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Quarterly Report

July 1 to September 30, 1969

Submitted by

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SEROLOGIC DIAGNOSIS OF MALARIA

PASA Control No. RA(HA) 5-68

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Amendment # 4

Purpose and Scope of Project

The project is designed to develop simple, rapid, accurate methods for the serologic diagnosis of malaria which would be applicable in the worldwide malaria eradication program.

Toward this end, this laboratory has concentrated its efforts on the evaluation and standardization of the indirect hemagglutination technique (IHA) for the detection of malaria antibody for epidemiological studies and the indirect fluorescent antibody test (IFA) for diagnostic and serologic speciation of malaria.

Studies on the indirect hemagglutination (IHA) test

I. Studies on the epidemiology of malaria in Haiti

With the collaboration of Dr. Hans Lobel, Chief Malaria Adviser, Malaria Eradication Program, a program to evaluate the IHA test for the prevalence of malaria antibody was initiated. A protocol for the study was drawn and, with the collaboration of the statistician in the Malaria Eradication Program, all data will be fed into the NCDC computer. The program will be carried out in three phases.

Phase 1. A collection of 100 sera from individuals with slide positive Plasmodium falciparum was made. This collection was tested by IHA and FA methods. The IHA test gave 46% positive when tested with a P. knowlesi antigen and the FA test 80% when tested with a P. falciparum antigen. The sera will be tested by IHA with a P. falciparum antigen as soon as one is available in an attempt to increase the sensitivity of the test.

Phase 2. Collections will be made in Haiti from three areas of known endemicity (high, low, and absent). Dr. Kagan will visit Haiti in October to confer with Dr. Lobel and to select the sites to be studied.

Phase 3. A country-wide collection will be made.

II. Stable, sensitized cells

Three types of stabilized cells are under investigation: formalin-treated, gluteraldehyde-treated, and pyruvic aldehyde-treated human group "O" cells. To date the formalin-treated cell is stable for at least six weeks if previously treated with tannic acid. Sensitization with antigen occurs optimally when carried out at 50°C, and this cell (tanned, formalized, sensitized) is stable for at least two weeks if frozen at -70°C.

Pyruvic aldehyde and gluteraldehyde as cell stabilizers are still under investigation. Gluteraldehyde treatment, at present, appears to be the better of the two, yielding a more stable and reproducible product.

III. Antigen stabilization

In an effort to stabilize malaria antigens, various lyophilization procedures have been investigated. Lyophilization prior to cell rupture in the Ribi Press does not materially affect the antigen, whereas lyophilization following cell rupture destroys the functional antigenicity of the plasmodia. Further work on the stability of the dried material under various storage conditions is being implemented.

IV. Seroepidemiologic studies

New Guinea	1,587 sera titrated
El Salvador	229 filter paper specimens drawn in conjunction with drug study program. This battery constitutes baseline data on 4 sectors in the country.
Ethiopia	852 filter paper specimens. A continuing study on the distribution of malaria in Ethiopia.
Haiti	98 serum specimens. First phase of pilot project - to test the efficacy of using the IHA test as a permanent adjunct to malaria eradication efforts in the field.
Uganda	372 sera. Collected from an endemic focus of malaria
Miscellaneous	31 sera from various sources

New studies being planned

Urban malaria, Karachi, Pakistan. A collaborative study with Dr. M. Rahman, National Epidemiologist. Initial studies to involve approximately 1,000 specimens, directed at determining focal endemic areas within the urban area of Karachi.

Ethiopia. In collaboration with Dr. Joseph Armstrong, NAMRU-3, Ethiopia Field Station. A study comparing IHA, IFA, and classical methods of determining endemicity. Pilot study to involve about 500 specimens.

Manuscripts cleared for publication

Mathews, Henry M., George U. Fisher, and Irving G. Kagan
The persistence of malaria antibody in Tobago following eradication as measured by the indirect hemagglutination test. Submitted to the American Journal of Tropical Medicine & Hygiene.

Manuscripts submitted for NCDC clearance

Mathews, Henry M., Janet A. Fried, and Irving G. Kagan
A seroepidemiologic study of malaria in the Republic of the Philippines by the indirect hemagglutination test.

Indirect fluorescent antibody studies

1. Plasmodium malariae was established in Aotus monkeys and antigen was prepared from it.
2. The study on duration of antibody in Viet Nam returnees (military personnel) has been completed. It was found that after curative treatment antibody levels drop precipitously within six months and are essentially negative within one year. A manuscript has been prepared from the data and has been accepted for publication in The American Journal of Tropical Medicine and Hygiene.
3. A comparison of titers of malaria antisera examined by IFA and IHA techniques has been completed. It was found that antisera from patients with repeated exposure, as compared with those experiencing a first attack, show significantly higher titers in the IHA test. There was no difference in the two groups in the IFA test. With patients experiencing their first attack, serology becomes positive by IFA before the IHA test. After curative treatment, IFA test results become negative before IHA results. Differences in the reactions of the two tests are attributed largely to differences in mechanism of the test reactions, coupled perhaps with anti-red cell factors that influence the IHA test. A manuscript is in preparation, and a report will be presented at the November meeting of The American Society of Tropical Medicine and Hygiene.
4. In the comparison of various antigens for similarities or differences in the IFA test reactions, Plasmodium falciparum and P. reichenowi have been found very similar. A slight difference as determined by reactions with P. vivax antisera has been found between P. brasilianum and P. malariae antigens. A report will be presented before the American Society of Parasitologists in November.
5. A sustained relatively high-level parasitemia fortuitously established in an Ateles verlerosus monkey has furnished the opportunity to study factors that may account for some phenomena of recrudescence of malaria in the presence of high antibody level. Briefly, the parasitemia was held above 1.0% for more than 250 days in the presence of antibody levels of 1:4096, as determined by the IFA test. There was also a sustained maintenance of an initial negative reaction in the test of 1:4 to 1:16 throughout the period. A report will be presented before the November meeting of the American Society of Tropical Medicine and Hygiene.
6. Routine diagnostic requests for fluorescent antibody titration of sera are steadily increasing. This may reflect the advent of summer rotation of troops from Viet Nam. However, we anticipate about 300 sera this year as compared to 268 received last year.

Future plans

1. Due to the use of baboons in liver shunt therapy in cases of hepatic coma in human patients, a test is under development to detect Hepatocystis infection. About 25% of baboons are known to be naturally infected with this malaria-like organism. Although transmission attempts to healthy human volunteers have been unsuccessful, patients with already almost non-functional livers may well be subject to infection under the conditions of intimate contact employed in liver shunt. Antigen has been prepared from Hepatocystis material, and anti-baboon sera awaits only tagging. This test will then be evaluated. Initial trials are very promising.
2. A project is underway to test the significance of high IFA antibody levels to malaria in persons from endemic regions. Students from such areas, resident at schools in the United States, will be used as subjects. Those found to have high antibody titer will be divided in two groups, one of which will be treated for malaria. A rapid drop in antibody titer, within 6 months, in the treated group and retention of antibody level in the untreated one, will indicate presence of infection in such cases. If no change occurs, then high-level antibody of long duration may be due merely to repeated exposure.
3. Further comparisons of the specificity and sensitivity of simian antigens will be carried forward.