PRELIMINARY REPORT ON THE USE OF THE ANTIGEN OF *DIROFILARIA IMMITIS* AS AN EPIDEMIOLOGIC TOOL AND AS A THERAPEUTIC AGENT IN *WUCHERERIA BANCROFTI* INFECTIONS IN FRENCH OCEANIA [1]

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I. Introduction

Interest in the antigen of *Dirofilaria immitis* as a laboratory aid in the diagnosis of *Wuchereria bancrofti* infections has existed since 1930 when Taliaferro and Hoffman (1) prepared an extract from the dog heart worm and observed that persons in areas endemic for human filariasis exhibited an intradermal sensitivity to small amounts of the antigen. Fairley (2) in 1932 reported the antigen to be a valuable laboratory tool. Lloyd and Chandra (3), Rodhain and Dubois (4) and Wright and Murdock (5), in investigations in India, Africa and Mexico, respectively, indicated cross reactions might occur between the filarial worm and other helminths. During World War II, King (6), Huntington (7), Michael (8), Bozicevich and Hutter (9), Dickson et al. (10), Saunders et al. (11) and Zarrow and Rifkin (12) reported the use of the antigen among American service men in the South Pacific. The consensus by 1947 was that *Dirofilaria immitis* antigen when used in skin tests with dilutions above 1 in 4,000 is fairly specific for human filariasis since a skin sensitivity to *D. immitis* is present in most persons infected with *W. bancrofti* or *Onchocerca volvulus* and is mainly absent in persons known not to have filarial infections.

The development of hetrazan (Notezine, Banocide), early trials in Puerto Rico (13), Virgin Islands (14), and Tahiti (15), (16), and an increasing interest in filariasis control among native

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populations in Fiji (17), Raratonga (18) and French Oceania (19) created a possible new application for skin testing with *D. immitis* antigen. This was the possible application of skin testing to measure the efficacy of human filariasis control programs.

Investigations in French Oceania conducted between 1949 and 1951 had this interest in mind. It is believed that the present data, although limited, are of sufficient general interest to include in a preliminary report.

The use of *D. immitis* antigen in the therapy of recurrent attacks of lymphangitis, believed to represent often an allergic phase of filariasis of *W. bancrofti,* was reported by Clearkin (20) in 1943. Among treated patients in British Guiana with histories of recurrent lymphangitis, he observed that the majority of the patients seemed to show definite improvement in general health, and the attacks appeared to be reduced in number, severity and duration, if they were not entirely eliminated.

Investigations on desensitizing patients with recurrent attacks of lymphangitis by the use of *D. immitis* antigen have been in progress for the past 3 years in Tahiti, and the preliminary observations on the antigen as a therapeutic agent are believed to be of sufficient interest for inclusion in this report.

The initiation of this experimental work in Tahiti was made possible by Dr. Willard H. Wright of the Laboratory of Tropical Diseases, National Institutes of Health, who furnished the antigen, and, who, as a member of the Advisory Committee of the Pacific Tropic Disease Foundation, manifested a keen and active interest in the work. We are indebted to Mr. J. Bozicevich of the Laboratory of Tropical Diseases for the preparation of the antigen.

II. Experience with the intradermal test as an epidemiological and public health tool

Materials and methods

The skin test technique consisted of the introduction of 0.01 ml of a 1-in-8,000 dilution of the extract intradermally into the volar surface of one arm. The antigen was prepared in accordance with the method of Bozicevich and Hutter (9). A similar amount of control solution prepared by Bozicevich was injected into the volar surface of the other arm. During the latter part of 1951, the control solution was injected into the same arm as the antigen as a precaution against variable readings which might result from differences in sensitivity. The antigenic reaction consisted of the formation of a wheal, with or without pseudopodia, and an area of erythema. The wheals were well defined, raised firm areas, usually with pseudopodia, surrounded by an area of erythema. The range in diameter of wheals in positive reactions was from 6 millimeters to 7 centimeters. Usually the control antigen elicited no wheal. If a wheal occurred at the site of the control injection, the skin test was considered questionable unless the wheal resulting from the *Dirofilaria immitis* extract was at least 3 millimeters larger. Responses were read at 20 minutes. At first the results were rechecked at 24 hours, but this practice was discontinued when it was observed that very few late reactions occurred.

Measured 20-mm^3 specimens of capillary blood were collected during the day by means of a Hellige hemoglobin pipette. The specimens were placed on a microscope slide as a thick smear about the size of a dime, dehemoglobinized, fixed in methyl alcohol, stained with

Giemsa and carefully examined for microfilaria.

Stool examinations were by direct smear. Usually only a single specimen was obtained.

Results and discussion

1. Data pertinent to the specificity of the intradermal reaction. (A) COMPARISON OF RESULTS OF SKIN TEST AND BLOOD EXAMINATION. Among 448 Polynesians [7] tested for the cutaneous reaction, and for microfilaria, 332 (74 per cent) gave a positive skin reaction, while 73 (17 per cent) exhibited microfilaria as judged by a single 20-mm^3 capillary specimen. Eight school children and 3 adults, representing 15 per cent of those with microfilaria, showed no reaction to the skin test. None of the 8 children with negative intradermal responses but with microfilaria gave a history of lymphangitis. One Tahitian with advanced elephantiasis had consistent negative responses during a period of 1.5 years.

(B) POSSIBLE INFLUENCE OF INTESTINAL NEMATODE INFESTATIONS ON THE INTRADERMAL REACTION. This was not thoroughly investigated, due to a number of factors. It was felt, moreover, that Bozicevich and others had fairly adequately established the specificity of the tests in the dilutions used, and in relation to *Ascaris lumbricoides.*

Data obtained in Tahiti consisted of a single stool examination on 26 elementary school children in the district of Mahina.

Among the 12 school children with ascaris infections, 7 (58 per cent) exhibited an intradermal reaction. One of these likewise was positive for microfilaria. Among the 14 children negative for *Ascaris lumbricoides,* 6 (43 per cent) exhibited an intradermal reaction to *D. immitis* antigen.

No school child in this group gave a history of recurrent lymphangitis.

(C) COMPARISON OF RESULTS OF SKIN TEST AND LENGTH OF RESIDENCE IN FRENCH OCEANIA. The results of skin tests among Europeans and Polynesians classified by their length of time in French Oceania are given in table 1.

It is to be noted that the grouping in this table includes both Europeans and Polynesians, as well as persons of all ages. The degree of transmission undoubtedly varies considerably within these islands but until more is known of this subject it seems reasonable to assume a relationship between lengths of residence and exposure to the parasite in such hyperendemic areas as Tahiti and the surrounding islands.

The increase in the percentage of positive reactors with increase in length of residence in French Oceania most probably indicates that intradermal sensitivity has developed to antigenic components in the extract of *D. immitis.* This increase can be interpreted as a sensitivity to *W. bancrofti,* to *D. immitis* or in a possibly much lesser degree to other helminths.

TABLE 1

Percentage of persons positive to skin test, by length of residence in French Oceania, 1949 through 1951

Length of residence (*years*)	Number tested	Number positive	Percentage positive
0–1.9	69	7	10.0
2–4.9	119	42	35.3
5 and over	625	409	65.0
Totals	813	458	56.3

[7] This group includes a large proportion of Polynesians under 14 years of age. For this reason, the number and percentage of persons positive for microfilariae are smaller than for the general population.

TABLE 2

Number examined and number and percentage of positive reactors to D. immitis *antigen, French Oceania, 1949 through 1951*

Group tested	Description	No tested	No. positive	Percentage positive
Tiarei	School children	30	21	70.0
Papenoo	School children	41	24	60.0
Mahaena	School children	30	20	66.6
Faaone	School children	36	26	72.2
Hitiaa	School children	35	26	74.3
Mahina	School children	32	19	59.4
Lower Paea	All ages	35	31	88.6
Infantry col.	Europeans and Polynesians	138	22	15.9
Marine	French marines in Tahiti from 5 months to 2 years	25	3	12.0
Clinic	New arrivals 2 weeks to 2 years	61	9	14.8
	Polynesians	103	83	80.6
Maiao	Sample all ages	64	51	79.7
Moorea	Sample all ages	73	65	90.4
Mahina	Preschool children	66	33	50.0
Arue	Preschool children	44	25	57.0
Totals		813	458	56.3

The respective roles of *W. bancrofti* and *D. immitis* in stimulating positive reactions to the antigen in Tahiti and elsewhere have yet to be elucidated.

2. Results of skin testing surveys among population of French Oceania. (A) GROUPS SURVEYED. In table 2 are listed the various groups skin tested during the years 1949 through 1951.

It is of interest to observe that the range in the percentage of positive reactors among preschool Polynesians (0 through 5 years of age) is from 50 to 57 per cent; among school children living in the districts, from 59 to 74 per cent; and among all age groups, from 80 to 90 per cent. It is likewise pertinent .that among new arrivals (marines, soldiers or tourists) who have been in Tahiti from a few weeks to 2 years, the percentage of positive reactors is from 12 to 15 per cent. Among 36 French and Americans tested within 6 months of their arrival, and who had never been previously in an area endemic for human filariasis, there were no positive reactors.

(B) SEX DISTRIBUTION OF POSITIVE REACTORS. Beye et al. (21) reported the microfilarial carrier rate in Tahiti to be greater among females in the ages under 15, but less than the rate among males in the ages over 15. There are indications that this difference may be entirely explained by variations in exposure between the two sexes on account of differences in clothing and work or social habits. Data obtained through use of the skin test may be useful in clarifying this problem. Among Tahitians there are observed to be no differences in the percentages of positive skin reactions in males and females at different ages.

(C) AGE DISTRIBUTION OF POSITIVE REATORS. The age distribution of persons with positive skin tests in the districts of Tahiti, and on Maiao and Moorea is given in table 3.

The increase in the percentage of persons giving a positive response with age is to be noted. It is of interest that whereas 31.9 per cent of the tested Polynesians in the districts under 2 years of age were positive to the skin test, reference to table 2 reveals that 14.8 per cent of new arrivals and residents of French Oceania for less than 2 years had a positive test.

(D) OTHER OBSERVATIONS. Twenty-seven Tahitians were skin tested first in September, 1949, treated with hetrazan, and then skin tested again in October, 1950, and in September, 1951. Twenty were originally positive and 7 were originally negative. A few changes in skin test response were observed to occur.

Of the 7 persons who were negative in 1949, 2 remained consistently negative, while 5 became positive either in 1950 or 1951. One person was positive in 1949, questionably positive in 1950, and negative in 1951. This was a 34-year-old woman whose microfilaria count averaged 62 per 20 mm^3 of blood during the period February to October, 1949. She had no history of lymphangitis. In October, 1949, she received a 7-day course of hetrazan with subsequent negative microfilaria counts. In early 1950, she moved to Papeete, and in January, 1951, she received another course of treatment with Notezine.

It is of interest that two Europeans who had lived in Tahiti for 1 year and then returned after an absence of 9 and 15 years gave a positive intradermal reaction, indicating that sensitivity may be of long duration in some people.

TABLE 3.

Number of Polynesians skin tested and number and percentage of positive reactors by age, French Oceania, 1949 through 1951

Age group	Number tested	Number positive	Percentage positive
0–1.9	22	7	31.9
2–4.9	93	58	62.3
5–7.9	66	41	62.1
8–10.9	85	62	72.9
11–12.9	63	45	71.4
13 and over	117	102	87.1
Totals	446	315	70.6

IIII. EXPERIENCE WITH USE OF *Dirofilaria immitis* ANTIGEN IN DESENSITIZING PERSONS WITH RECURRENT ATTACKS OF LYMPHANGITIS

Materials and methods

Thirty-eight patients selected because of well established histories of recurrent attacks of filarial lymphangitis have either completed or are in process of receiving injections of extract of *Dirofilaria immitis* antigen.

The majority of these patients were observed in the central clinic for a minimum period of 6 months before the initiation of desensitization in order to establish an accurate history of the frequency of attacks. The cases selected at first were not previously treated with hetrazan, but when it was observed that the drug frequently had little effect on modifying the frequency of such attacks (16), 22 people so treated were also included in the desensitization group.

By the method of selecting the persons for treatment, the majority of the group were above 40 years of age. Some were affected with elephantiasis in a single part, but more often a number of parts were involved.

Before treatment each person received a physical examination, a skin test with *D. immitis* antigen, and a blood test of 20 mm^3 of capillary blood for microfilariae.

The desensitization schedule consisted

of subcutaneous injections of *D. immitis* antigen commencing first with 0.05 ml of a 1-to-1,000,000 dilution and then with a 0.1-ml weekly increase in dosage through various dilutions until 1 ml of a 1-to-1,000 dilution was administered. Intradermal skin tests and collections of capillary blood for search for microfilariae were done at periodic intervals.

Every person selected had had at least one attack of lymphangitis per month for the preceding 6 months before treatment. The patients were questioned weekly during the desensitization period and then at periodic intervals, preferably monthly, after the cessation of treatment.

Results

Of the 38 persons so treated, 29 showed definite lessening in the frequency of attacks, 7 revealed no significant change in frequency, but subjectively felt the attacks were not so painful or severe, while two evidenced no observable change in either frequency or severity. No changes were observed in density of microfilariae or in skin test response during the course of therapy.

As indicated, the patients were seen once a week during the period of therapy, and were studied for 6 months prior to therapy in an effort to make the observations on the frequency and severity of attacks as objective as possible.

The apparent stability of the skin test response during therapy is interesting and in conjunction with the observations on the frequency and severity of attacks implies a densitization involved in the lymphangitis without a skin desensitization.

Observations have continued on these patients and will be included in a later paper.

IV. Summary and conclusions

Preliminary data are presented which indicate that a relation exists between exposure to *Dirofilaria immitis* or *Wuchereria bancrofti* and the development of human reactors to the antigen of *D. immitis*. The relation is not entirely specific, but might be utilized, in time, to measure changes in the degree of transmission of filariasis in French Oceania.

Preliminary clinical evidence obtained by using the antigen of *D. immitis* as a desensitizing agent in Tahitians suffering from recurrent lymphangitis is encouraging, and is suggestive of an allergic factor in precipitating the attacks.

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