

SEATO MEDICAL RESEARCH STUDY ON "THE IMMUNO-EPIDEMIOLOGY OF FILARIASIS"

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GENERAL INFORMATION

One difficulty in performing serologic tests is the collection of microfilariae for antigen preparation. Preliminary trials have been carried out using separation of Dirofilaria immitis microfilariae by albumen gradient centrifugation. The microfilariae seemed to concentrate at the 35% albumen level but unfortunately the rbc also concentrate at this band. Further improvement of the technique is being attempted.

Some preliminary results have been obtained in applying the indirect haemagglutination test to filariasis serology. Antigen was prepared by thoroughly grinding 3 grams of Dirofilaria immitis in 5 ml. phosphate buffered saline pH 7.2. The supernatant was used as antigen at optional dilution of 1/1200. The sera of two filariasis patients were tested. One patient infected with W. bancrofti gave a titer of 1/25,600 and the other infected with B. malayi gave a titer of 1/12,800. The control serum was negative. Further refinement of the IHAT is in progress and eventually it is planned to determine whether there is any correlation between skin test positivity and IHAT titer.

An Armigeres subalbatus colony was brought from Singapore and in collaboration with the Entomology Department has been successfully established here. A. subalbatus is an excellent "selective" vector for Brugia pahangi. Since it is very difficult to distinguish B. malayi from B. pahangi morphologically it is planned to use A. subalbatus for xenodiagnosis.

STUDY REPORT

Title: Immuno-Epidemiology of Filariasis

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Objective - Relatively little is known regarding the immune response in humans infected with filaria. There have been several methods, notably skin tests and complement fixation test, applied for diagnostic purposes but in general the results have been inconsistent. The aim of this study is to apply existing methods such as the skin test to whole populations in endemic areas and to correlate the results with such factors as age, microfilaraemia, and clinical signs of infection. It is hoped that these populations can be studied over a period of years so that clinical and immunologic changes, particularly in children, can be followed.

Attempts will also be made to develop and apply other immunologic methods so that a more logical picture might be presented by a battery of techniques.

New Guinea Surveys: Data obtained from the immuno-epidemiological survey carried out in New Guinea in May 1965 were analyzed and prepared for publication. Although the field work for these studies was not done under the auspices of this organization, the data were analyzed and prepared for publication during the period of the study report. Further, they form the basis for the subsequent work reported herein. In this survey the skin test antigen prepared from Dirofilaria by Prof. Sawada was used. Two endemic areas, the Trobriand Islands and Cape Gloucester, New Britain, were studied as well as a population in the Eastern Highlands (6500-7500 ft) where filariasis is not supposed to occur. The results may be summarized as follows. Firstly, in both endemic areas the microfilaraemia rate increases gradually with age. Secondly, in both endemic areas the skin test positivity rate increases gradually with age until in the adult group over 90% give positive reactions. Thirdly, at all ages there is about a 3 fold greater skin test positivity rate than microfilaraemia rate. Fourthly, very young children who have a demonstrable microfilaraemia rarely give a positive skin test; about half the infected children in the 9-13 year old group show a reaction. The co-positivity of adults however is almost 100%. Fifthly, skin test reactions in the non-endemic area were invariably negative except for a few adult males who had been to the coast to work on the plantations. The burden of intestinal helminths was quantitatively and qualitatively similar in the populations living in endemic and non-endemic areas. Further evidence of at least group specificity of the test was indicated by negative reactions in experimental animals infected or immunized with such helminths as Strongyloides and hookworm.

The problems arising from this investigation are as follows. What is the significance of the large numbers of adult reactors in whom no microfilaraemia can be detected? Does it mean that in endemic areas virtually every individual has been infected by the time he reaches maturity and that the majority of these infections are of a subpatent nature? The other possibility is that they represent an immune reaction to a non-human filaria such as Dirofilaria. Why do young children seem to be immunologically unresponsive at least as evidenced by the skin test? Obviously other measures of the immune reaction must be devised before further work can proceed on this problem.

Thailand Surveys: The survey methods used in New Guinea have been repeated in studies of two Thai populations. It is of interest to determine whether the same age relationship to skin test reactivity obtains in Thailand, particularly in areas where B. malayi infections are present. In view of WHO's reliance on the skin test as an assessment of filariasis control programs, information as to its reliability in this part of Asia is of considerable practical importance.

The first area chosen was Phuket. Little or nothing is known regarding filariasis in Western Thailand, but it is known that the periodicity of Brugia malayi differs in Eastern and Western Malaya.

Skin tests with Sawada's antigen and 20 cumm. night blood films were done on 31 patients in Phuket General Hospital (all hospitalized for conditions other than filariasis) and on 81 people living in a typical village. All the hospital patients were adults. 12 (38.7%) showed a positive reaction with wheals ranging from 8 mm. to 17 mm. No microfilaria were found in any blood films. Most of the reactors were not from the city but lived in the outlying villages.

Of the 81 villagers, 19 (23.4%) gave positive skin test reactions. With the exception of one 13 year old, all positive reactors were adults. No microfilaria were detected in any of the thick films. Percentage of eosinophils ranged from 2% to 17% but there was no apparent correlation between high eosinophilia and a positive skin test.

Chumporn Survey: A survey of two villages near Chumporn was carried out in January 1966. A total of 375 people of all ages were examined. The results are summarized in tables 1 and 2. The pattern of increasing skin test positivity rate with age is evident in both villages. As in New Guinea, the co-positivity ratio of skin tests and microfilaraemia was low in Children. This was true for Bangluk village, however none of the people skin tested in Paknamchumporn were microfilaraemia positive. A survey of this village in 1964 gave an infection rate of 3.5% (5/145) and another recent blood survey since gave a rate of 2.4% (3/121). Despite the low microfilaraemia rate, five cases of elephantiasis were seen in Paknamchumporn, 4 of which gave positive skin test reactions. In Bangluk, 6 cases of elephantiasis were seen of which 3 were skin test positive.

These results again show the contradiction between skin test positivity and demonstrable microfilaraemia. Long term clinical, parasitological and immunological investigations in filariasis are required before the significance of our results can be determined.