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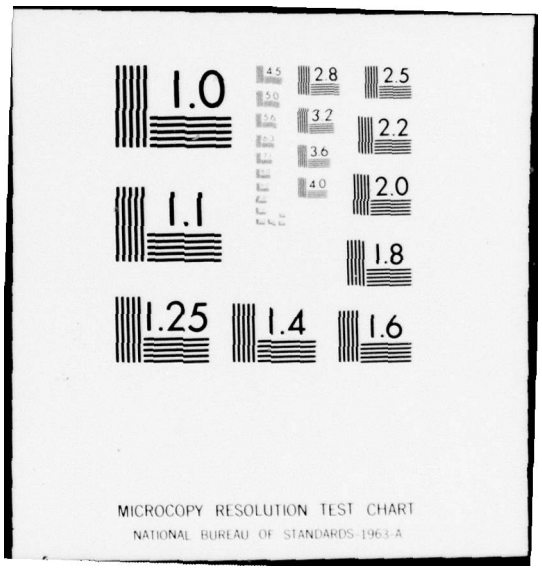
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IMMUNOLOGY OF MALARIA

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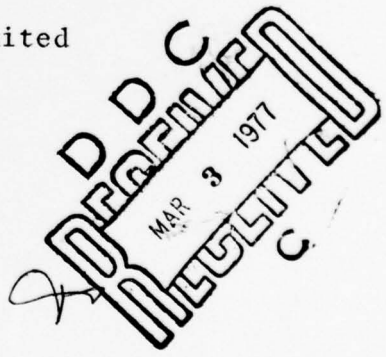

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Clinical investigations were conducted to determine whether non-immune persons can be immunized against vivax or falciparum malaria after exposure to X-irradiated sporozoites of Plasmodium vivax or Plasmodium falciparum. Batches of Anopheles stephensi mosquitoes were infected with malaria and, 12 to 18 days later, they were irradiated with X-rays until they had received a dose of at least 12 Krads. They were then allowed to feed on adult male volunteers, the concurrent inoculation of sporozoites serving as

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an immunizing dose.

Three volunteers were exposed to irradiated sporozoites of the Ethiopian (Tamenie) strain of P. falciparum on 6 to 8 occasions at intervals of 2 weeks or longer. The period of immunization extended over a period of 10 to 38 weeks and the volunteers were bitten by a total of 500 to 1000 irradiated mosquitoes carrying sporozoites. After completing the immunization schedule, the volunteers were challenged by exposure to 7 to 45 infected mosquitoes on one or more occasions. At the time of challenge, the mosquitoes were divided into 2 groups and allowed to feed intermittently on immunized and control volunteers by the interrupted bite technique. Control volunteers invariably developed patent infections, but immunized volunteers were protected for at least 8 weeks after the last immunizing exposure. It is noteworthy that immunization with the Ethiopian strain also induced protection against challenge with a heterologous strain from Vietnam. Although protection was observed for a 2-month period, it was no longer evident 4 months later.

In other studies, 8 volunteers were exposed to infected irradiated mosquitoes on 1 to 4 occasions over a period 1 to 4 months. They were bitten by a total of fewer than 200 irradiated mosquitoes containing sporozoites; three of the volunteers were bitten by irradiated mosquitoes infected with P. vivax and 5 of them by mosquitoes infected with P. falciparum. This less frequent exposure to a smaller number of attenuated sporozoites failed to protect any of the volunteers against subsequent challenge with the homologous strain.

The findings indicate that protection against malaria can be obtained by inoculation of human malaria sporozoites attenuated by X-irradiation. It is unclear at present whether the inoculation of larger number of sporozoites on one or two occasions might induce long-term protection against malaria. This question can only be answered when pure sporozoite preparations, free of mosquito tissue and contaminating microorganisms, become available for human evaluation.

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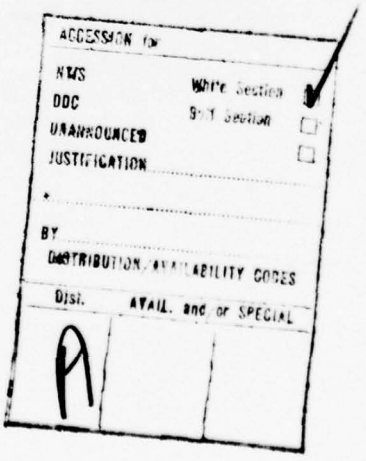

I. BACKGROUND

A. Location

The studies were performed by the Laboratory for Tropical Diseases, Rush-Presbyterian-St. Luke's Medical Center located at the Stateville Branch of the Illinois State Penitentiary, Joliet, Illinois. The general conditions and methods used have been described in detail elsewhere. [1,2] Precautions employed at Stateville included extremely close medical and nursing care on one floor of the prison hospital, where routine laboratory determinations including parasite counts, electrocardiograms, and emergency medical cart, etc., were immediately available. In the event that special equipment available only at Presbyterian-St. Luke's Hospital were required for the treatment of a patient at Stateville his transfer to the hospital could have been accomplished easily. Insofar as testing new drugs, vaccines, etc., was concerned, because the Stateville facility was part of the Department of Medicine, these items were submitted to the Committee on Human Investigation. This committee reviewed and approved clinical use of all drugs for the Department of Medicine in accordance with FDA regulations and ethical medical practice. Untoward reactions were also reported via the Committee on Therapeutics and Pharmacology to the Department of Medicine and thence to government and national registries as required.

Clinical studies with human malaria had been carried

out with inmate volunteers at this center for 25 years. During this time, the center became a poineer in establishing the principles and necessary safeguards for volunteers. The use of written and informed consent and the right to terminate participation in any given program were established at this facility long before general concern resulted in such expressions as the Declaration of Helsinki.

The work of the facility at Stateville was of course dependent on the continued full cooperation of the Warden and the Director, Department of Corrections, State of Illinois. During 1975 the Department of Corrections decided to close the Malaria Research Project at Stateville. The decision was taken because logistical considerations associated with inmate rehabilitation and was not related to medical or ethical reasons.

B. Experimental

Immunity to malaria had been induced in animals after inoculation of X-irradiated parasites. Inoculation of irradiated erythrocytic parasites of Plasmodium berghei attenuated the course of infections, but did not significantly reduce the percentage of patent infections upon challenge. [3,4] Studies by Richards with P. gallinaceum suggested that sporozoites might be more effective as immunizing agents than inactivated erythrocytic parasites.[5]

More recent studies with A/J mice had suggested that

a solid immunity could be obtained after injection of X-irradiated sporozoites of P. berghei.[6,7,8] In these investigations, Nussenzweig et al. had found that:

1) The higher the dose at which sporozoites were irradiated, the smaller the percentage of animals that became infected. The following results were obtained when 5,000 to 10,000 irradiated sporozoites were injected into susceptible mice: 4 Krads, 59% (20/34) infected; at 6 Krads, 12% (7/58) infected; at 8 Krads, 1% (1/53) infected; at 10 Krads, 2% (1/64) infected; and at 12.5, 15, or 20 Krads, none (0/37) infected. Nonirradiated control sporozoites infected 88% (30/34) of injected mice. The presence of subpatent parasitemia in these animals were excluded by consistently negative results upon subinoculation or after splenectomy.

2) In addition to a lower infection rate brought about by irradiated sporozoites, the animals that did become infected had significantly prolonged pre-patent periods. Liver biopsy specimens suggested that this was due to arrest or retardation in the development of pre-erythrocytic tissue schizonts.

3) After an irradiation dose of 8 or 10 Krads, a single inoculum of 7.5×10^4 sporozoites protected 75% of 20 subsequently challenged mice against a lethal infection, whereas a single inoculum of 5.0×10^3 sporozoites protected only 42% of 36 animals (mean of 3 experiments). Only 10 or 30

control animals failed to succumb to the infection.

4) In comparing the effects of irradiation doses, a single inoculum of 1 x 10^4 sporozoites at 15 Krads failed to protect any animals.

5) A single immunizing dose of even 7.5 x 10^4 sporozoites (8-12 Krads) usually produced only partial protection. A minimum of 4 boosters (5 x 10^3 sporozoites), administered every 2 weeks were frequently required to produce close to total protection. In animals which were not protected, a partial suppressive effect was observed and was characterized by a prolongation of the pre-patent period, peak parasitemia and survival time.

During studies with P. cynomolgi in Macaca mulatta, Warren and Garnham [9] irradiated infected mosquitoes with 5 to 10 Krads dosages. Ten Krad doses apparently inactivated all sporozoites while lesser doses procuded graded responses as reflected by morphologic changes in developing tissue schizonts in liver biopsies. However, monkeys which received irradiated sporozoites showed "no" significant protection after subsequent challenge with non-irradiated sporozoites.[10,11]

II. OBJECTIVE

To determine whether immunity to malaria could be induced in human volunteers after being bitten by irradiated mosquitoes infected with malaria parasites.

III. PROCEDURES

In view of the encouraging results obtained with irradiated sporozoites of P. berghei, investigations were started to determine whether a similar protection could be obtained with P. vivax or P. falciparum in human volunteers. Because of ethical and other considerations, sporozoites removed from the mosquito host could not be inoculated directly into any volunteers. Consequently live infected mosquitoes were irradiated and allowed to bite non-immune volunteers to determine whether the released sporozoites exerted any immunogenic effects. It was realized, of course, that only a small proportion of sporozoites in the salivary glands would be released by mosquitoes and that the number of sporozoites to which these volunteers would be exposed was considerably less than in the animal experiments.

Healthy male inmate volunteers of Stateville Penitentiary, Joliet, Illinois, between the ages of 21 and 40 years, were selected for the studies. In addition to a thorough history and physical examination, volunteers had to pass the following laboratory determinations: urinalysis, including fasting morning specific gravity and microscopic examination of the sediment; hematocrit; hemoglobin; WBC; serum bilirubin; SGOT and SGPT; BUN; serum alkaline phosphatase; serum creatinine; blood grouping; chest X-ray. In addition, sickle-cell positive or glucose-6-phosphate dehydrogenase (G6PD) and glutathione reductase deficient black

volunteers were identified and excluded from these studies. All volunteers were non-immune, i.e., they had not experienced any previous exposure to malaria.

One-to two-day old mosquitoes (Anopheles stephensi) at the Stateville insectary were infected by feeding on gametocyte "carriers" in the Malaria Ward. Eighty-four "carrier" volunteers participated in these studies and about one-third of them developed a sufficient number of gametocytes to infect mosquitoes. After their blood meal, mosquitoes were placed in an incubator maintained at 20° C and fed on a sucrose solution. After 6 days, the guts from 5 mosquitoes in each batch were examined for oocysts. At 10, 12, 14, and 16 days, the salivary glands of 5 additional mosquitoes were examined for sporozoites. Mosquitoes from cages that were heavily infected (at least 25% of mosquitoes with more than 1000 sporozoites per gland) were irradiated with 12,000 rads between 12 and 18 days after their blood meal.

Irradiation of mosquitoes was performed by Professor A. Chung-Bin, Chief of the Section of Radiation Physics, Presbyterian-St. Luke's Hospital. A Westinghouse 220 KVP Orthovoltage X-ray machine was used for this purpose. The surface of the sample to target distance was 10 cm. The tube was operated at 220 KVP and 18 millamperes and the quality of the beam was half mm copper. Dose rate was about 2100 roentgen/min. The exposure time was determined by measurement with a Victoreen R meter prior to the exposure of the sample

and, in addition, a Lithium fluoride disk was used to confirm the dosage of each sample irradiated. The space containing the mosquitoes during the irradiation was 1.0 cm in thickness and 9.4 cm in diameter. 13.0 cm thickness of water was provided as backscatter material.

After X-irradiation of mosquitoes, with about 12,000 rads, mosquitoes were immediately transported back from the hospital to the penitentiary (30-40 minutes drive) and were allowed to bite volunteers who had never been exposed to malaria for about 20 minutes. After the blood meal, each engorged mosquito was dissected to determine whether its salivary glands contained any sporozoites. Control volunteers were bitten by non-infected irradiated mosquitoes. In addition, one or more volunteers were usually exposed to a small number of non-irradiated infected mosquitoes to verify mosquito infectivity. This procedure was repeated on 2 to 8 different occasions at intervals of 2 or more weeks.

The following baseline and at least twice-weekly follow-up values were obtained from all volunteers participating in these studies: hemoglobin, hematocrit, WBC, differential count, reticulocyte count, platelet count, thrombin and prothrombin times, fasting blood glucose, BUN, SGOT, SGPT, CPK, serum bilirubin, urinalysis, microurine, and electrocardiograms. These observations were continued for 4 weeks after the last exposure to irradiated mosquitoes.

About 2 weeks after their last exposure to irradiated

mosquitoes, volunteers were challenged with non-irradiated infected mosquitoes to determine whether they had developed any immunity against malaria. Throughout these studies, a control volunteer was exposed to the same batch of mosquitoes in order to verify the transmission potential of that particular batch of A. stephensi mosquitoes. The mosquitoes were divided into 2 groups and allowed to feed intermittently on immunized and control volunteers by the interrupted bite technique. After completion of this procedure, they were dissected to determine the actual number of mosquitoes that were infected. Blood film examination was started 7 days after challenge. Depending on the development of patent parasitemia after challenge, blood films were made daily for 3 weeks, every second day for the next 2 weeks and every third day for another 4½ months if indicated. If parasitemia did not develop after challenge in the immunized volunteers they were rechalleneged several weeks later with either the same strain or a different one.

Volunteers were given radical curative treatment either after developing a clinical attack of malaria or after completion of the study (whether they developed a patent infection or not). Volunteers exposed to mosquitoes infected with P. vivax received the standard chloroquine-primaquine treatment. Volunteers exposed to mosquitoes infected with P. falciparum received standard chloroquine treatment if the strain was drug-sensitive or amodiaquine-tetracycline treatment [12] for a drug-resistant strain.

IV. RESULTS

A. Toxicity Studies

Before initiation of the efficacy studies 5 volunteers from the Navy Medical Research Institute participated in preliminary studies to assure prison volunteers that the inoculation of irradiated sporozoites had no adverse side-effects in man. These 5 volunteers were bitten by several hundred irradiated mosquitoes (both infected and non-infected) once each week for a period of 6 weeks. Thorough medical and laboratory examinations failed to show any evidence of systemic side-effects.

B. Efficacy Studies

1. Plasmodium vivax

Studies were initiated with the Chesson strain of P. vivax. The 3 persons who were exposed to infected irradiated mosquitoes on 4 occasions at 2 to 4 week intervals developed acute attacks of malaria after challenge with the homologous strain. The pre-patent period, development of parasitemia and clinical course of infection were similar in the control volunteers and the volunteers who were bitten by infected irradiated mosquitoes.

2. Plasmodium falciparum

Further studies were conducted with either the Ethiopian (Tamenie) strain or the Vietnam (Marks) strain of P. falciparum. Five persons were exposed to infected irradiated mosquitoes on 1 to 4 occasions over a period of 1 to 4 months

and three persons were exposed to such mosquitoes on 6-8 occasions over a period of 3 to 9 months.

Volunteers who were immunized 1 to 4 times were bitten by a total of 200 or fewer irradiated mosquitoes containing sporozoites. Two of the volunteers were treated once with chloroquine during the course of immunization after the development of patent parasitemia following exposure to irradiated mosquitoes. None of the 5 volunteers were protected against challenge with the homologous strain and the course of infection was similar to that observed in control volunteers in every respect.

In contrast to the above findings, each of the 3 volunteers who was exposed to irradiated sporozoites on 6 or more occasions was protected against malaria. The course of these volunteers - L.A., D.S., and W.D. are illustrated in the Figure. They were all immunized with the Ethiopian (Tamenie) strain of P. falciparum and the immunizing doses were given at intervals of 2 weeks or longer. During the course of these studies, immunization and challenge schedules could not be carried out at well-defined intervals or with a constant number of infected mosquitoes. In relying on volunteer gametocyte carriers to infect mosquitoes, it is virtually impossible to predict the course of an infection, the extent to which an infection has to be suppressed by drugs to prevent the development of dangerously high levels of parasitemia, the duration and intensity of the subsequent gametocyte wave and the infectivity

of gametocytes to vector mosquitoes.

Volunteer L.A. was exposed to irradiated mosquitoes on 6 different occasions and the interval between each exposure was exactly two weeks. Dissection of blood-fed mosquitoes over the 12-week period revealed that he had been bitten by a total of 440 mosquitoes infected with sporozoites. Two weeks after the last immunizing exposure, the volunteer was challenged with 13 non-irradiated mosquitoes infected with the same strain of P. falciparum. He did not become infected, whereas the control volunteer developed patent parasitemia 12 days later. However, after being challenged again 16 weeks after the last immunizing dose with 45 infected mosquitoes, he developed an infection during the normal prepatent period. The results of these preliminary findings were published in 1974.[13]

Volunteer D.S. was exposed to irradiated mosquitoes on 8 different occasions and was bitten by a total of 954 irradiated mosquitoes containing sporozoites. The first four immunizing exposures were given at regulary biweekly intervals. Then, there was an interval of about 13 weeks between the fourth and fifth exposures. A period of six weeks elapsed before the next exposure, and the last two exposures were given once more at biweekly intervlas. The course of immunization extended over a period of 33 weeks. Two weeks after the last immunizing dose, he was bitten by 14 non-irradiated mosquitoes infected with the Ethiopian strain. He was

protected and did not develop a patent infection. About 8 weeks after the last immunizing dose, he was rechallenged by 12 mosquitoes infected with the same strain and, again, he was fully protected. On both occasions, the control volunteers developed patent infections within 3 weeks after challenge. The immunized volunteer was not protected after being challenged by 7 mosquitoes infected with the Vietnam (Marks) strain of P. falciparum 17 weeks after the last immunization. He was also not protected against challenge with the Ethiopian (Tamenie) strain 25 weeks after the last immunization.

Volunteer W.D. was exposed to irradiated mosquitoes 7 different occasions and was bitten at irregular intervals over a period of 40 weeks by a total of 910 irradiated mosquitoes containing sporozoites. About 8 weeks after the last immunizing dose, he was challenged with 9 mosquitoes infected with the Vietnam (Marks) strain and was completely protected against infection with this heterologous strain. Two control volunteers became patent within 11 and 13 days. The immunized volunteer was challenged again 18 weeks after his last immunizing dose by mosquitoes infected with the Ethiopian strain, but developed patent parasitemia 13 days later.

V. CONCLUSIONS AND RECOMMENDATIONS

The results of these investigations in man indicate that protection against malaria can be obtained by inoculation of irradiated malaria sporozoites. These findings are supported by the results of other investigations.[14] Under the conditions of our studies, 6 to 8 exposures with a total of about 500-1000 irradiated mosquitoes were required before protection was obtained. Complete protection lasted for at least 8 weeks after the last immunizing exposure, but it was no longer evident 16 to 18 weeks later. The observation that the protection induced by this immunization procedure was effective against challenge with a strain of P. falciparum from another continent is most encouraging and it should stimulate further investigations to develop a malaria vaccine containing attenuated sporozoites.

It is recommended that:

(1) Investigations be pursued to obtain pure human sporozoites, free of mosquito tissue and contaminating microorganisms, in order to determine whether administration of a sizeable inoculum of attenuated sporozoites on 1 or 2 occasions can induce long-term protection against malaria.

(2) In view of the development of patent parasitemia in 2 unprotected persons after they were bitten by mosquitoes irradiated with 12 Krads, further studies should be carried out with mosquitoes irradiated at a higher dose to ensure that no sporozoites remain infective after irradiation.

(3) Various strains of Plasmodium falciparum and/or P. vivax be established in non-human primate models to obtain sporozoites for immunization, antigenic, and serologic studies.

(4) Optimum conditions be determined for gametocyte production in primates to obtain consistently high levels of infection in mosquitoes, and for the collection, separation and storage of human sporozoites.

(5) Pathological and toxicological studies be conducted in primates immunized with sporozoite antigens.

(6) The value of primates, primarily A. trivirgatus, be explored as a model for assessing the efficacy of sporozoite antigens.

(7) The specificity of sporozoite antibody tests, e.g. the indirect fluorescent sporozoite inhibition test, be investigated for various strains of P. falciparum and P. vivax.

(8) Demographic and epidemiologic data be collected in population group(s) who will eventually participate in field trials involving sporozoite-specific serologic surveys and vaccination studies.

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APPENDIX A

STATE OF ILLINOIS
DEPARTMENT OF CORRECTIONS
SPRINGFIELD

The Malaria Project at Stateville Penitentiary, Joliet

Introduction

Clinical studies of human malaria, with the participation of inmate volunteers, have been in progress at the Stateville Branch of the Illinois State Penitentiary in Joliet for 28 years. The project was a clinical unit of the Department of Medicine at the University of Chicago until June, 1971. Since then, it has been a clinical unit of the Department of Medicine of Rush-Presbyterian-St. Luke's Medical Center, Chicago. The Malaria Project currently receives its financial support solely by contract with the U.S. Army Medical Research and Development Command and the Office of Naval Research. The work of the Project depends on the cooperation of the Director of the Department of Corrections and the Warden of the Penitentiary.

The Malaria Project has made important contributions to the health and welfare of millions of people throughout the world. Initial evaluation of drugs which are now widely used in the treatment and prevention of malaria was carried out here, this evaluation has contributed significantly to the reduced prevalence of malaria. Despite these achievements, about 200 million persons suffer from malaria each year and drug-resistant malaria parasites have spread to many countries of South America and Southeast Asia. In many African villages entire populations are sometimes stricken with malaria and it is the single most important disease on the Continent. The malaria problem is also of immediate concern to this country when one considers that more than 3 million Americans travelled abroad to malarious or potentially malarious areas during 1971. It will undoubtedly become more acute as international travel to and from the United States increases. Malaria has also been a major problem in military and civilian personnel returning from Southeast Asia.

In addition to the outstanding contributions in the field of malaria, the human deficiency of the red cell enzyme, glucose-6-phosphate dehydrogenase, was discovered at this project as a result of studies with antimalarial drugs. It is known that this hereditary deficiency is found in at least 200 million people of all races, including 10-15% of all black Americans, a prevalence greater than that of sickle cell trait. One of the clinical consequences of this disorder is that affected persons develop hemolytic anemia when given certain types of medication. This important discovery has alerted the medical profession to the possible adverse effects of commonly used drugs in susceptible people and has undoubtedly already saved many thousands of lives. The Project and its staff continues to be consulted, nationally and internationally, about this condition which in a more severe form is also prevalent in people of Mediterranean and Oriental ancestry.

Selection and consent procedures for volunteers

Inmates who wish to volunteer forward a request to the Malaria Project indicating their desire to participate in the program. Their names are sent to the prison administration for approval. The administration approves or disapproves their participation on the basis of their past record, conduct, and length of time remaining in their sentence before they go to the Parole Board. Volunteers must be 21 years or older to participate in the program. Inmates who are approved by the prison administration are screened at the Project by chest X-ray, electro- cardiography, urinalysis, blood sugar, complete blood counts and blood tests for liver and kidney function. After a review of this preliminary information, each person is interviewed and given a complete physical examination by one of the physicians at the Project. Only men who show no signs of any physical impairment are allowed to participate in the malaria program.

During the personal interview the purpose and procedures of the study are explained to the potential participants. They are told in layman's language the details of the study as fully as possible. They are also advised that without appropriate treatment malaria can become a serious and potentially lethal illness. Incidentally, most of them seem to be aware of this because they have seen highly dramatized versions of the illness in movie and television performances. Potential volunteers are encouraged to ask any questions. Special emphasis is placed on the voluntary nature of the individual's involvement and especially on his right to terminate his participation at any time during the course of the study. He may ask to be cured at any time and will be released from the hospital after curative treatment and convalescence. He is told that, after becoming infected, the course of his illness will be handled very conservatively and the malaria infection is heavily suppressed by appropriate medication. Nevertheless, if the infection is not well-tolerated for any reason, the physician reserves the right to cure the patient at any time. Potential volunteers are also told that blood and urine samples will be collected and that they may be bitten by mosquitoes during the course

of the study. *All potential volunteers are assured that they will be cured of malaria* and that in the history of the Malaria Project there have been no long term ill effects from exposure to this illness. It is pointed out, nevertheless, that it is not possible to guarantee absolute safety in any medical situation. Potential volunteers are also encouraged to visit the malaria ward and talk with current participants if they wish. After a thorough discussion, the men who want to participate are given the consent form in which the points of the proposed study are written in simple language. After assurance that they understand the contents, those who wish to participate sign their names in the presence of a witness.

Compensation

Many inmates work at assignments for which they are paid and absence from their regular work during participation in the malaria program usually means a loss of remuneration. Consequently, the volunteers receive about $30 a month during their participation. It is felt that this amount is reasonably related to compensation for other services in the prison and is not high as to contribute undue inducement to participate in the program. Although they forfeit the privilege of going to the yard for daily exercise, the boredom of inpatients is relieved by the availability of television and a good selection of current magazines on the ward. At the conclusion of the study, a letter is sent to the Warden stating that they have participated in the program. Each volunteer, however, understands that his participation is no more advantageous when his case is considered by the Parole Board than good behavior elsewhere in the penitentiary.

Types of study

Once a volunteer has entered the malaria program he may either be hospitalized on the malaria ward as an inpatient or be involved with the program as an outpatient and continue with his usual activities. His in- or outpatient status varies with the nature of the study. In any case, a volunteer may not be an outpatient if he has malaria parasites in his blood or if he shows any signs or symptoms of not being in good health.

Over a period of a year between 80 and 120 volunteers participate in the program. The number of individuals participating at any one time averages about 25, approximately half of them being outpatients. Their length of participation varies from a few weeks to a few months.

Volunteers may participate in a number of different ways in the malaria program. They are given a choice of being involved in, 1) Phase I studies to determine the tolerance in man of a new antimalarial drug, 2) Phase II studies to determine the antimalarial efficacy of a new drug, 3) studies to determine the value of new regimens of known antimalarial drugs against human malaria, 4) becoming a short-term malaria "carrier".

Some important features of the malaria program

Volunteers may be infected with either vivax or falciparum malaria. Only untreated falciparum malaria may be fatal and this does not usually occur until 10 to 50% of the red cells are infected with malaria parasites. In volunteers participating in the malaria program, never more than 1% of the red cells are infected (usually less than 0.5%) and this represents a wide margin of safety in these studies. Furthermore, all strains of malaria to which volunteers may be exposed, must have been proven previously to be curable by antimalarial drugs available to the staff; this includes "drug-resistant" strains of malaria such as those resistant to chloroquine or to other known antimalarials.

The design of the study will vary according to its primary purpose. Determination of the *therapeutic* efficacy of a drug against malaria means that a volunteer will be admitted to the malaria ward upon the appearance of parasites in his blood and that he will remain in the hospital until his blood shows no parasites. He may then be discharged from the malaria ward, but his blood will be examined daily for at least 60 days after treatment. The drug has effected a cure if no parasites are seen during this follow-up period as an outpatient. On the other hand, if parasites reappear during this time, the patient is immediately readmitted to the ward and appropriate treatment with a known curative drug is instituted. A new drug is initially given to volunteers who have already acquired a marked degree of immunity to malaria. Such a drug is given to nonimmune volunteers only after its effect against parasites in immune volunteers have been carefully ascertained. The antimalarial effect of a new drug is supplemented with well-documented effective antimalarials if the need should arise. The clinical condition and safety of the patient are always the prime consideration. Determination of the *prophylactic* (or protective) efficacy of a drug may not require admission to the malaria ward unless the drug is ineffective and parasites appear in the peripheral blood stream during his subsequent observation period of at least 60 days after last drug administration. A follow-up period of 6 months is necessary if the antirelapse value of a drug is being determined against infections with vivax malaria.

A malaria "carrier" implies that the volunteer "carries" or has parasites without experiencing any symptoms. Small amounts of parasitized blood from such patients can be used for *in vitro* culture studies and for infecting mosquitoes. During the first month of the infection, fever and symptoms are controlled by medication with suppressive drugs. The average volunteer experiences about 3 febrile episodes during this time. Each episode lasts 2-3 days and in the intervening time he is virtually free of symptoms. This low-grade infection, however, enables the volunteer to become progressively more immune and, at the end of about a month, he usually requires no further suppressive treatment for alleviation of symptoms. He maintains this low level of parasites (about 0.1 to 0.5% of red cells with parasites) for a few weeks. Thereafter, when he becomes even more immune to malaria and very few parasites are present in his blood stream, he is treated with an appropriate drug regimen to cure him of his infection.

It should be noted that because there are several types of study in which the volunteers may participate by no means are all of the volunteers for the malaria program actually infected with malaria. Furthermore, many of those who are infected with malaria never develop symptoms or fever. All volunteers, however, are accepted on an equal basis and a sense of participation with the medical and inmate staff is encouraged.

Patient care

Clinical and laboratory examinations vary with the type of study. Volunteers participating in these studies are followed according to carefully-outlined protocols. All inpatient volunteers are seen by a physician at least once a day. Nurses observe and monitor the clinical condition of each patient and the physician on 24-hour duty is promptly alerted of any change in the patient's condition. Patients are weighed and their blood pressures are taken at least once a day. Pulse and temperature are measured several times a day and every 30 minutes during febrile episodes. Careful attention is paid to their fluid intake and output and any imbalance is promptly corrected. Finger-tip specimens of blood from each patient are examined daily for the presence of malaria parasites and repeated more often if indicated. In addition, a complete profile of the blood picture, and liver and kidney functions are performed at least twice a week.

The medical staff maintains complete records on each volunteer. These include an initial history and physical examination sheet, full nursing notes, temperature charts, laboratory report sheets, individual drug cards, and physicians' daily progress notes. These records are kept on file and can be referred to by authorized persons at short notice.

If a particular patient requires special care or facilities beyond the capacity of the Malaria Project, special personnel or equipment may be brought to the Project from Rush-Presbyterian-St. Luke's Medical Center or the patient may be transferred to that Center. The Medical Center is committed to treat any patients for as long as necessary if the patient's illness is a result of his participation in a malaria study.

If a medical or surgical condition unrelated to malaria arises during his stay on the malaria floor, the volunteer is cured of malaria and transferred to the care of the prison physician. He, in turn, may call on consultants to see the patient or transfer him to a nearby nonprison hospital in Joliet if required.

Administration of drugs

Inmates may receive medication only after it has been ordered by a doctor. Drugs are given under the supervision of a nurse and the patient is observed swallowing the medication. No drugs are allowed to leave the floor. Supplies of frequently-used drugs are kept in locked cabinets in the nurses' room. Only the civilian registered nurse or the physicians have access to them. Small quantities of drugs that may possibly be ordered by the physician via telephone at night are kept in another locked cabinet in the nurses' room and can be only dispensed under the supervision of an experienced officer. Larger stocks of drugs or seldom-used drugs are stored in locked cabinets in the storeroom. Strict supervision is also maintained with regard to needles and syringes and a careful inventory is kept.

Institutional guidelines and monitoring for volunteer studies

Throughout the years of its existence, the Malaria Project has endeavored to utilize procedures designed to protect the well-being and freedom of choice of prison volunteers which are equal, if not superior, to those utilized in volunteers who are not confined. The use of written and informed consent and the right to terminate participation of any given program were established here long before general concern resulted in such expressions as the Declaration of Helsinki,

1966. Nevertheless, because of, 1) rapidly advancing medical knowledge and discoveries, 2) reports of instances of apparent abuses of the volunteer system (not at this project), 3) the dramatic and tragic recognition of possible difficulties with the use of new drugs, e. g., thalidomide, and, 4) increasing public awareness of these matters, our program is under continous review. Additional safeguards and support from the Department of Medicine of the Rush-Presbyterian-St. Luke's Medical Center have been provided. The Dean, Chairman of the Department of Medicine, and members and attending physicians of the Department of Medicine have subscribed fully to this program.

Protocols for the evaluation of new drugs in volunteers at the Malaria Project are submitted to the Committee on Human Investigation at Rush-Presbyterian-St. Luke's Medical Center. This committee reviews and approves or disapproves all proposed clinical investigation at the Medical Center in accordance with FDA regulations and the "Institutional Guide to DHEW Policy on Protection of Human Subjects, 1972." Members of this committee include clergymen, lawyers, as well as physicians. No member who is involved in a specific clinical investigation participates in the review of that investigation. Furthermore, all new drugs submitted from the Malaria Project have had prior approval by the Army Investigational Drug Review Board and the Federal Drug Administration. Frequent progress reports during Phase I and II of a drug study are also submitted to the Walter Reed Army Institute of Research and the Army Investigational Drug Review Board. This exacting Board follows strict guidelines and may halt an investigation at any stage of its development.

A series of workshops will be held shortly under the auspices of the National Institutes of Health to develop guidelines to strengthen the protection of subjects having "limited civil freedom." This agency of the Department of Health, Education and Welfare has already initiated "an examination of possible methods of compensation for subjects who, in spite of all precautions, are harmed by research activities". Although, investigations at Stateville have not resulted in any serious or permanent after-effects, there is a very remote possibility of such an occurrence. Consequently, the outcome of the deliberations at NIH will serve as a guideline for establishing methods of compensation in the event it is needed. In this context, it should be recalled that guidelines for "compensation" of any volunteers regardless of their civil status have not been established.

Staffing

The Malaria Program at Stateville is one of the outstanding Malaria Centers in the world and, as such, it attracts a competent and dedicated staff. They hold appointments at the Rush-Presbyterian-St. Luke's Medical Center and two of the physicians are assigned to the project from the Walter Reed Army Institute of Research. The staff includes Dr. F. E. Carson, Director of Pharmacogenetics and Professor of Medicine, Dr. K. H. Rieckmann, Chief of Tropical Disease and Associate Professor of Medicine, Dr. H. Frischer, Chief of Clinical Hematology and Associate Professor of Medicine, Major R. L. Williams, Board certified internist and Adjunct in Medicine, Captain G. M. Trenholme, Board certified internist and Adjunct in Medicine, Mr. D. L. Allen, administrative assistant, and Mrs. P. A. Wynn, Registered Nurse. In addition, official consultants are Dr. R. D. Powell, Professor of Medicine at the University of Iowa and Dr. J. Bowman, Professor of Pathology and Medicine at the University of Chicago.

No member of the staff is engaged in any investigations for any pharmaceutical company and no one derives any financial remuneration or other benefits from any drug company, either directly or indirectly. Neither is any secret work performed at the Project.

Rehabilitation

Much of the technical work at the Malaria Project is performed by 18 qualified inmates who are trained by the staff of the Project. Through the years many of these technicians have studied during their training, have obtained certification in medical technology and, after release, excellent positions in this field. More recently with the improved development of rehabilitation programs in other prison areas increased recognition of the Malaria Project in rehabilitation has led to active discussions with Mr. Leon Dingle, Dean of the School of Allied Health Sciences, Rush University, Rush-Presbyterian-St. Luke's Medical Center, to provide even further training and accreditation for inmates working on the Project both directly with Rush University and in conjunction with educational release programs at Lewis College. At present, several technicians have progressed sufficiently to apply for this program and are submitting their resumes to Dean Dingle. Mr. Dingle, Mr. William Donahue, Dean of Allied Health Sciences at Central YMCA College and Mr. Peter Carruthers, Dean of Allied Health Sciences at Malcolm X College, recently visited the Project to discuss programs with the technicians personally.

National and International reputation of Malaria Project

Various committees and scientific groups of the World Health Organization have stated repeatedly that the value of studies at human malaria research centers cannot be overemphasized and that such centers be given every possible

encouragement and assistance. In 1967, Dr. L. J. Bruce-Chwatt, outstanding British malaria authority and Director of the London School of Tropical Medicine and Hygiene (formerly, Director of the Malaria Division of the World Health Organization, Geneva) wrote the following in *Science*, 155: 1617.

". . . I should like to stress how much medical science, and thus the human society as a whole, has benefited from investigation carried out on volunteers, deliberately infected with malaria, with the aim of assessing the value of various new drugs.

"First of all it must be stated that no animal, with the exception of apes and monkeys, could be infected with human malaria parasites. Various experimental models on birds and rodents are useful only for preliminary screening of potentially valuable antimalarials. The decisive evaluation of drugs for the prevention and treatment of human plasmodia can be done only on man. For a number of years studies of antimalarial drugs were based upon results of treatment of cases of malaria seen in hospitals. These observations were useful but the variability of the clinical response to natural malaria infections limited their scientific value and much difficulty has been experienced in the interpretation of data from different countries.

"In 1942-43 when the acute shortage of quinine showed the vital need to develop new synthetic drugs, a number of experimental studies were carried out on human volunteers in Britain and the United States. The most famous of these experiments were those by Fairley in Australia on approximately 1,000 army volunteers deliberately infected with malaria. This work was taken up in the United States by two outstanding malaria research projects that started in 1944 and is still continuing. One was set up at the Federal Penitentiary at Atlanta, the other in the *Illinois State Penitentiary near Chicago*. The stated objective of both projects was to assess the value of promising drugs for the prevention of sporozoite-induced malaria and for the clinical and radical cure of established infections. *Those who are acquainted not only with the rules governing the acceptance of the service of volunteers in these two research units, but also with the way the medical and ethical principles are adhered to, can bear testimony to the fact that the health, the dignity, and the freedom of choice of these subjects are protected.*

"The World Health Organization expressed its appreciation of these studies in terms that are not often used in sober scientific reports: At the present time, human malaria research centers employing nonimmune volunteers exist only in the U.S.A. The amount and quality of scientific data obtained in these centers on the characteristics of drug-resistant strains of malaria parasites and on their response to drugs is invaluable, and *medical science owes an immense debt of gratitude to the administrators of these institutions, to the research workers concerned, and above all to the courage and devotion of the volunteers.*"

Many other noted visitors to the Project of national and international reputation have been impressed with the safety, clinical care and ethical considerations at the Stateville Project and have held it up as a model for others to follow.

RUSH-PRESBYTERIAN-ST LUKE'S MEDICAL CENTER
1753 West Congress Parkway, Chicago, Illinois 60612

APPENDIX B

COMMITTEE ON HUMAN INVESTIGATION
NOTICE OF APPROVAL FOR EXPERIMENTATION INVOLVING HUMAN BEINGS

PRINCIPAL INVESTIGATOR Karl H. Rieckmann, M.D. and Paul E. Carson, M.D.

TITLE OF PROJECT Immunology of Malaria

SOURCE OF FUNDS Office of Naval Research

The above application for approval of clinical investigations involving human beings has been reviewed by the Committee on Human Investigation. It is approved as appropriate and ethical with regard to:

1. Your guaranteed protection of the rights and welfare of the human beings involved.

2. An appropriate method for obtaining informed consent of the participants, and

3. Potential risks and medical benefits.

In the details of separate protocols, the investigator has the individual responsibility to secure the above rights and consent, to use procedures that allow minimum risk, and by his conduct to adhere to and sustain:

1. "The Recommendations Guiding Physicians in Clinical Research," known as THE DECLARATION OF HELSINKI, adopted by the World Health Organization within the Proceedings of the XVIII World Medical Association, and

2. "The Recommendations for Experimentation on Normal Volunteers" in accordance with the guidelines adopted by the Committee on Human Investigation, Rush-Presbyterian-St. Luke's Medical Center, February 1, 1972."

APPROVAL IS GRANTED FOR ONE YEAR. Projects extending beyond one year must be resubmitted annually for the approval of the Committee on Human Investigation. Any anticipated changes in protocol during the course of the project must receive the prior approval of the Committee on Human Investigation.

Member _____
Atty. L. Edward Bryant, Jr.

Member _____
Max E. Rafelson, Ph.D.

Member _____
Paul E. Nielson, M.D.

Member _____
Harold A. Paul, M.D.

Member _____
Rev. Bernard R. Pennington

Member Out of town
Rev. Christian A. Hoyde

Member _____

Acting Chairman _____
A. William Holmes, M.D.

Date July 24, 1973

RUSH-PRESBYTERIAN-ST. LUKE'S MEDICAL CENTER

Committee on Human Investigation

APPLICATION FOR REVIEW AND APPROVAL

PRINCIPAL INVESTIGATOR:	Karl H. Rieckmann, M. D.
ASSOCIATE INVESTIGATOR:	Paul E. Carson, M. D.
DEPARTMENT:	Department of Medicine - Section of Pharmacogenetics
TITLE OF PROJECT:	Immunology of Malaria
SOURCE OF FUNDS:	Office of Naval Research - Naval Medical Institute
PROJECT PERIOD:	July 1, 1973 through June 30, 1974

1. The procedures and practices for this project are essentially the same as those already approved for the project titled "Chemotherapy of Malaria" a copy of which is attached. The patients involved in this study are male inmate volunteers at Illinois State Penitentiary at Stateville, Joliet, Illinois. The Stateville Project occupies the 3rd floor of the prison hospital with all facilities, i.e., ward, laboratories, and offices contained within this integrated space. The procedure for volunteering is as follows: Inmates send a request to our physicians asking to be a volunteer on the Project. Each week the list of these potential volunteers is sent to the prison administration for approval on the basis of their past record, conduct, and length of time remaining in their sentence before going to the Parole Board. Currently, also, the age range permitted for volunteering for malaria, particularly falciparum malaria, is 20 to 42 and, for toxicity or metabolic studies, 21 to 52. Inmates who are approved by the administration receive a complete physical examination by one of our physicians, extensive laboratory studies, and a chest x-ray. If all of these examinations are within normal limits, the volunteers are then called to the hospital for a personal interview by a physician on our staff.

2. During the interview, the general purpose of the project is explained to the volunteer and then the nature of the particular study in which the volunteer will be involved. Each study may or may not involve being infected with malaria; any given study may involve only a study of drug toxicity and/or metabolic effects associated with the administration of a given drug. If infection with malaria is a part of the particular study, the volunteer is told that this disease can be a serious and potentially lethal illness and that possible complications may occur. Each volunteer at this interview is encouraged to ask questions, and it is emphasized to each volunteer that while participating he may ask to be cured at any time and to be released from the hospital as soon as medically possible. He is also told that the physicians on the

Project reserve the right, at their own discretion, to cure any volunteer with malaria whether or not the particular study is completed. At the end of the interview the physician notes on the chart in writing that the interview has been held, and that the volunteer has understood the material discussed. Subsequent to this interview, the volunteer is asked to sign a volunteer agreement like that which is being used for the Chemotherapy of Malaria Project in which informed consent and the date involved, etc., are indicated. A sample copy of the form currently being used for the Army is enclosed. This form may be further revised during the course of the year in which event a substitute of this form will be submitted. Currently two instead of one witness sign these forms and it is to be noted that the volunteer is asked to read the form and again to ask questions. If it is not clear that the volunteer understands what he is signing, he is not allowed to volunteer.

If, during the period of study, a volunteer contracts a complicating illness other than related to malaria or to the antimalarial drugs used, e.g., appendicitis requiring an appendectomy, we are required to initiate cure of the malaria and transfer the patient to the prison hospital for definitive care by the prison physician or another physician as directed by the Warden and the prison physician. If a complication should occur during the course of any given study which is related to the study and/or to e.g., malaria, or secondary to a drug effect which might require intensive care beyond that available at the prison hospital, transfer to the Rush-Presbyterian-St. Luke's Medical Center, e.g., for intensive care may be required. Such a transfer is now legally within the prerogative of the Warden who may grant permission for this on medical advice and at his discretion.

3. Except that each volunteer is given a complete medical examination including history, physical and laboratory studies which might reveal a condition previously unknown to him, there is no initial potential diagnostic and/or therapeutic benefit to the volunteer insofar as these studies of malaria are concerned. In fact, every effort is made, as indicated above, to make certain that each volunteer is healthy prior to the initiation of any given study. Successful study of malaria depends on having available non-immune volunteers and is one reason why centers for studies of human malaria are located in non-malarious areas.

   During his tenure as a volunteer on the project, the volunteer may be required to stay within the facilities of the malaria project throughout the period of study or, depending on the nature of the study the volunteer may be allowed to participate as an ambulatory patient.

4. The scientific and medical benefits of this study in general are as indicated in the application for Chemotherapy of Malaria. The specific purpose of this project is related to the immunology of malaria with the work being directed to determining if a vaccine against sporozoites (the form of the malarial parasite delivered from the salivary glands of infected mosquitos) can be developed. If in pilot studies a vaccine against the sporozoites could be proven effective then the benefit would presumably be the possibility of immunizing large populations against malaria.

5. The initial phase of this study involves irradiating mosquitos infected with malaria and have developed sporozoites with 12,000 to 15,000 rads. This level of radiation inactivates or kills the sporozoites but not the mosquitos and it is hoped that when these mosquitos bite the volunteers the volunteer will develop immunity against sporozoites. Extensive animal work using this technique has been done in at least three laboratories without ill effect to the animal. Nevertheless, to determine that this would be safe for human beings five investigators from the Naval Medical Research underwent this protocol before its approval for use at the Stateville Project. They were bitten once a week for five weeks without ill effect. The investigators from the Naval Medical Research Institute during the coming year propose to obtain permission from the Food and Drug Administration to use sporozoites after their removal from mosquitos in order to inject them intravenously. If this Food and Drug Administration approval is obtained before the end of this project period we will notify the committee.

Principal Investigator

Department Chairman

Associate Investigator

Institutional Official

I, _____, having attained my twenty-first (21st) birthday, and otherwise having full capacity to consent, do hereby volunteer to participate in a medical investigation entitled: Immunization Against P. falciparum Malaria under the direction of _____.

The implication of my voluntary participation in this medical investigation, its nature, duration and purpose, the methods and means by which it is to be conducted, and the inconvenience and hazards which may be expected have been thoroughly explained to me by _____. These implications are set forth in the statement below this agreement. I have been given an opportunity to ask questions concerning this medical investigation, and any such questions have been answered to my full and complete satisfaction.

I understand that this medical investigation and/or my participation in it may be terminated at any time at the direction of the attending physicians. I understand that I may at any time during the course of this medical investigation revoke my consent, and withdraw from participation freely and without prejudice. I understand that in the event of my withdrawal or termination the attending physicians may find it necessary for me to undergo further medical examination or treatment if, in the opinion of the attending physicians, such examinations or treatment are necessary for my health or well being.

_____ _____
(Signature) (Date)

I was present during the explanation referred to above, as well as the Volunteer's opportunity for questions, and hereby witness his signature.

_____ _____
(Witness' Signature) (Date)

The program in which you are participating has been set up by competent investigators to develop a malaria vaccine and is sponsored by the U.S. Government. Although there is little malaria in this country, it is a very important disease overseas and at least 100 million people in different parts of the world are sick with malaria each year.

In this medical investigation you will be bitten a number of times by a few hundred mosquitoes. These mosquitoes were put under an X-ray machine for a few minutes in order to kill the malaria parasites. When these mosquitoes bite you, they will release some of these malaria parasites into your body. Because the parasites were killed beforehand, you should not come down with malaria. Many studies like this in animals and in volunteers have shown that this procedure is safe and that it will not cause you any harm.

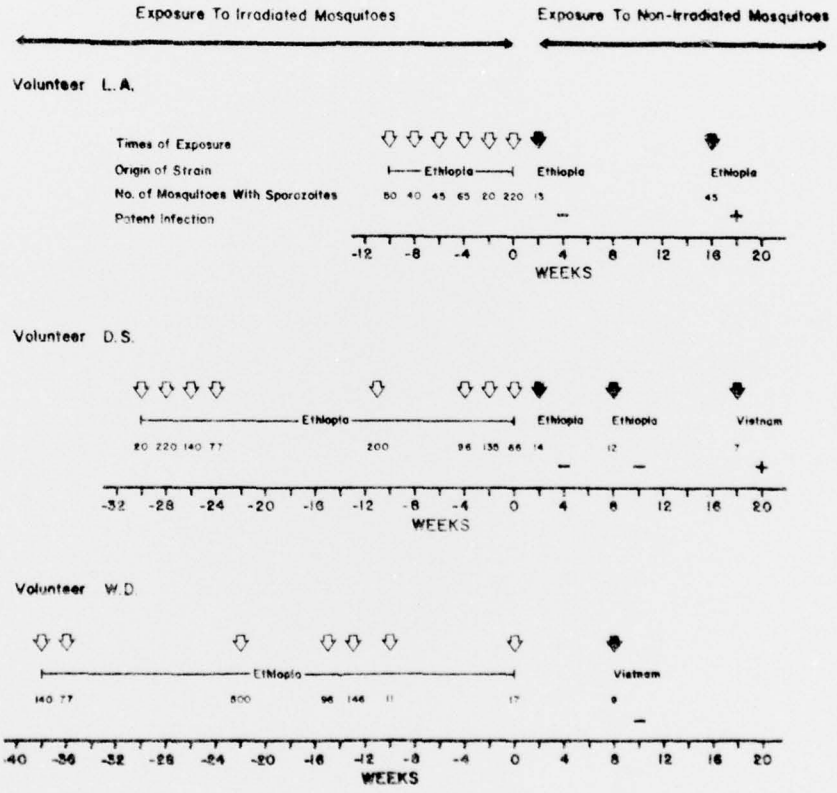
After you have been bitten a number of times by such mosquitoes, you will be bitten on one or more occasions by 10 to 20 infected mosquitoes which have not been x-rayed. We hope that the previous immunization with x-rayed mosquitoes will protect you against malaria and that you will not develop malaria after being bitten by the infected mosquitoes.

If you are protected against malaria, you will not become sick.

If you are not protected against malaria, you will get chills, fever, headache and probably be sick to your stomach one to three weeks after being bitten by infected mosquitoes. After you become sick with malaria you will be given drugs which will cure you permanently of malaria.

Malaria can be a serious disease and can even endanger life. However, under the conditions of these medical investigations, there is little chance that you will suffer any permanent ill effects as a result of your participation.

FIGURE


Exposure To Irradiated Mosquitoes
Exposure To Non-Irradiated Mosquitoes
Volunteer L. A.
Times of Exposure
Origin of Strain
No. of Mosquitoes With Sporozoites
Patent Infection
Ethiopia
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Ethiopia
80 40 45 65 20 220 13
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WEEKS
Volunteer D. S.
Ethiopia
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