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REPORT NUMBER 4

INFECTIOUS AND COMMUNICABLE DISEASES AFFECTING POPULATIONS
INTRODUCED INTO ENDEMIC AREAS (U)

ANNUAL PROGRESS REPORT

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George A. Thurber, M.S.

and

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Joseph H. Miller, Ph.D.	Jorge E. Velasco, B.S.
Margaret I. Nickle, M.S.	Rodrigo Zeledón, Sc.D.

27 October 1971

(For the period 1 October 1970 to 30 September 1971)

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Washington, D. C. 20314

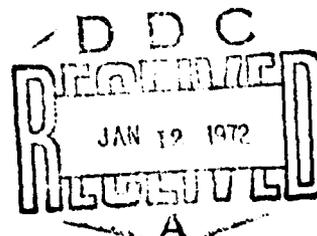
Contract No. DADA 17-68-C-8023
Louisiana State University
New Orleans, Louisiana 70112

DDC AVAILABILITY STATEMENT

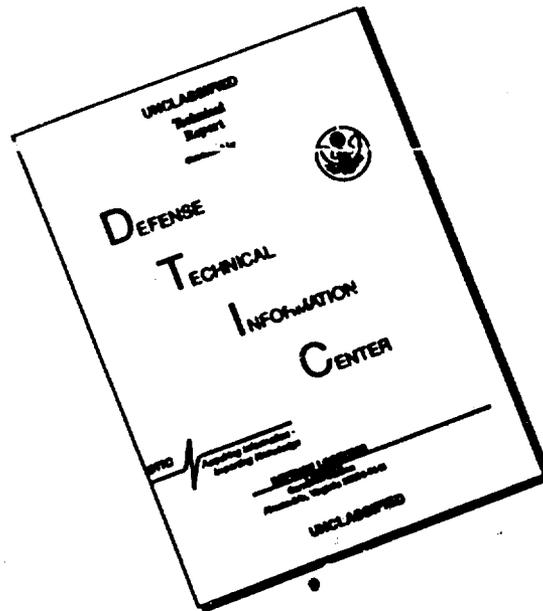
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DOCUMENT CONTROL DATA - R & D		
<i>(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)</i>		
1. ORIGINATING ACTIVITY (Corporate author) Louisiana State University School of Medicine 1542 Tulane Avenue, New Orleans, La. 70112		2a. REPORT SECURITY CLASSIFICATION Unclassified
		2b. GROUP
3. REPORT TITLE INFECTIOUS AND COMMUNICABLE DISEASES AFFECTING POPULATIONS INTRODUCED INTO ENDEMIC AREAS		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) Annual Progress Report for 1 October 1970 through 30 September 1971		
5. AUTHOR(S) (First name, middle initial, last name) John C. Swartzwelder, Víctor M. Villarejos, George A. Thurber, and 12 professional colleagues.		
6. REPORT DATE 27 October 1971	7a. TOTAL NO. OF PAGES 46	7b. NO. OF REFS 3
8a. CONTRACT OR GRANT NO. DADA 17-68-C-8023	8b. ORIGINATOR'S REPORT NUMBER(S) 4	
9. PROJECT NO.	8c. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
10. DISTRIBUTION STATEMENT Approved for public release; distribution unlimited.	11. SUPPLEMENTARY NOTES	
	12. SPONSORING MILITARY ACTIVITY U.S. Army Medical Research and Development Command Washington, D. C. 20314	
13. ABSTRACT The title of this contract implies a direct relationship to specific military function and operation. All of the studies have a direct relationship to medical problems of military importance with particular reference to the tropics. The medical problems which are being studied include the etiologies of diarrheal diseases in the tropics, pathogenesis of amebic lesions, etiology of infectious hepatitis, biological control of Chagas' disease vectors, reconnaissance for a possible Venezuelan equine encephalitis enzootic focus in the Mississippi Delta region, and taxonomy, distribution, and ecology of phlebotomine flies in Bolivia and of black flies in Costa Rica. In accordance with the stated aims of Project THEMIS, the LSU/THEMIS program has: (1) Conducted multidisciplinary research by groups of faculty members and research associates; (2) Made substantial progress in research projects involving several biomedical disciplines; (3) Involved at least 15 faculty members in research activities; (4) Presented papers at six scientific meetings; two papers were published during the period of this report; (5) Collaborated in research with personnel of two federal agencies; and (6) Continued our activities in research on problems in tropical medicine of importance to the military.		

DD FORM 1 NOV 68 1-73

REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Security Classification

Security Classification

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Infectious hepatitis, etiology Diarrheal diseases of tropics Amebiasis, pathogenesis Hartmannella sp., pathogenesis Encephalitis, amebic Encephalitis, Venezuelan equine Chagas' disease, biological control Sandflies, Bolivia Black flies, Costa Rica						

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SUMMARY

1. All studies under this contract have a direct and apparent relationship to specific military function and operation. This is apparent from the project title "Infectious and Communicable Diseases Affecting Populations Introduced into Endemic Areas." Our studies are being conducted in Central America, the Caribbean islands, and in Louisiana, which is a subtropical area of this country.

2. The medical problems which are being studied include the etiologies of diarrheal diseases in the tropics, pathogenesis of amebic lesions, etiology of infectious hepatitis, biological control of Chagas' disease vectors, reconnaissance for a possible Venezuelan equine encephalitis enzootic focus in the Mississippi Delta region, and taxonomy, distribution, and ecology of phlebotomine flies in Bolivia and of black flies in Costa Rica.

3. Two scientific papers based upon research supported by project THEMIS have been published during the period covered by this report. Papers have been presented at six scientific meetings by staff members engaged in project THEMIS.

4. Collaboration has been established with five Peace Corps groups in Ponce, Puerto Rico; St. Thomas and St. Croix, Virgin Islands; Tegucigalpa, Honduras; and in San José, Costa Rica. Dr. Cyril J. Hodapp, NASA Lunar Receiving Laboratory, Houston, Texas, is collaborating in the study on the etiology of infectious hepatitis.

5. During the past year, studies on the etiology of diarrheas, experienced by North Americans entering and living in tropical environments, have involved five groups of Peace Corps volunteers and a group of Goshen College students.

Extracts have been prepared from 502 fecal specimens from Peace Corps volunteer groups. These have been inoculated into cultures of the human malignant cell line, HEP-2. A virus isolation rate of 55 percent (207 isolates) was obtained. Virus identification procedures will be initiated upon completion of passages confirming isolation. Of 502 extracts inoculated into newborn mice, 324 (65 percent) produced clinical signs; extracts from paralyzed mice will be passaged to cell culture to facilitate identification. The high isolation rates, in culture and in

newborn mice, are noteworthy since very few of the specimens were obtained from acute cases of diarrhea.

Serologic investigations were carried out with paired serum samples collected from Peace Corps volunteers in training. Data revealed that a substantial number of the volunteers were already immune to one or more Cocksackie Group B virus types when training in the tropics commenced. Although the serologic results which show low rates of infection by the Cocksackie Group B types do not appear, at present, to correlate with the high rates of isolation of paralysis-producing agents in newborn mice, such data do appear to correlate with the low rates of diarrhea which were experienced within these groups of volunteers. The Goshen College students in Costa Rica experienced more diarrhea. Data being derived from the study of the latter group will be reported in greater detail when the virologic aspects have progressed further.

6. The project on the pathogenesis of amebic lesions includes studies on (a) Entamoeba histolytica, which may produce intestinal lesions with extension to other organs, and (b) amebae of the genus Hartmannella which can produce fatal encephalitis in man and experimental animals. a) The surface activity of trophozoites of E. histolytica is being studied by electron micrographs of amebae in intestinal lesions in guinea pigs. Acid phosphatase activity was present in large amounts in both the cytoplasm and at the periphery of the trophozoites. The cytoplasmic activity seems primarily associated with membranous structures of the endoplasmic reticulum and lysosomes. Numerous phagocytic vacuoles demonstrated activity at their interior surface and within their contents. Primary lysosomes with significant activity are associated with such phagocytic vacuoles. Host cells adjacent to the amebae are devoid of such reaction deposits. Acid phosphatase activity at the amebal surface is associated with blebs and branching filiform pseudopods which appear to be involved in a process of apocrine release. These surface projections appear to be breaking off into the space surrounding the amebae. Occasionally this space contains necrotic host tissue and reaction product in the interstices. Alkaline phosphatase activity was not detected in the amebae using present methods. b) In order to characterize and confirm peroxisomes in pathogenic strains of Hartmannella sp. observed cytochemically and ultrastructurally, density gradient centrifugation is being employed. Amebae are washed, homogenized, and the soluble enzymes removed. The centrifuged sediment, containing membrane-bound enzyme systems, are placed on a

density gradient, centrifuged, and fractions are collected and prepared for electron microscopy. The staining reactions in the trophozoites of Hartmannella sp. suggests that there are three enzyme systems inducing the oxidation of DAB, one in the peroxisomes and two in the mitochondria. The enzyme system on the inner and outer membranes of the mitochondria is enhanced by the addition of H₂O₂ and the second system on the cristae is not. Neither system appears to be a peroxidase. These enzyme systems are cyanide sensitive, slightly azide sensitive, ATZ insensitive, and heat labile. c) A method for the rapid fixation, infiltration, and imbedding of hartmannellid and other amebae has been developed. The new procedure permits a suspension of amebae to be prepared and micrographs to be obtained in a 24-hour period.

7. Studies are continuing on attempts to isolate the agent or agents of infectious hepatitis (IH). The plan of this investigation is based upon the hypothesis that conventional host-cell systems found suitable for the isolation and propagation of the majority of the enteric virus types should be equally suitable for the propagation of the agent or agents of infectious hepatitis, provided that the specific cultural requisites are met. The major investigator of this study employed a similar hypothesis for the first isolation of a Rhinovirus. A Costa Rican isolate (C.R.076) which showed a definite CPE under specific cultural conditions was studied. It was obtained from a case of IH in 1969. Paired sera from a contact case of IH in 1964 were challenged with this agent. No increments in neutralizing antibody were detected in convalescent serum. The existence of more than one IH virus type cannot be ignored. Further studies showed that this agent could infect Rhesus monkey renal cells as well as human malignant cell HEp-2. Identification with available antisera to known virus types has not been successful to date; however, a number of antisera have yet to be employed for this purpose. Attempts to isolate similar agents from specimens of feces collected from Air Force personnel having IH, with HEp-2 cells with this host cell system under modified cultural conditions, and serial blind passage were unsuccessful.

8. In the study on the biological control of Chagas' disease vectors, with the parasitic microhymenopteran Telenomus fariai, the triatomid population has been reduced by 75 to 80 percent. The preference of Telenomus for fertilized Triatoma dimidiata eggs over unfertilized eggs has been confirmed.

Parasitism of T. dimidiata by the mite Pimeliaphilus zeledoni in the laboratory has resulted in molting and teratological defects in recently molted nymphs. Difficulty in molting, including death of nymphs, incomplete formation of legs, and failure of the cuticle to harden have been observed.

Experiments on the dynamics of the camouflage phenomenon in T. dimidiata have shown a definite relationship between the physiological condition of the nymphs, such as repletion and degree of starvation, and the phenomenon. Light intensity also has a significant influence on this activity.

9. In recent years it has been found that enzootic foci of Venezuelan equine encephalitis (VEE) exist in what appear to be small, sharply circumscribed foci. In the enzootic foci which have been studied in some depth, there is indication that the basic virus cycle involves rodents and rodent-feeding Culex of the subgenus Melanoconion. Several species of this subgenus occur in the United States.

A reconnaissance for a possible enzootic VEE virus focus in the Mississippi River Delta Region has been undertaken. Ecological situations similar to enzootic areas in South America are being examined. Culex (M.) aikenii, the vector in enzootic foci in South and Central America, has not been found in the preliminary work; but C. erraticus, one of its associates in an enzootic focus in Panama, has been identified.

10. There are no published studies of the Lutzomyia (=Phlebotomus) of Bolivia. The flies are medically important in the transmission of leishmaniasis and bartonellosis. In recent years several new arboviruses have been found associated with Lutzomyia. Evidence also has been accumulated that in some situations they may be vectors of vesicular stomatitis virus.

A project to determine the composition, distribution, and ecology of Lutzomyia in the Yungas Valley of Bolivia has been undertaken. From the latter part of June to early August 1971, 207 specimens of Lutzomyia were collected at various altitudes in different habitats. The material is being subjected to analysis.

11. Work on the identification of black flies (Simuliidae) collected during field studies in Costa Rica is continuing. It will be necessary for Dr. B. V. Travis to return to Costa Rica in 1972 for six months to work intensively in order to complete the major portion of the identifications with Dr. M. Vargas, if full fruition of these studies is to be achieved. A relatively small amount of additional funding will be required to accomplish this.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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BODY OF REPORT

INTRODUCTION

The title of this contract "Infectious and Communicable Diseases Affecting Populations Introduced into the Tropics" implies a direct relationship to specific military function and operation. Diarrheal diseases and infectious hepatitis are major problems of the military. A wide array of arbovirus infections in the tropics represent hazards to newcomers especially in rural, swamp, and forested areas. The prevalence of amebiasis in some communities in Latin America may exceed 50 percent and this poses a problem for civilian and military personnel entering such areas. Chagas' disease, with its complication of myocarditis and the absence of suitable therapeutics, is endemic in wide areas of Latin America. Biting flies, particularly black flies (Simuliidae), produce bites with serious reactions, in addition to being vectors of diseases of man and animals. Phlebotomine flies (sandflies) are vectors of cutaneous, mucocutaneous, and visceral leishmaniasis which are endemic in many areas of Latin America. Often leishmaniasis is a major hazard for persons entering sylvan areas for agricultural or military endeavor. They also transmit bartonellosis.

In accordance with the stated aims of Project THEMIS (DOD Brochure, November 1968), the LSU/THEMIS program, during the current year, has:

- (1) Continued multidisciplinary research by groups of faculty members and research associates in the field of tropical medicine;
- (2) Conducted and made substantial progress on research projects involving several biomedical disciplines;
- (3) Involved at least 15 faculty members from several departments, who represent competence in several disciplines, in the research and training activities;
- (4) Included young and developing staff members in the program;
- (5) Published two scientific papers and presented results of current research at six scientific meetings;

- (6) Collaborated in research with personnel of two Federal Agencies in six geographic areas;
- (7) Provided training in tropical medicine for medical officers of the Armed Forces.

FACILITIES AND COLLABORATIVE INSTITUTIONS

Research and training activities of the THEMIS program of the Louisiana State University Medical Center are conducted at the School of Medicine and William Pitcher Plaza (Florida Avenue) in New Orleans and at the offshore facility in San José, Costa Rica. These facilities complement each other and provide unusual opportunities for research and training and field studies on infectious diseases of the tropics and subtropics. Excellent research, training, and library facilities are available at the New Orleans and offshore facilities.

Scientists from several institutions, listed below, are collaborating with the staff of the LSU School of Medicine in the research and training projects of this program.

Louisiana State University Medical Center, New Orleans
University of Costa Rica School of Medicine and School of Microbiology, San José, Costa Rica
Hospital San Juan de Dios, San José, Costa Rica
Ministry of Health of Costa Rica
National Children's Hospital, San José, Costa Rica
NASA Lunar Receiving Laboratory (Dr. Cyril J. Hodapp), Houston, Texas
Peace Corps Training Center, Ponce, Puerto Rico
Peace Corps Training Centers, St. Thomas and St. Croix, Virgin Islands
Peace Corps Volunteers, Tegucigalpa, Honduras
Peace Corps Volunteers, San José, Costa Rica
Goshen College (Indiana) Volunteers in Costa Rica

PUBLICATIONS AND MEETINGS

Papers Published

- Childs, G.E., J.H. Miller. 1971. Peroxidase localization in Hartmannella trophozoites. 29th Ann. Proc. Electron Microscopy Soc. Amer., pp. 346-347.
- Travis, B.V., and M. Vargas V. 1970. Bionomics of black flies in Costa Rica. II. An ecological consideration. Proc. 57 Ann. Meet. New Jersey Mosq. Exterm. Ass'n., pp. 111-112.

Papers Presented at Scientific Meetings *

- Childs, G.E., and J.H. Miller. Ultrastructural localization of peroxidases in Hartmannella trophozoites. 8th Ann. Symp., La. Soc. for Electron Microscopists, New Orleans, La., March 5, 1971.
- Childs, G.E., and J.H. Miller. Ultrastructural localization of peroxidases in Hartmannella trophozoites. Southwestern Ass'n. of Parasitologists, Texas A&M Univ., College Station, Texas, April 24, 1971.
- **Childs, G.E., and J.H. Miller. Peroxidase localization in Hartmannella. 29th Ann. Meet. Electron Microscopy Soc. of Amer., Boston, Mass., August 11, 1971.
- Trapido, H. Geographic distribution and ecological settings. Pan American Health Organization Workshop-Symposium on Venezuelan Encephalitis Virus, Washington, D.C., September 14-17, 1971.
- Trapido, H., and C. Sanmartin. Studies of survival of Venezuelan equine encephalitis virus in Colombian Simulium. Ann. Meet. Amer. Soc. Trop. Med. & Hyg., San Francisco, November 1-4, 1970.
- Travis, B.V., and M. Vargas V. Bionomics of black flies in Costa Rica. II. An ecological consideration. 57th Ann. Meet. New Jersey Mosq. Exterm. Ass'n., Atlantic City, March 11-13, 1970.

*This travel was at no expense to the contract.

**This paper won an EMSA Presidential Scholarship.

ETIOLOGY OF DIARRHEAL DISEASE IN PERSONS INTRODUCED INTO A
TROPICAL ENVIRONMENT

Introduction

During the past year, studies on the etiology of diarrheas, experienced by North Americans entering and living in tropical environments, have involved two groups of youths: (1) Peace Corps volunteers, and (2) Goshen College students.

The Peace Corps volunteers had been sent into such tropical surroundings in areas of the Caribbean for training and orientation before being assigned to posts in various Latin American countries. The Peace Corps Training Centers which collaborated with our investigations were located in Puerto Rico, St. Thomas, St. Croix, and in Honduras.

The Goshen College, located in Indiana, has established programs of social studies in Costa Rica and other countries. Groups of college students go to Costa Rica for periods of 2 to 3 months. These are highly motivated young people, for whom this usually represents their first experience of travel outside of the United States. The vigorous cooperation of Dr. Theron Schlaback, Local Director of the Goshen College Study Program, was of great value in the conduct of the study.

Results

In Puerto Rico, five groups of volunteers were observed during their stay at the Training Center at Ponce. These groups comprised a total of 71 individuals. Fecal and blood samples were collected from the trainees upon their arrival and prior to their departure following the completion of their training at the Center. A total of 136 fecal samples was collected. Stools were obtained also from seven volunteers at the time they were experiencing diarrhea. Paired blood samples for serologic investigations were obtained from 70 of the volunteers.

In the Virgin Islands, 59 volunteers were followed at the Training Center at St. Croix, while 40 were observed at the Center at St. Thomas. The St. Croix train-

ing group submitted 91 fecal samples, while 76 specimens of feces were collected from those at St. Thomas. No diarrheas were experienced among the St. Croix volunteers. Seven persons in the St. Thomas group reported episodes of diarrhea and submitted acute disease samples for etiologic studies. As in the case of the Puerto Rican trainees, paired serum samples were collected from both groups prior to and following completion of training.

Our studies were broadened further through the cooperation of the Peace Corps Training Center in Honduras. Four volunteer groups were followed in this area, comprising a study population of 49 individuals. A total of 199 fecal samples was submitted for etiologic examination. In contrast to the Puerto Rico-Virgin Island groups, 27 of the individuals followed in Honduras experienced episodes of diarrhea.

Our studies were complemented also by the arrival of a small group of eight Peace Corps volunteers in San Jose, Costa Rica, for duty in that country. Baseline blood and fecal specimens were obtained from these individuals. Acute fecal specimens were obtained also, from the volunteers when attacks of diarrhea were experienced during their stay in Costa Rica. A total of 15 acute disease samples has been collected.

As indicated earlier, it was the good fortune of this investigation to have the Peace Corps volunteer study populations supplemented with yet another group of North Americans. Through the enthusiastic cooperation of Dr. Theron Schlaback, the Local Director of the Goshen College Study Program, 18 blood specimens and 36 control fecal specimens were collected from a group of 18 Goshen College students. In addition, 16 acute fecal specimens were submitted by members of this group experiencing diarrhea during a 6-week period. The Goshen College students have proved to be the most disciplined and cooperative group which we have encountered in this investigation.

Extracts have been prepared from 502 fecal specimens collected from the Peace Corps volunteer groups in Puerto Rico, in the Virgin Islands, and in Honduras. The fecal extracts have been inoculated into cultures of the human malignant cell line, HEP-2. Cytopathic effects have been observed in cultures inoculated with 275 of the fecal extracts, resulting in a virus isolation rate of 55 percent. One hundred and twenty-five of the isolates have

been confirmed by passage in the same cell line. Virus identification procedures will be initiated upon completion of passages confirming isolation. Fecal extracts were inoculated into newborn mice (<18 hours old) also, utilizing the intra-cerebral, intraperitoneal and intrascapular routes (into the brown fat area) simultaneously. Of the 502 specimens, 324 produced clinical signs, a rate of approximately 65 percent. Extracts from paralyzed mice will be passaged into cell culture to facilitate the identification of such agents. The high rate of virus isolation in cell culture, as well as in newborn mice, is noteworthy, since only a very few of the specimens were obtained from acute cases of diarrhea.

Serologic investigations were carried out with the paired serum samples collected from the Peace Corps volunteers who were in training in Puerto Rico and in the Virgin Islands. One hundred and twenty-eight paired sera were screened against the first five Coxsackie Group B virus types, while 102 serum pairs were tested against Coxsackie Group B, Type 6 virus. Studies with this particular virus group was of immediate interest, since earlier investigations of children with and without diarrhea in Costa Rica had shown an etiological relationship between infection with members of this virus group and the diarrhea syndrome.

The serologic data revealed that a substantial number of volunteers were already immune to one or more Coxsackie Group B virus types when training commenced. The prevalence of neutralizing antibody to the various virus types in the initial serum sample was as follows: Coxsackie Group B Type 4 (16%), Type 1 (24%), Type 5 (25%), Type 6 (26%), Type 2 (38%), and Type 3 (53%). Infection, as evidenced by significant increments in antibody levels in paired sera, was found to be minimal: Coxsackie Group B, Type 4 (0%), Type 1 (1%), Type 5 (5%), Type 6 (5%), and Type 2 (7%). Although the serologic results which show low rates of infection by the Coxsackie Group B types occurring during the training period do not appear, at present, to correlate with the high rates of isolation of paralysis-producing agents in newborn mice, such data do appear to correlate with the low rates of diarrhea which were experienced within these groups of volunteers.

Bacteriologic studies conducted on the 16 acute diarrhea fecal samples submitted by the Goshen College

students resulted in the isolation of Salmonella (species not identified) from two specimens. Direct examination of stools for parasites revealed Giardia lamblia in one case, which was cleared by specific chemotherapy. Virologic investigations are in progress. A new group of Goshen College students is expected to arrive in one month. Arrangements have been made for its inclusion in the study.

PATHOGENESIS OF AMEBIC LESIONS

Part I. Studies on Entamoeba histolytica

Several strains of Entamoeba histolytica were cultured on polyxenic egg slant-saline-rice starch medium described by Boeck and Drbohlav. Two of these strains were found to be pathogenic for guinea pigs.

Guinea pigs (150 gm) were maintained on a calorie-deficient diet for several days prior to inoculation. Trophozoites were washed in saline and injected directly into the caecum by laparotomy. Ulcers were produced with inocula containing more than 200,000 trophozoites of strain J-22 and more than 1,000,000 amebae of strain LB. Strains 200, J-27, and ML did not produce significant caecal ulcerations.

From gross examination, the ulcers produced by strain J-22 at 7-10 days post-inoculation were larger, more deeply indurated, and more hemorrhagic than those produced by strain LB in the same period of time. Week-old ulcers produced by strain LB resembled those caused by J-22 in about 3 days.

Lesions produced by both strains were prepared for general electron microscopy by the following method. Ulcerated areas of the caecum were excised and cut into 1 mm. cubes. Tissue was fixed in cold 3-6% glutaraldehyde buffered in 0.25M cacodylate-HCl (pH=7.4) for 2 hours. Then it was washed overnight in three changes of cold 1M cacodylate-HCl buffer (pH=7.4) with 7% sucrose. Post-fixation was in 0.25M cacodylate-HCl buffered 1% OsO₄ for 2 hours. Next the tissue was washed three times in deionized water, dehydrated through a graded series of ethyl alcohols to propylene oxide (2 changes, 10 minutes each), and embedded in Araldite 501. Paraffin-embedded sections of adjacent ulcerated tissue were stained with hematoxylin and eosin and viewed by light microscopy.

Ultratome sections were cut on an LKB Ultratome I, placed on naked 200-mesh grids, stained with uranyl acetate and lead citrate, and viewed at 50 KV in a Hitachi HU-11A electron microscope.

Trophozoites of strain J-22 treated in the above manner had an increase in vacuolization and in the amount of endo-

plasmic reticulum. There was stacking of elongate cisternae reminiscent of a Golgi apparatus. Autophagic and phagocytic vesicles associated with primary lysosomes were often observed. Branching filiform pseudopods, which appear to be involved in an apocrine process, were present. Older, senescent appearing, or dying amebae within the ulcer may be surrounded by a walling-off process. Perhaps endo-enzymes liberated from such amebae do aid in the invasive process. Ultrastructural examination of ulcers produced by strain LB is as yet incomplete.

Lesions produced by strain J-22 were examined for acid and alkaline phosphatase activity. Excised tissue was fixed in 2% glutaraldehyde in 0.1M cacodylate buffer (pH=7.4) with 7% sucrose for 2 hours, washed briefly in 1M buffer, sectioned on a Smith-Farquhar Tissue Chopper at 50 μ , and rinsed overnight in 0.1M cacodylate buffer with 7% sucrose. This tissue was then incubated for acid phosphatase localization according to the method of Barka and Anderson or for alkaline phosphatase localization after Mayahara, et al. Control tissue was incubated in complete medium with sodium fluoride, in complete medium without substrate, and in complete medium without the lead-containing constituent. Incubated tissue was post-fixed in 1% OsO₄ and embedded as described above.

No alkaline phosphatase activity was found in the J-22 strain of E. histolytica by these methods.

Acid phosphatase activity in strain J-22 was localized in large amounts in the cytoplasm and at the periphery of the trophozoites. The cytoplasmic activity seems primarily associated with membranous structures of the endoplasmic reticulum and lysosomes. Numerous phagocytic vacuoles showed activity at their interior surface and within their contents. Primary lysosomes with significant activity were associated with such phagocytic vacuoles. Host cells adjacent to the amebae in section were devoid of such reaction products.

Acid phosphatase activity at the amebal surface was associated with blebs and branching filiform pseudopods which appear to be involved in a process of apocrine release. These surface projections seemed to be breaking off into the space surrounding the amebae. Occasionally this space contained necrotic host tissue with reaction product in its interstices.

The study of ulcers produced by the LB strain of E. histolytica after 15-20 days in the guinea pig is planned. It is thought that this strain is considerably less invasive than strain J-22 and their comparison may prove fruitful. Further histochemical tests for specific nucleotide phosphatase, and employing colloidal iron, ruthenium red, lanthanum nitrate, and methenamine silver, are contemplated for study of both strains. Such tests will enable us to characterize the superfluous molecular moiety present at the external surface of the bimolecular leaflet in tissue-phase E. histolytica.

Part II. Studies on Hartmannella

Certain strains of small free-living amebae of the genus Hartmannella are the etiologic agents of a fatal encephalitis in man and experimental animals. These amebae are able to penetrate the nasal mucosa and migrate along the olfactory nerve to the brain. It is hoped that an investigation of the cell biology of these amebae may provide an insight into the mechanism of invasion of these and, perhaps, of intestinal amebae.

Biochemical and density gradient centrifugation studies have revealed that the oxidative enzymes of Acanthamoeba castellanii, a related species, are localized in two subcellular particles, the mitochondria and peroxisomes (microbodies) (Muller and Moller, 1969).

Although hartmannellid amebae have been the subject of several ultrastructural studies, peroxisomes have not been identified morphologically in these organisms. This study is a preliminary attempt to localize catalase activity in the peroxisomes and to characterize the cytochemical properties of the peroxidase enzyme systems in Hartmannella sp. (A-1, Culbertson), a pathogenic strain grown in axenic culture of trypticase soy broth. Cytochemical tests using 3,3'-diaminobenzidine tetra HCl (DAB) and hydrogen peroxide were employed for localization of peroxidase (Novikoff and Goldfischer, 1969). The oxidized product of DAB forms osmiophilic deposits at sites of peroxidase activity.

One-week cultures of Hartmannella sp. were fixed, washed, and divided into 10 experimental groups. The complete medium, described by Novikoff and Goldfischer (1969)

for the optimal staining of peroxisomes, contained 5.6 mM DAB and .02% H_2O_2 in 50 mM 2-amino-2-methyl-1,3-propanediol buffer. The amoebae were incubated at 30°C for 60 min.

The effects of pH were tested by substituting acetate buffer, pH 6.0, and Tris buffer, pH 7.5, in the complete medium. The role of H_2O_2 in the reaction was determined by omitting the substrate and, also, by adding catalase to the complete medium to eliminate the possible effects of endogenously produced substrate. The effects of inhibitors, 3-amino-1,2,4-triazole (ATZ), sodium cyanide, and sodium azide, were tested by incubating the trophozoites in propanediol buffer plus the inhibitor for 30 min prior to incubation in the DAB-containing medium with the inhibitor. Heat lability was determined by incubating the amoebae at 56°C for 30 min, and at 100°C for 1 min, in propanediol buffer prior to incubation in the complete medium. All sections were observed unstained in a Hitachi HU-11A electron microscope operating at 50 KV.

In amoebae incubated in the complete medium, both mitochondria and peroxisomes were strongly stained. The peroxisomes of *Hartmannella* sp. are smaller than the mitochondria and more irregular in shape. The reaction in the peroxisomes appears as a fairly homogeneous deposit over the entire organelle with no apparent membranous substructure on which the peroxidase is localized. In the mitochondria the stain was localized on the fine, branched, tubular cristae and on the inner and outer membranes. No reaction was observed in the matrix, intracristae spaces, and between the inner and outer membranes.

At pH 6.0, the optimal pH for the staining reaction in the mitochondria (Novikoff and Goldfischer, 1969), all membranes of the mitochondria were strongly stained. In addition, deposits of reaction product obscured the intracristae spaces and the spaces between the inner and outer membranes. Only a slight reaction was seen in the peroxisomes. The staining of the mitochondria and peroxisomes in the trophozoites incubated at pH 7.5 was essentially the same as in the complete medium.

The reactions in the group incubated without H_2O_2 were similar to those in the group incubated with catalase added. No reaction was observed in the peroxisomes and only a slight reaction was seen in the inner and outer membranes of the mitochondria. The cristae, however,

showed a strong reaction.

The peroxisomes appeared sensitive to ATZ, a specific inhibitor of catalase activity, while the mitochondria were insensitive.

Both peroxisomes and mitochondria showed no reaction when incubated with NaCN and a moderate reaction when incubated with NaN_3 .

The reaction in the cristae and peroxisomes is only slightly heat sensitive at 56°C , although the inner and outer membranes of the mitochondria appeared to be more sensitive. No reaction was seen in either the mitochondria or peroxisomes in amebae incubated at 100°C .

A summary of results appears in Table 1.

The staining reactions in the trophozoites of Hartmannella sp. suggests that there are three enzyme systems inducing the oxidation of DAB, one in the peroxisomes and two in the mitochondria.

The enzyme system on the inner and outer membranes of the mitochondria is enhanced by the addition of H_2O_2 , and the second system on the cristae is not. Neither system, however, appears to be a peroxidase since there is a reaction in the absence of the peroxide substrate or with the addition of catalase. These enzyme systems are cyanide sensitive, slightly azide sensitive, ATZ insensitive, and heat labile. Optimal activity occurs at pH 6.0.

The staining reactions of the peroxisomes are similar to those described for peroxisomes of multicellular plant and animal tissues. The dependency of the reaction on H_2O_2 as a substrate indicates that the enzyme system of this organelle is a peroxidase. Specific inhibition of the reaction by ATZ further suggests that the enzyme is catalase. The enzyme system is cyanide sensitive, slightly azide sensitive, and heat labile. The morphology of these peroxisomes differs from those described from higher plants and animals. In contrast to the usual round or oval structures with crystalline cores, peroxisomes of Hartmannella are irregular in shape; a crystalline core is seen only rarely. Vacuoles or indentations are a common morphological feature.

Table 1. Summary of reactions of mitochondria and peroxisomes of Hartmannella with diaminobenzidine

Group	Mitochondria			Peroxisomes
	Cristae	Intracristae spaces	Membranes Matrix	
Complete	+++	-	+++	+++
pH 6.0	+++	+	+++	-
pH 7.5	+++	-	+++	+++
No H ₂ O ₂	+++	-	+	-
Catalase	+++	-	+	-
ATZ	+++	-	+++	-
NaCN	-	-	-	-
NaN ₃	++	-	++	-
56°C, 30 min.	++	-	+	++
100°C, 60 min.	-	-	-	-

- No reaction
 + Slight reaction
 ++ Moderate reaction
 +++ Strong reaction

In order to characterize further and confirm the identity of these peroxisomes, density gradient centrifugation studies are being undertaken. The amebae are washed, homogenized, and the soluble enzymes removed. The sediment, containing membrane-bound enzyme systems, is placed on a density gradient, centrifuged, and the fractions collected and prepared for electron microscopy. At present, efforts are being made to determine, by biochemical, cytochemical, and morphological studies, which fractions contain the peroxisomes.

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STUDIES ON THE ETIOLOGY OF INFECTIOUS HEPATITIS

Introduction

In the 1969-70 Annual Progress Report encouraging findings were described which were obtained when fecal extracts from cases of infectious hepatitis (IH) were inoculated into cell cultures. Since the observations were made near the close of the report period, additional data were not available. As stated previously, the plan of this investigation was based upon the hypothesis that the conventional host-cell systems found suitable for the isolation and propagation of the majority of the enteric virus types should be equally suitable of the isolation and propagation of the agent or agents of IH, provided that specific cultural requisites are met. The same approach which led to the initial recognition of the Rhinovirus group, by the researcher engaged in this project, establishes a precedent for such a concept. Hence, the problem then has become one of defining the cultural requisites.

Results

Since it was uncertain whether fecal samples collected from individuals who were diagnosed as having IH actually contained excreted virus, preliminary investigations were carried out with multiple specimens. As previously reported, subtle changes were noted first in cultures inoculated with the fecal extracts and incubated for a prolonged period. Such effects tended to disappear with a change of medium. On the other hand, continued incubation did not make the cellular alteration more obvious.

Even though the changes observed were barely perceptible, the decision was made to pursue this aspect further. The approach employed was that of modifying the medium as well as the environment of the host cells. When these avenues were explored, an unusual phenomenon was observed. Cultures which were inoculated with four of the seven fecal extracts under test, rapidly and completely degenerated simultaneously, while control cultures remained intact. Upon incubation for an additional 24 hours, similar changes were noted in the control and remaining inoculated cultures. This was attributed to the modified medium. At the present time, the changes ob-

served in inoculated cultures prior to that seen in the control cultures are not interpreted as being due to the direct effect of a possible agent upon the cells, but rather to an indirect effect of such an agent. To distinguish these changes from distinct viral cytopathic effects, such cellular destruction will be temporarily referred to as "IH effects." The IH effect may be attributed to toxic factors; however, inoculations of cell cultures previously with the same fecal extracts did not produce such changes.

When serial passages were made of the harvested culture fluids, the IH effect was observed once more in the inoculated cultures. The third subpassage fluid was titrated through a dilution of 10^{-4} , and the IH effects were noted in cultures inoculated with each of the dilutions. This would represent a dilution of 1:100,000,000 of the original inoculum. It is recognized that this does not necessarily obviate the possibility of the presence of a potent toxic factor in the fecal extract affecting the cells. It does raise speculation that a replicating agent may be present.

A small double-blind study was conducted with fecal samples collected from cases of IH and from a control population showing no evidence of IH. Although the results obtained upon decoding seemed encouraging, the differences in the rates of the IH effect observed between the control fecal extracts and those for cases of IH did not appear significant. However, the issue may have been clouded by the possibility that some individuals within the control population may have been shedding IH agents and still have appeared asymptomatic. High rates of enteric virus isolations have been obtained from the general population sampled in Costa Rica, which ranged from approximately 45 percent in the 40-year old or greater age group to almost 80 percent in the 1-2-year old age group. If one considers the high incidence of IH in Costa Rica and if the IH virus parallels, to any degree, the distribution of enteric viruses within the population, then there is reason to assume that the IH agent is ubiquitous there and the clinical cases of IH observed merely represent the "top of the iceberg." In addition, there is no evidence that the IH effect may be the result of the action of a single suspect agent, and, in reality, may be indicative of a number of types, just as a single common cold agent was sought by the writer, with more than 60 types ultimately being uncovered. It is obvious that additional efforts are required

before interpretations may be made on a clear-cut basis. It should be noted that fecal extracts from three IH cases yielded agents which produced a conventional viral CPE. These are believed to be common enteric virus types, since they were recovered under normal cultural conditions.

One specimen, (CR69-076), collected from a case of IH as part of the double-blind study described above, produced the characteristic IH effect encountered. However, when cultures of similar cells were inoculated with this specimen, but incubated under somewhat different cultural conditions, cytopathic effects characteristic of many enteric virus types were observed. Attempts to identify this agent against a spectrum of virus-type specific antisera have been unsuccessful to date; however, a number of antisera have yet to be employed for this purpose.

Efforts to detect increments in neutralizing antibody levels in paired sera from a contact case of IH collected in 1964, employing the 076 agent as antigen, were unsuccessful. As mentioned, the existence of more than a single IH virus type cannot be ignored. Further studies have shown that the 076 agent was capable of infecting Rhesus monkey renal cells, as well as the human malignant cell HEp-2. Attempts to isolate similar agents from specimens of feces collected from Air Force personnel and their dependents with IH, by the procedure employed, were unsuccessful. Although the possibility that this agent may represent a genetic variant of the IH virus which has become adapted to the host cells cannot be ignored, it is currently felt that this agent is either merely a common enteric virus type involved in a concurrent infection with IH and was being excreted in minute quantities, or that it represented a "traveler" virus, accidentally ingested and then detected in the course of its journey through the gastrointestinal tract.

Although a number of findings have been made, the fundamental question which must be answered is whether the IH effect is, in reality, the indirect effect of a proliferating infectious agent which ordinarily replicates without accompanying host cell disruption. This should be followed by the establishment of those conditions which are necessary to permit optimal viral proliferation and the detection of the virus thus propagated. Support for such thinking is obtained from a negative aspect, namely the high incidence of this disease, some 37,000

cases in the United States alone through August 14 of this year, in addition to the failure of numerous investigators, employing a wide variety of host systems, to demonstrate the etiologic agent for this disease. Further, the ability of agents to replicate without disruption of the host cell system is not unique. One may cite a number of specific examples, including the Simian Viruses 5, 40, and 41, rubella virus, parainfluenza virus types 1, 3, and 4, as well as an attenuated strain of Newcastle virus. Furthermore, the composition of the maintenance medium employed for the host cells was found to influence the type of cytopathic effects observed with some agents. Measles virus, as an example, proliferating in cells being maintained in a complete medium, produced spindle-shaped cells, while in the presence of a glutamine-deficient medium, virus-infected cells took on the appearance of multinucleated giant cells.

Studies have been hampered by the need for large quantities of actively growing host-cell stocks as seed for tube cultures, and by the sudden unexplained reduction in the rates of cell proliferation in the stock cultures after a relatively few passages. Efforts to overcome this problem will include the obtaining of cells from alternate sources as well as the use of outgrowth media other than that employed in the past.

BIOLOGICAL CONTROL OF CHAGAS' DISEASE VECTORS

Parasitism of triatomid eggs by Telenomus fariai

The following is a summary of studies on the possibility of biological control of two populations of Triatoma dimidiata by the wasp Telenomus fariai. The experiment was begun in October 1969 by liberating about 7,000 wasps in each of two houses (nos. 26 and 44) in an endemic area of Chagas' disease (San Rafael de Ojo de Agua, Province of Alajuela, Costa Rica) every 2 - 3 weeks (in a few instances at longer periods). Each time the wasps were liberated, the triatomid bugs present in the houses were counted during a standard period of time (60 min.). Starting in December 1970 another house in the same area, with natural infestation by T. dimidiata, was included in the experiment as a control. In all three houses the population of triatomids was counted each time that the wasps were released in houses 26 and 44 (Tables 1, 2, and 3). The data indicate that house 26, which originally had about 40 insects of different instars (easily visible after a careful search of their favorite hiding place), started decreasing in number gradually with a tendency of the first instars to disappear. After the 7th treatment, the triatomid population decreased by 75 to 80 percent. In house 44 there was a marked decrease after the initial treatments followed by an increase after treatment number 5; then there was an apparent disappearance of the insects at the 11th treatment. This apparent eradication coincided with the efforts of the inhabitants to destroy them. The insect population was again evident after treatment number 16. Since then, it has been very low with no first or second instars present. In house 27, the control, the original population of insects was 21 and has remained more or less constant through nine counts in a period of about 7 1/2 months (Table 3).

The preference of Telenomus for fertilized T. dimidiata eggs when exposed at the same time to unfertilized eggs was confirmed by an additional experiment (Table 4).

Due to a contamination of laboratory colonies of triatomids (including T. dimidiata) with Telenomus at the end of last year, we had a reduced number of eggs available for some other experiments which are planned. At present some of the studies are being conducted and in-

clude: a) determination of the effect of dusting the eggs with dirt on the ability of the wasp to find them; this explores the theory of masking the odor of the egg by dust in an effort to render detection by the parasite more difficult; b) investigation of the possibility of making eggs from other species of triatomids (not normally parasitized by Telenomus) more attractive by covering them with powder from macerated T. dimidiata eggs (further study related to the odor theory); c) determination of the number of hours or days Telenomus can live once they are liberated. At the conclusion of these experiments, we shall publish the accumulated information on the behavior of the wasps under different circumstances and on the feasibility of biological control with this microhymenopteran.

Parasitism of triatomids by Pimeliaphilus mites

Experiments to observe further the host specificity of Pimeliaphilus zeledoni were conducted. Results again confirm the difficulty that this mite has in parasitizing a heterologous host like Triatoma barberi (Table 5). Furthermore, the effect of the mite on its natural host, T. dimidiata, in the laboratory has been observed through several experiments. Difficulty in molting, including death of nymphs, and morphologic defects, such as absence or incomplete formation of the legs and failure of the cuticle to harden, have been observed in recently molted nymphs. A note on the studies with this mite will be prepared for publication soon.

Camouflage activity of the triatomids

The publication of the paper "The camouflage phenomenon in several species of Triatominae (Hemiptera: Reduviidae)" was delayed in order to clarify varied results in the study of the behavior of some instars of the same species. Additional experiments were designed to study the dynamics of the phenomenon in relation to the physiological state of the nymphs. Specimens of all instars of T. dimidiata were tested for the presence of the camouflage instinct immediately before and after a blood meal and every 3rd day thereafter. Observations were made 15 min. and 24 hours after placing the nymphs in earth-filled Petri dishes. The results of these experiments indicate that the physiological condition of the insect

is important in its manifestation of the camouflage phenomenon. Before a blood meal the instinct is very marked but persists at a slower rate after feeding, as judged by the high percentage of nymphs that remain uncovered after 15 min.; however, a high percentage are covered within 24 hours. After 2 or more weeks there is a marked decrease in activity at both the 15-min. and 24-hour observations. This phenomenon seems to appear earlier in the smaller nymphs than in the larger. This is probably related to the physiological changes that accompany the molting process. Other experiments are in progress to observe the effect of prolonged starvation of nymphs immediately after molting and of light on the camouflage instinct.

Table 1. Effect of the release of Telenomus fariai on the population of Triatoma dimidiata of house no. 26

Date (days)	9-16-69 (0)	11-6-69 (51)	12-16-69 (40)	1-24-70 (39)	3-5-70 (40)	4-4-70 (30)	5-5-70 (31)	6-6-70 (32)	6-27-70 (21)	7-23-70 (30)	8-25-70 (33)	
Treatment no.	1	2	3	4	5	6	7	8	9	10	11	
1st instar	0	0	0	0	0	0	0	1	0	0	0	
2nd instar	3	5	2	0	0	1	0	1	2	0	0	
3rd instar	5	9	3	3	2	2	0	0	0	1	2	
4th instar	12	16	4	2	6	0	2	2	3	1	1	
5th instar	15	4	2	16	5	4	2	1	2	1	2	
Adults	4	6	1	3	1	5	3	0	1	3	4	
TOTAL	37	40	12	24	14	12	7	5	8	6	9	
Date (days)	9-19-70 (25)	10-17-70 (28)	11-14-70 (28)	12-12-70 (28)	2-10-71 (60)	3-8-71 (26)	4-1-71 (24)	4-27-71 (26)	5-21-71 (24)	6-16-71 (26)	7-15-71 (29)	8-8-71 (24)
Treatment no.	12	13	14	15	16	17	18	19	20	21	22	23
1st instar	0	0	0	0	0	0	0	0	0	0	0	0
2nd instar	0	0	0	0	0	0	0	0	0	0	0	0
3rd instar	1	2	1	0	0	0	0	0	0	0	0	0
4th instar	2	1	3	3	3	1	1	1	2	2	3	1
5th instar	1	3	2	3	4	1	0	3	3	1	2	3
Adults	3	2	2	3	3	2	2	5	4	3	1	2
TOTAL	7	8	8	9	10	4	3	9	9	6	6	6

Table 2. Effect of the release of Telenomus fariai on the population of Triatoma dimidiata of house no. 44

Date (days)	11-25-69 (0)	12-16-69 (22)	1-24-70 (39)	3-5-70 (40)	4-4-70 (30)	5-5-70 (31)	6-6-70 (32)	6-27-70 (21)	7-23-70 (30)	8-25-70 (33)	9-19-70 (25)
Treatment no.	1	2	3	4	5	6	7	8	9	10	11
1st instar	0	0	0	5	7	8	6	5	3	3	0
2nd instar	0	0	0	1	2	4	4	4	4	5	0
3rd instar	3	0	1	0	1	3	2	1	2	1	0
4th instar	6	2	1	0	1	0	2	1	0	2	0
5th instar	4	3	1	1	0	0	0	1	1	1	0
Adults	5	5	1	0	1	0	1	1	1	1	0
TOTAL	18	10	4	7	12	15	15	13	11	13	0
Date (days)	10-17-70 (28)	11-14-70 (28)	12-12-70 (28)	2-10-71 (60)	3-8-71 (26)	4-1-71 (24)	4-27-71 (26)	5-21-71 (24)	6-16-71 (26)	7-15-71 (29)	8-7-71 (24)
Treatment no.	12	13	14	15	16	17	18	19	20	21	22
1st instar	0	0	0	0	0	0	0	0	0	0	0
2nd instar	0	0	0	0	0	0	0	0	0	0	0
3rd instar	0	0	0	0	0	1	0	1	0	0	0
4th instar	0	0	0	0	0	2	1	1	2	3	2
5th instar	0	0	0	0	0	1	2	2	2	0	1
Adults	0	0	0	0	0	1	2	1	3	1	1
TOTAL	0	0	0	0	4	4	5	5	7	5	4

Table 3. Triatoma dimidiata population in a control house (27) after a search of 1 hour

Date (days)	12-28-70 (0)	2-10-71 (44)	3-8-71 (26)	4-1-71 (24)	4-27-71 (26)	5-21-71 (24)	6-16-71 (26)	7-15-71 (29)	8-7-71 (24)
No. of counts	1	2	3	4	5	6	7	8	9
1st instar	2	3	1	0	0	0	0	0	0
2nd instar	3	1	2	3	0	0	0	1	1
3rd instar	5	4	1	2	3	2	3	4	4
4th instar	4	3	3	3	7	8	6	3	8
5th instar	5	4	2	4	6	5	6	4	5
Adults	2	4	5	6	8	9	8	9	7
TOTAL	21	19	14	18	24	24	23	21	25

Table 4. Parasitism of fertilized eggs (from couples) and unfertilized eggs (from virgin females) from Triatoma dimidiata by Telenomus fariai

Condition	No. of eggs exposed	No. of eggs parasitized (%)
Fertilized	30	29 (96.6)
Unfertilized	30	14 (46.6)

Table 5. Parasitism of two species of triatomids by Pimeliaphilus zeledoni *

Species	No. of days after exposure to the mites	Insect no. (1-5) and number of mites (below)				
		1	2	3	4	5
<u>T. dimidiata</u>	0					
	15	17	10	6	5	6
	29	34	12	10	5	9
	43	30	10	21	9	-
	54	36	0	31	5	-
	68	49	0	-	-	-
	82	58	1			
	96	64	5			
	110	87	15			
	124	4	10			
	1	-				
<u>T. barberi</u>	0					
	15	0	0	0	0	2
	29	1	2	0	0	0
	43	2	7	3	0	-
	54	-	5	0	2	
	68		5	1	3	
	82		6	2	0	
	96		0	1	3	
	110		5	2	2	
	124		7	1	3	

* Five 3rd-instar nymphs of each species were exposed to 100 mites in a Petri dish at 26.5°C.
 - Dead

RECONNAISSANCE FOR A POSSIBLE VENEZUELAN EQUINE
ENCEPHALITIS ENZOOTIC FOCUS IN THE
MISSISSIPPI RIVER DELTA REGION

During the past decade, epizootemics of Venezuelan equine encephalitis (VEE) have been intense and wide-spread. In the Venezuelan outbreak of 1962-64, 30,000 human cases with 190 fatalities were reported. In Colombia, there were an estimated 200,000 to 400,000 human cases and 100,000 to 150,000 equine deaths in the epizootemic of 1967-68. In 1969 the virus was active in equine and human populations in Central America, Ecuador, and Peru; in 1970 in southern Mexico; and 1971 on the Gulf coast of Mexico and southern Texas. The reasons for the periodic appearance of VEE in epizootemic form across northern South America and its spread into Central America, Mexico, and Texas for the first time are not understood.

In recent years it has been found that enzootic foci of VEE virus exist in what appear to be small, sharply circumscribed foci. Such foci have been found in Brazil, Trinidad, Colombia, Panama, Central America, Mexico, and southern Florida in the United States. The relationship of these enzootic foci to the periodic epizootemic episodes in man and equines is obscure, although there is evidence that there are several strains of the virus in nature which differ in antigenicity, pathogenicity, and the viremia levels they reach in different hosts.

During epizootemics in man and equines, VEE virus has been recovered from virtually all genera of mosquitoes commonly feeding on these animals. In the enzootic foci which have been studied in some depth, there is indication that the basic virus cycle involves rodents and rodent-feeding Culex of the subgenus Melanoconion. The Culex subgenus Melanoconion includes numerous species in the neotropical region and several occur in the United States. Characters for the recognition of species within the subgenus Melanoconion are found principally in the males and larvae; the females are mostly difficult to distinguish, and in some cases recognition characters for females at the species level have not yet been found.

In long-term studies of an enzootic VEE focus in Panama by workers at the Gorgas Memorial Laboratory, it was found that, although VEE virus could readily be recovered from triturated specimens of wild caught Culex (Melanoconion), transmission by bite to laboratory animals could not be accomplished. Most recently, it has been found that naturally infected Culex (Melanoconion) of one species, aikenii, are capable of readily transmitting the virus by bite (Science, 172: 594-595, 1971). This species has been found to breed in association with water lettuce, Pistia stratiodes, in the Chagres River at a Panama enzootic focus. Culex (Melanoconion) aikenii is not now known from the United States, but Pistia is distributed north to the Gulf Coast states. Sigmodon hispidus, the cotton rat, which has been found to have a high incidence of antibodies to VEE virus at enzootic foci in Mexico, Central America, and Panama, also occurs along the Gulf Coast in the United States.

The evidence for the possible involvement of birds in the natural cycle of VEE virus is contradictory. Nevertheless, in an arbovirus study carried on in a swamp near Lake Pontchartrain in the Mississippi delta region, neutralizing antibodies for VEE virus were found in a low proportion of bird sera collected in 1952 (Amer. J. Hyg. 62: 233-254, 1955). Rodents were not examined in this study.

With this much background information, it has seemed worth beginning exploration of the possibility that an enzootic VEE virus focus may exist in the Mississippi River delta area.

Although there are Pistia herbarium specimens from as far north as the latitude of Baton Rouge, 60 to 80 miles from the coast, a severe winter in the early sixties is reported by local botanists to have killed off much of the Pistia at the northern limit of its range. During the winter of 1970-71, field trips were made to sites in the Delta region from which herbarium specimens are known. During these cold months, Pistia was found in only scattered small patches and no mosquito larvae were found sheltered among them. By June there was a vigorous proliferation of the plants covering water surfaces and associated mosquito larvae were moderately abundant. Of the numerous Culex (Melanoconion) collected, all examined thus far have been C. (M.) erraticus. While Culex (M.) aikenii has not yet been found, it is noteworthy that

Culex (M.) erraticus is one of the species associated with aikenii in the Chagres River enzootic focus in Panama.

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THE SPECIES COMPOSITION, DISTRIBUTION, AND ECOLOGY
OF THE LUTZOMYIA (=PHLEBOTOMUS) OF THE
YUNGAS VALLEY OF BOLIVIA

Introduction

There are no published studies of the Lutzomyia (=Phlebotomus) of Bolivia. These small Diptera are of importance as vectors of leishmaniasis in much of the neotropical region including Bolivia, and also as vectors of bartonellosis in the Andean regions of Peru and Ecuador. In recent years several new arboviruses have been found associated with Lutzomyia and evidence has accumulated that in some situations they may be vectors of vesicular stomatitis virus.

The study is designed to give information on the Lutzomyia fauna in an altitudinal transect from above 4000 meters to nearly 200 meters. The field work was carried on during the period June through August 1971 in the Yungas valley, which in its 200 km length provides access to semi-arid habitats ecologically similar to those in which bartonellosis is known further north in the Andes and also, at lower elevations, to tropical forest areas in which leishmaniasis occurs. Collecting was done by several methods which will yield information on both the anthropophilic and zoophilic elements of the Lutzomyia fauna, the altitudinal distribution of the species, and the habitats and microhabitats in which they occur. Collections are being preserved in the field. Species identifications is being done at the home laboratory in New Orleans.

Results

At the time of writing, data are available on collections made between 22 June and 4 August 1971. The following tabulations provide preliminary information on the altitudes and habitats from which Lutzomyia have been obtained.

Table 1. Numbers of Lutzomyia by habitat

Tree holes and hollows	29
Tree crevices	2
Tree buttresses	2
Chicken coops	107
Pig pens	14
Sheep corral	19
Mine shaft	3
Cemetery	2
Masonry wall	4
Human habitation (thatched roof)	1
Total	<u>183</u>

N.B. An additional 24 specimens were taken in CDC miniature light traps.

Table 2. Numbers of Lutzomyia by altitude

500 meters	8
501-1000 meters	21
1001-1500 meters	20
1501-2000 meters	155
2001-2500 meters	3
Total	<u>207</u>

Comments

Data on effort expended in making these collections will not be available until later; but the present impression is that Lutzomyia populations are small, both at the higher and lower elevations. Of the several domestic livestock-associated habitats examined, chicken coops have been most productive, suggesting that there may be an ornithophilic species present. Comparisons of the species composition of Lutzomyia collections taken in association with pigs, sheep, and chickens will be of particular interest. In light of the known human-feeding predilection of Lutzomyia further north in the Andes in Peru, it is surprizing that only a single specimen has so far been found in a human habitation. The taxonomic study of the Yungas material will reveal whether there may not be major differences in the species composition of the Lutzomyia occurring in Bolivia as compared with Peru.

BIONOMICS OF BITING DIPTERA OF COSTA RICA

Work on the identification of black flies (Simuliidae) collected during the field studies in Costa Rica is continuing. However, it will be very desirable for Dr. B. V. Travis to go to Costa Rica in 1972 for 6 months to work intensively with Dr. M. Vargas in order to complete the major portion of the identification of specimens. This will be necessary if full fruition of the studies is to be achieved. Additional funds to cover travel to Costa Rica and return, per diem, and a small amount for technical assistance and supplies, will be needed to do this.

During the year a paper on the "Bionomics of Black Flies in Costa Rica II. An Ecological Consideration" was presented and published in the Proceedings of the 57th Annual Meeting of the New Jersey Mosquito Extermination Association, Atlantic City, March 11-13, 1970.