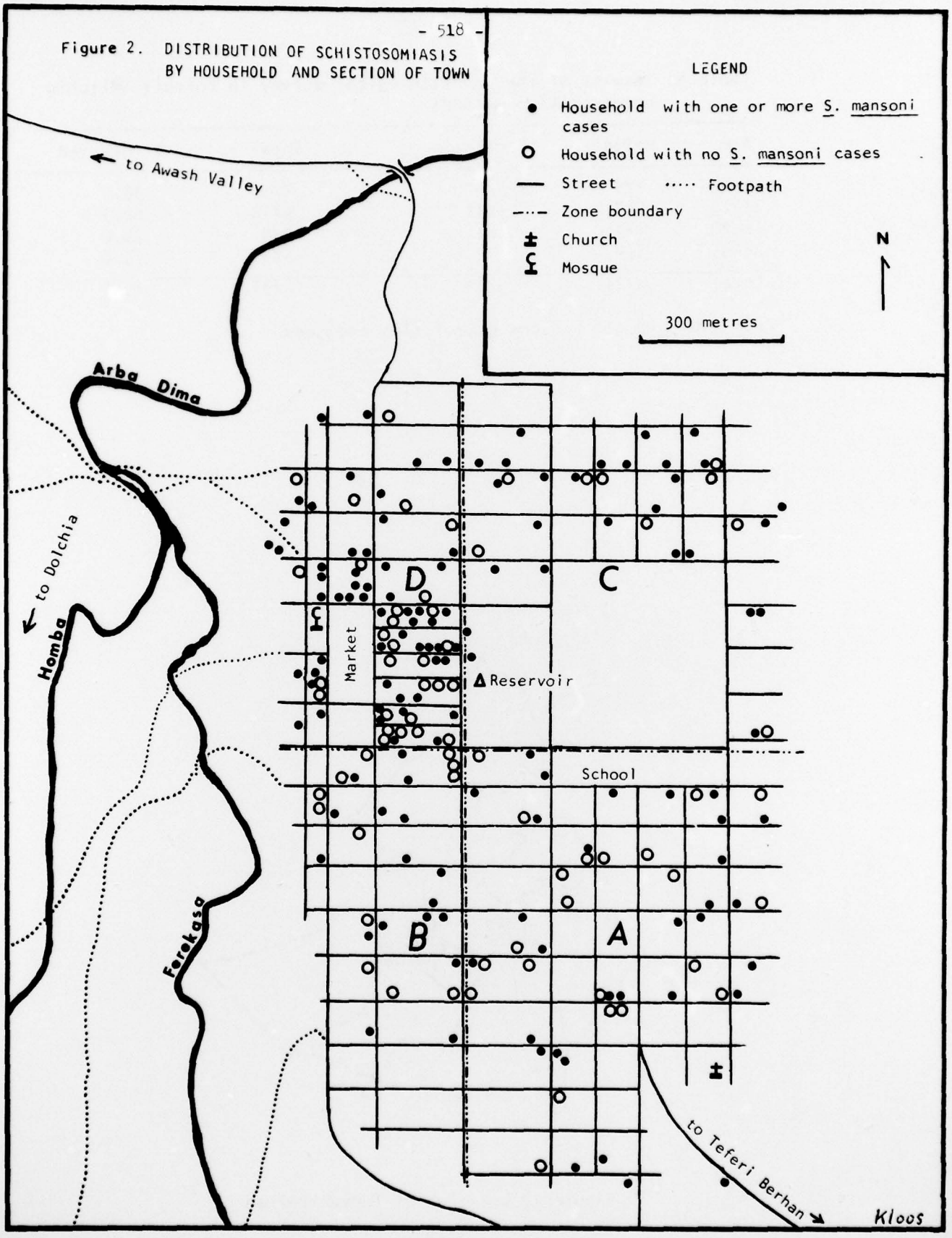


Figure 1. Location of Tensae Berhan

Figure 2. DISTRIBUTION OF SCHISTOSOMIASIS BY HOUSEHOLD AND SECTION OF TOWN



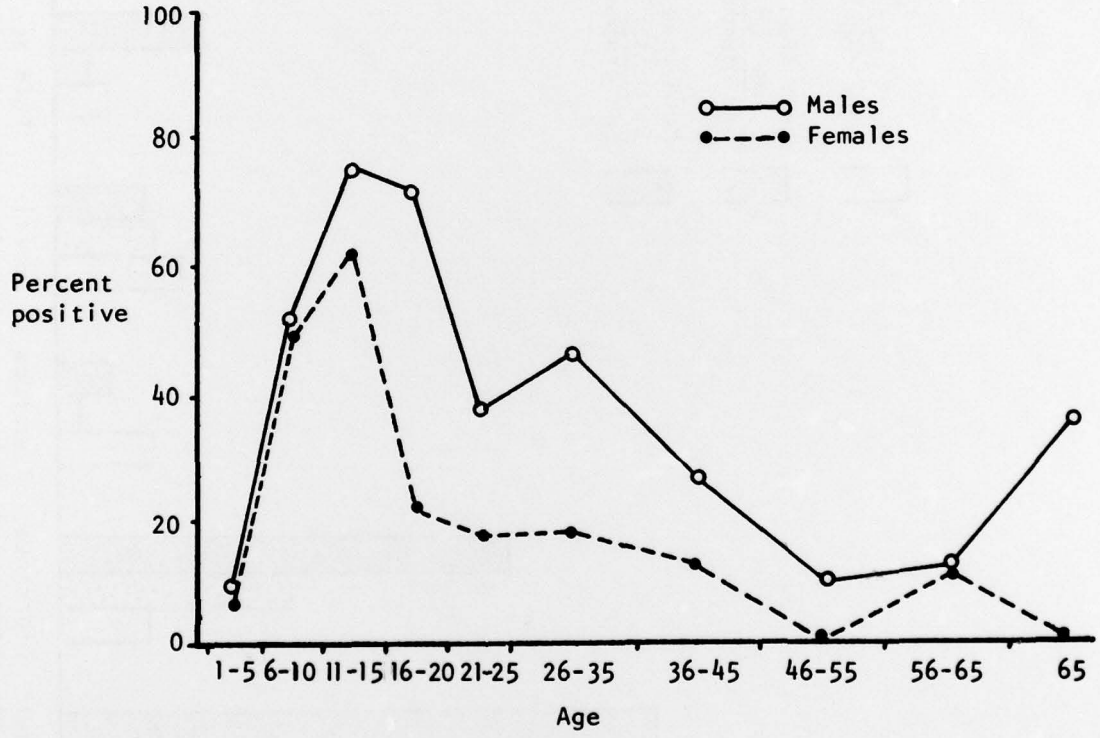


Figure 3. Prevalence of S. mansoni infections, by sex and age group, in Tensae Berhan

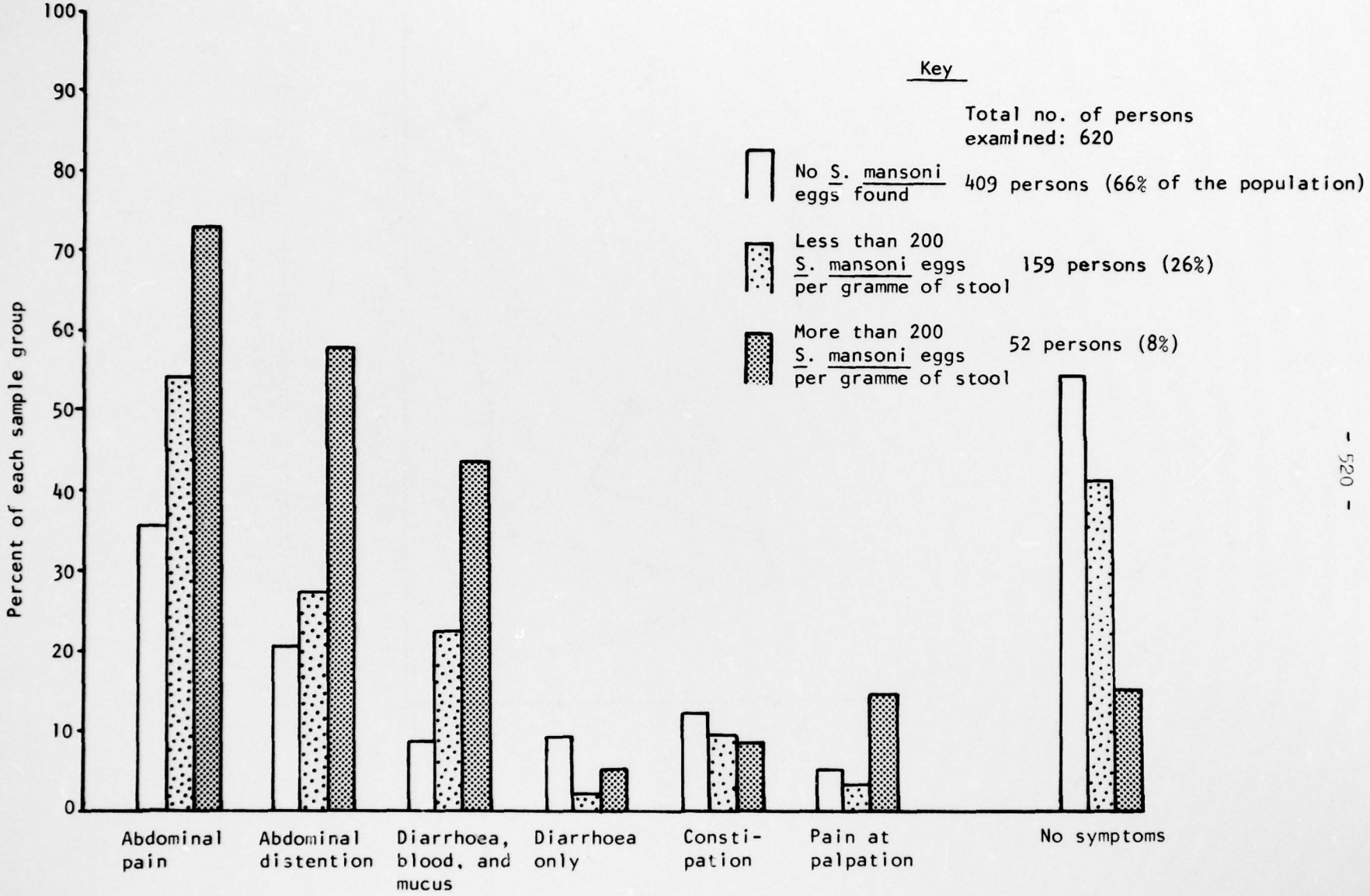


Figure 4. Relationship between symptoms and egg output

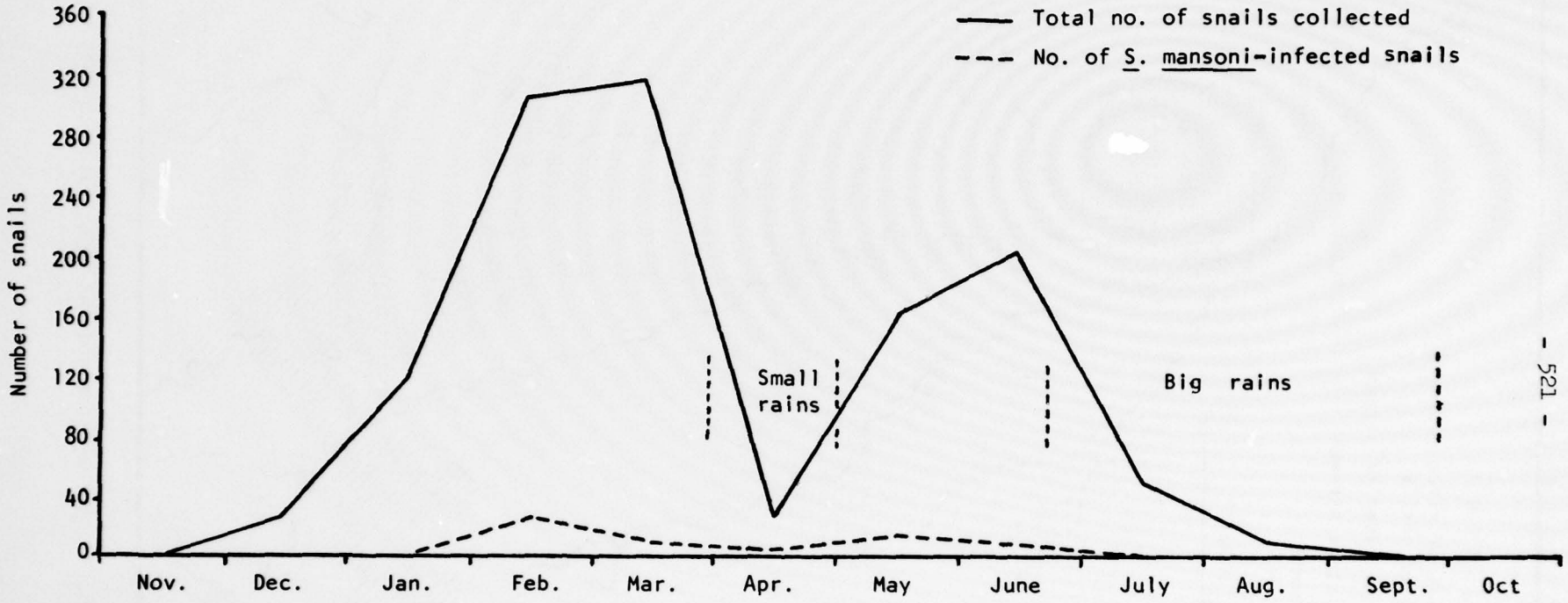


Figure 5. Seasonal distribution of B. pfeifferi in the Ferekasa, Homba, and Arba Dima rivers, June 1974-Aug. 1976

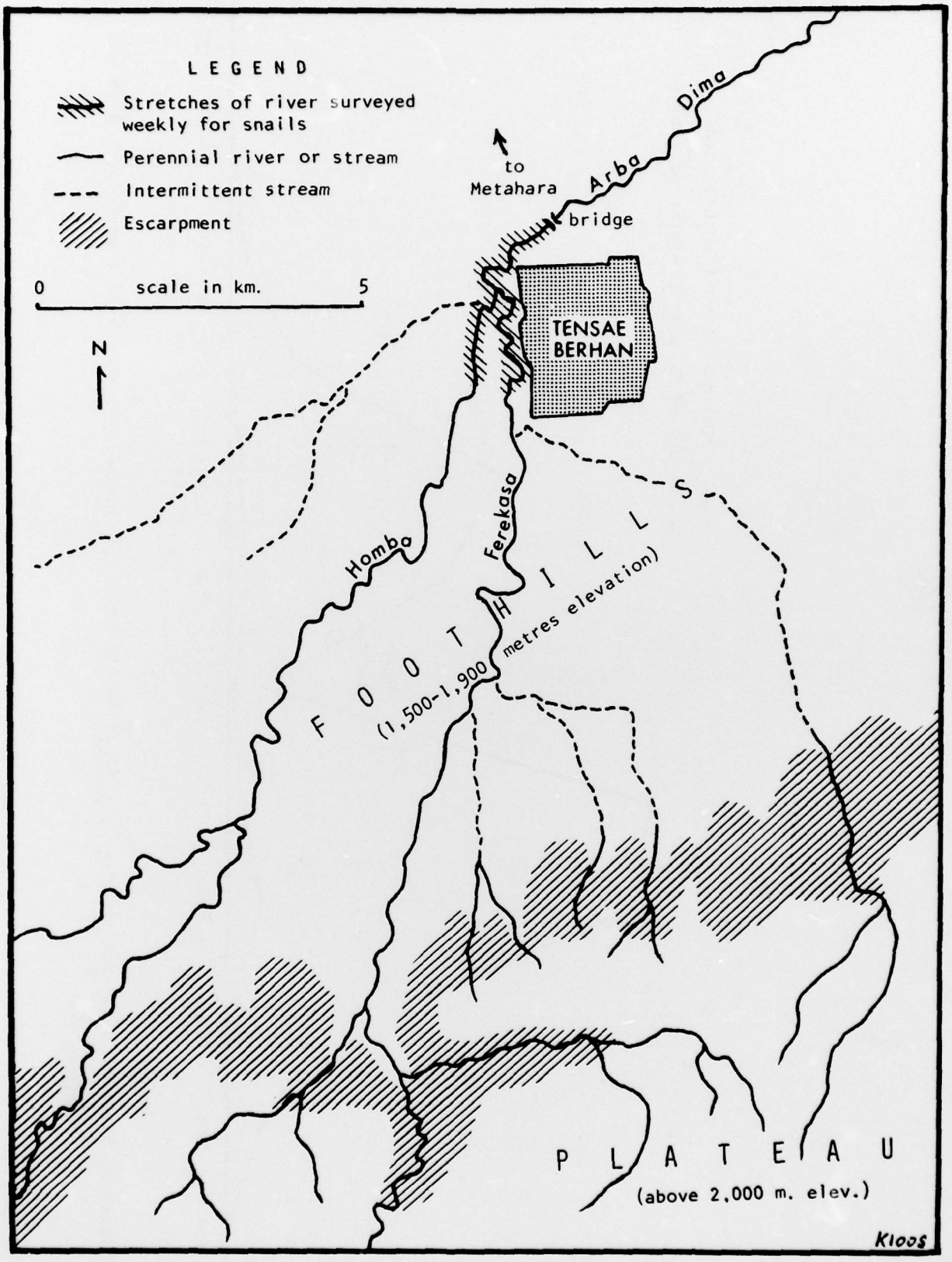


Figure 6. The survey area and the major water courses of the Arba Dima watershed