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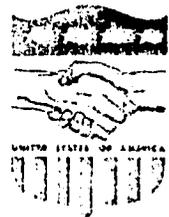
HOPE BRIGHTENS FOR MALARIA VACCINATION

by Dr. Paul H. Silverman

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THE TECHNICAL FRONT

HOPE BRIGHTENS FOR MALARIA VACCINATION

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On July 1, 1966 work on immunological aspects of malaria was initiated at the University of Illinois under a contract with the Agency for International Development. The general long range objective of the research is the eventual development of a vaccine for the prevention and treatment of human malaria.

The contract represented a farsighted realization by AID that additional tools besides insecticides and chemo-therapeutic drugs would be needed in the future struggle against malaria—a view which is daily confirmed by reports of resistance to insecticides by malaria mosquito vectors and by the increasing evidence of the ineffectiveness of malaria drugs which were formerly so useful in treating the disease in humans.

The decision to support the program at the University of Illinois, in addition to being farsighted, required courage. The contention of the Illinois group that malaria vaccination was theoretically feasible was not a view that was universally held by malariologists.

It is well known that once an individual has recovered from an infection of mumps or whooping cough, he is resistant to a recurrence of the disease even if reexposed to the infection. It was observations of this sort that led to the development of vaccines against a whole range of bacterial and viral diseases from cholera to yellow fever.

But malaria is not caused by bacteria or viruses. It is a parasitic disease caused by a protozoan, a minute pretoplasmic acellular or unicellular animal. Observations in malarious areas indicate that it fails to induce immunity, particularly in children, for about 10 years. In

other words, those who survive 10 years of constant exposure to malaria acquire some natural immunity to the infection. This is hardly a very promising basis on which to plan a vaccine!

But the Illinois group was convinced that the failure to develop immunity after natural infection could be overcome artificially if due consideration is given to the changes which the protozoan parasite goes through in the human body.

Encountering Malaria

The first encounter that man experiences with malaria is at the time that the mosquito injects sporozoites (infective protozoa) into his blood. The number of sporozoites injected by a mosquito is highly variable and good estimates of daily exposure in endemic areas are not available. However, analysis of sporozoites indicates that the amount which a person is likely to acquire by mosquito bites is not sufficient to stimulate an immune reaction.

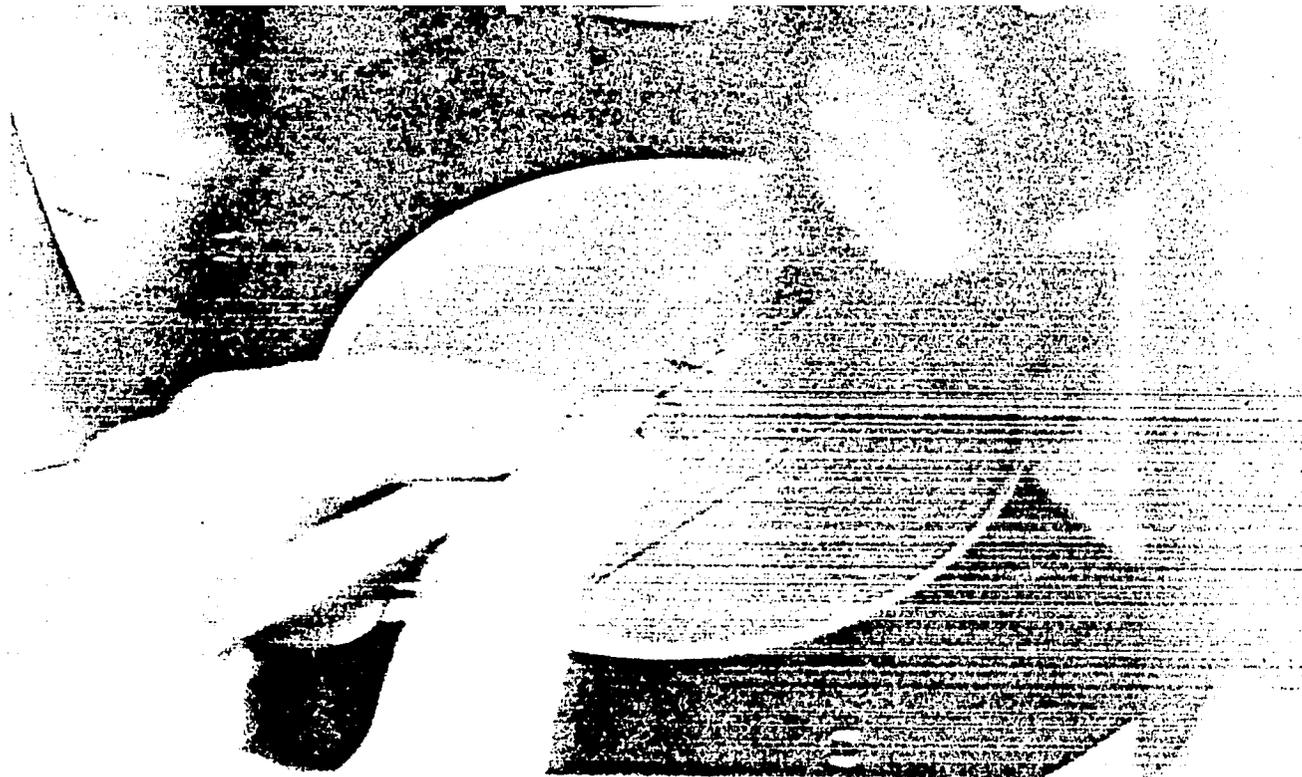
Nor is there sufficient time for the sporozoite to induce immunity. In less than an hour it changes into an exoerythrocytic stage (in the tissue rather than in the blood) in the liver, where it is no longer available to the body's immune system.

Under experimental laboratory conditions, however, scientists at the University of Illinois and elsewhere have shown that a high degree of immunity can be induced artificially by the sporozoite stage and after it enters the blood stream before the parasite enters the liver and transforms into the trophozoite or vegetative stage.

We have here in malaria immunity a situation which contrasts sharply with the experience of microbial immunologists. It appears that it is possible to induce a *better* immunity to malaria by artificial means than that which normally occurs as a result of exposure to *natural* infection.

It has been found that sporozoites which have been weakened by ultra violet and X-ray or killed by freeze-thawing or careful dehydration

Presently the project director of AID's malaria vaccine research program at the University of Illinois, Urbana, Dr. Silverman is one of the pioneers in developing concepts of immunological responses to parasitic diseases. He currently serves as advisor to the World Health Organization's Committee on Malaria Immunology and as consultant for the Armed Forces Commission on Malaria.



These microdissections of mosquitoes provide researchers with valuable information about the malaria-carrying insect.

can be used to vaccinate against infection. The amount of sporozoite antigen (the chemical that produces the immune reaction) required, the method of antigen preparation, and the immunizing schedule of vaccine injections are all critical factors in achieving a successful immunity.

In general, the body-parasite relationship described for the sporozoite stage also applies, with much modification, to the trophozoite (blood) stages.

Without belaboring the details, which are beyond the scope of this article, the implications for the future development of a vaccine are several. It is necessary to determine which of the various stages of the life cycle are potentially useful as sources of antigen and which are susceptible to the immune response. Once specific antigens have been prepared and demonstrated to be effective immunizing agents, the problem becomes one of developing the practical technology for the production of adequate amounts of antigen.

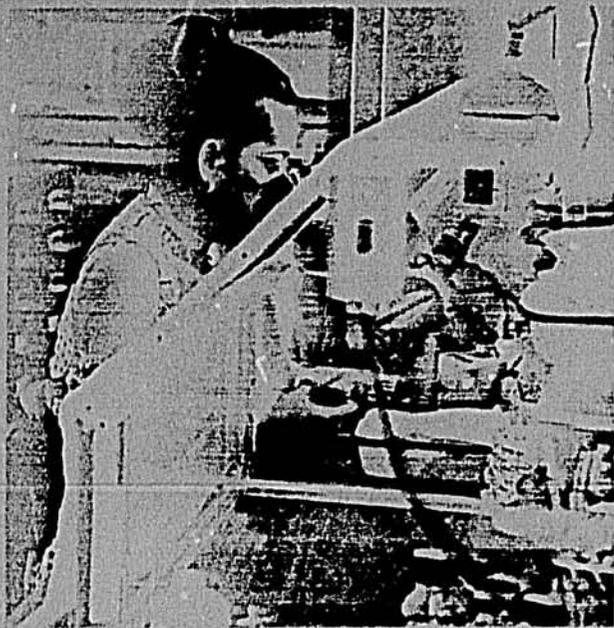
At the University of Illinois, sporozoite antigens have been prepared and tested in mice and found to be effective in stimulating resistance to infection in 99 percent of the immunized

mice. Trophozoite antigens have also been prepared in a partially purified form and demonstrated to be highly effective in mice. A single injection stimulates resistance in over 90 percent of the immunized mice, enabling them to survive and recover from what otherwise would have been a fatal infection.

The work with trophozoite antigens in mice has been translated into tests to protect monkeys against a virulent strain of primate malaria. This work has been successful and gives confidence that results obtained in the mouse-malaria system can eventually be applied to man on an experimental basis—perhaps within five years.

But there are a number of problems which must be overcome.

The task of preparing adequate amounts of sporozoite antigen involves the laborious and difficult maintenance of anopheles mosquitoes, infecting them by feeding on animals whose blood contains gametocytes (infective cells), storing the infected mosquitoes in a healthy condition until oocysts (the developing stages of malaria in the mosquito midgut) and sporozoites have developed, then manually dissecting out the infected mosquitoes' salivary glands and



Dr. M. R. Ronquillo scrutinizes an *in vitro* tissue culture of the anopheline mosquito.

preparing them for antigen injections or using them for the infection of mammals.

This tedious process has been routinized sufficiently to provide material for the experimental studies which have established the potential immunizing ability of the sporozoite antigens. It is obvious, however, that the method is not suitable for the large scale production needed for vaccines.

In order to solve this bottleneck, *in vitro* (i.e. in the test tube) tissue cultures of anopheline mosquitoes were undertaken in 1969. Methods have been developed to rear mosquitoes bacteria-free (aseptically) through their entire life cycle including blood feeding. Aseptically reared mosquito larvae have provided a source of tissues which are maintained in an antibiotic free nutrient medium where they undergo considerable development and proliferation.

These tissue cultures appear, from preliminary results, to provide an adequate environment for the development of the oocyst and sporozoite stages. An immediate task is to scale up *in vitro* production of the sporozoite related antigens and to develop similar methods for trophozoite and gametocyte production.

Currently, the method for preparing trophozoite antigens requires a source of infected blood.

Animals are bled at a time when a high percentage of their red blood cells are infected. The infected red cells are treated by passage through a controlled low pressure system. This

disrupts the red cell membranes and releases the parasites. After appropriate washing and centrifugation, the trophozoites are subjected to a second treatment at high pressure. The second treatment disintegrates the parasites and enables their separation from contaminating hemoglobin and other materials to take place by passage through a chromatographic column.

Although each of the two vaccines has been tested by itself and found to be effective, they have not yet been tested together. We are now working on methods to combine both antigens in one polyvalent vaccine which would both stimulate resistance to the infection at the time it enters the body and suppress an infection after it has reached the blood stage.

Using Multiple Antigens

Immunologists have known for some time that immunization with multiple antigens injected at the same time is less effective than presenting each antigen by itself. On the other hand, the effectiveness of the antigen can be improved markedly by purifying the active substance so that it is free of extraneous matter. Purification, therefore, is an important step in the preparation of a polyvalent vaccine. A number of methods are being investigated as possible purification steps.

Another complicating factor encountered during the monkey trials is the finding that protective immunity was induced only in those monkeys which had been injected with a combination of antigen mixed with adjuvant. Adjuvants are catalysts which enhance antigenic activity. Though not required for the stimulation of immunity in mice, they may be needed for primate immunity. A wide variety of adjuvants are used in human vaccines and trials to find safe and effective adjuvants for use with malaria antigens will have to be undertaken.

It is exceedingly difficult to predict, with any confidence, when an effective human malaria vaccine will be available. Our research group has already fulfilled the conditions of the AID contract, which provides financial support for the testing of vaccines in monkeys after encouraging protectivity is induced in laboratory rodents. On the basis of laboratory results and the theoretical considerations outlined above, a human vaccine appears feasible. The final accomplishment will depend on our success in solving the practical problems of antigen production and effective immunization.