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<td>Katamine, Daisuke</td>
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Skin Reaction of Bancroftian Filariasis with a Purified Antigen, FPT, Prepared from Canine Filaria, *Dirofilaria immitis*

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(Director: Prof. Daisuke KATAMINE)

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Filaria is one of the most important tropical diseases in the world. As in Japan elephantiasis, chyluria and hydrocele patients are still found in some areas. Many studies have been made in attempt to obtain information on the mechanism of filarial immunity and to find better antigens to be used for serologic tests and skin tests.

The author has been making studies of this subject with aid of his co-workers and the results have been published in Japanese.

Present paper attempts to summarize briefly some of their recent experiences concerning skin reaction for filariasis and its practical application, illustrating them on the tables and figures.

**Specific activity of FPT antigen to filarial infection**

For skin test the author uses a new refined antigen, FPT, which is a polypeptide fraction prepared from canine filaria, *Dirofilaria immitis* by Yamamura’s method for isolation of tuberculin-active peptide from tuberculi bacilli. (Tada et al., 1962) The detailed procedure of isolation and purification of the antigen is presented on the table. (Table 1)

Intradermal injection of about 0.01 ml. of the antigen solution containing 1.0 microgram of FPT antigen is sufficient to arise a wheal of 3.0 mm. diameter. If positive, the wheal increases rapidly in the size accompanied with surrounding erythema. Intensity of the reaction is interpreted by increase in wheal diameter measured 15 minutes after injection. This skin reaction was carried out on 541 microfilaria carriers and 264 control indi-
Table 1. Isolation and purification of FPT antigen

<table>
<thead>
<tr>
<th>Step</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult worms (male and female)</td>
<td></td>
</tr>
<tr>
<td>Washed with physiological saline several times</td>
<td></td>
</tr>
<tr>
<td>Ground with tissue grinder</td>
<td></td>
</tr>
<tr>
<td>Added aceton and filtrated (repeated same procedure)</td>
<td></td>
</tr>
<tr>
<td>Dried in a vacuum desiccator</td>
<td></td>
</tr>
<tr>
<td>Extracted with N/10 HCl at room temperature for 48 hrs. under stirring</td>
<td></td>
</tr>
<tr>
<td>Centrifuged (3,000 r. p. m. 10 min.)</td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>Precipitate</td>
</tr>
<tr>
<td>Adjusted to pH 7.0</td>
<td></td>
</tr>
<tr>
<td>Centrifuged</td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>Precipitate</td>
</tr>
<tr>
<td>Added saturated picric acid solution and stood at room temperature over night</td>
<td></td>
</tr>
<tr>
<td>Centrifuged</td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>Precipitate</td>
</tr>
<tr>
<td>Added and extracted with 3% HCl-Alcohol for 2 hrs. under stirring</td>
<td></td>
</tr>
<tr>
<td>Centrifuged</td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>Precipitate</td>
</tr>
<tr>
<td>Concentrated to 1/10 volume under reduced pressure</td>
<td></td>
</tr>
<tr>
<td>Aceton added, repeated same procedure several times</td>
<td></td>
</tr>
<tr>
<td>Centrifuged</td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>Precipitate</td>
</tr>
</tbody>
</table>

In control group, nevertheless, it ranged from 0 to 5.0 mm. with a peak situated at the smallest side, which resembles the reaction with physiological saline solution tested on people of the endemic communities. These two different curves crossed each other at the point of 4.0 mm. Therefore, if the reaction of 4.0 mm. or more increase in wheal

viduals of non-endemic areas who were proved to have had no filarial infection.

Frequency distribution of intensity of the reaction in microfilaria carriers group showed nearly a normal curve with a peak in the larger side around 9.0 mm. There was found a close correlation between final size of the wheal and that of the surrounding erythema.
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### Table 2. Comparison of the results of skin test and CF-test

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>Antibody titer</th>
<th>CF-test(+) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(−)</td>
<td>5 X</td>
</tr>
<tr>
<td>10 mm−</td>
<td>132</td>
<td>13(9.8)</td>
</tr>
<tr>
<td>7−9</td>
<td>67</td>
<td>18(26.9)</td>
</tr>
<tr>
<td>4−6</td>
<td>44</td>
<td>19(43.2)</td>
</tr>
<tr>
<td>0−3</td>
<td>*</td>
<td>6(46.2)</td>
</tr>
<tr>
<td>Total</td>
<td>256</td>
<td>56</td>
</tr>
</tbody>
</table>

* mf (+)

Diameter was regarded as positive, 94.8% of microfilaria carriers had the positive reaction. (Fig. 1)

The cross or non-specific reactions were examined on 15 ascariasis, 24 ancylostomiasis, 19 gnathostomiasis, 2 heterophyisis, 6 paragonimiasis, 7 schistosomiasis, 1 taeniaisis, 10 trichocephalasis, 27 various allergic diseases and 23 tuberculosis patients. Among them, only one gnathostomiasis and one asthoma patient showed positive reactions. (Tada, 1962, Yoshimura, 1963) It may be concluded, therefore, that cross reactivity of this antigen is very low.

Further investigations were made to study the specific activity of this antigen. 243 skin test positive residents in highly endemic community were examined for complement fixation test using a crude extract of adult canine filaria in order to compare the results of those two tests. Among them 193 were positive for CF-test. The positive rate and antibody titer by CF-test rose in accordance with the increase of intensity of the skin reaction. Namely, 119 or 90.2% among 132 persons who had the strong positive skin reactions over 10 mm. were also positive for CF-test. On the other hand, 6 or 46.2% among 13 microfilaria carriers who had the negative skin reactions were also negative for CF-test. (Table 2)

In a highly endemic community, Gusukube, Miyako Island, 166 microfilaria negative inhabitants who have shown the positive skin reaction over 9.0 mm. were examined again for microfilaria ten months later. As the results, microfilaria turned to be positive in 10 or 6.0% of them.
Epidemiological aspect of filariasis from a view point of skin test survey in the endemic areas

The skin test with FPT antigen shows a high specificity for filarial infection as mentioned above. On the assumption that its positive reaction means the successful infection with filarial worm or at least the entrance of infectious larvae in the past, analysis of the results of skin test survey on the community inhabitants may reveal far more epidemiological characteristics which could not be studies by simple blood survey and clinical observations. The author aimed to clarify the present condition of epidemicity of bancroftian filariasis in Japan and to study the dynamic aspect of prevailing filariasis in the communities from these results obtained by the skin test survey and night blood examination for microfilaria.

1. Incidence of microfilariaemia and rate of positive skin reaction

In the Ryukyu Islands and Nagasaki Prefecture of Japan, 14,053 inhabitants including junior and senior high school students were tested with FPT skin test combined with night blood examination. The results are shown on the figures.

In Miyako Island which has more than 25.0% of microfilaria rate, nearly all inhabitants showed positive reactions even among microfilaria negative individuals. In moderate and low endemic communities in Nagasaki Prefecture, the positive rates were as follows: Nagata 37.1% (mf-rate 2.9%), Kurosaki 54.8% (mf-rate 6.5%), Sakiyama 68.3% (mf-rate 13.8%) and so on. No microfilaria carriers were demonstrated in the villages where positive rates of the skin reaction were under 30% of the population. As a whole, the rates of positive reaction in the endemic communities were usually several times as high as the microfilaria rates and these two rates correlated closely with each other in every community. Positive rate and intensity of the skin reaction form upward curves as microfilaria rate in the population increases. (Fig. 2)

Thus, the rate and intensity of positive reaction indicate the real expansion and chance of the infection among inhabitants in the community.

2. Age distribution of the positive skin reaction

Fig. 3, 4 and 5 show age distribution of the positive skin reaction and of its intensity in Miyako Island, Sakiyama and Kurosaki, each of which had a microfilaria rate of different degree. In the lowest endemic area, Kurosaki, the positive skin reaction did not appear before 7 years of age and then it gradually increased up to 46.8% at the age of 13 years. In Saki-
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Yama, the earliest positive reaction appeared in the group of 3 years of age in 20.0% and it reached to 70.0% at 13 years age-group. Whereas, in Miyako, 66.7% of 2 years age-group and 98.3% of 13 years of age-group turned out to be positive. The first appearance of microfilaria in these three areas were 10, 6 and 2 years of age respectively. Generally in every community, the rate of positive reaction increase with age up to its peak at the age of 30 to 40 and then gradually declines.

In Miyako Island, intensity of the reaction was 5.0 mm. in the average at 2 years of age and 8.0 to 10 mm. at the age group of 6 or more. While in Kurosaki, the group of 13 years old has only 3.0 mm. in the average and it does not increase any further than 6.0 mm. even in the older age group. (Fig. 3, 4, 5)

As for the infants under 6 years of age in these three villages, the rate of positive reaction was 80.2% on the average in the highly endemic communities of Miyako Island, while it was only 17.6% in Sakiyama and none in Kurosaki and Nagata. The positive rates among infants seems to increase with accelerating rate as microfilaria incidence of the community elevates. On the assumption that the positive skin reaction of infants below 6 years of age represent the recent infection in these six years, it can be said that the increase of microfilaria rate and average microfilaria density of the community promote the infection, especially in the youngest generation. In Miyako Island, spread of the disease will be so rapid that almost all of the inhabitants receive the contamination of filarial worm by
3. Frequency distribution of intensity of the skin reaction

Distribution curve scaled with degree of intensity of the skin reaction in several communities are shown in the figures.

In every figure, there are found two peaks, one of which is situated at low intensity zone and another at higher. The first peak is similar to the exponential curve which is usually obtained from a control group of non-endemic areas or a sample group injected with physiological saline solution. Those first and second peaks may indicate the non-infected and infected groups respectively.

Location, height and shape of these two peaks depend on the epidemicity in the community. It is noted that the two peak are clearly separated with a deep ravine situated at 2 or 3 mm. zone and that the second one is situated at intenser zone of 7.0 to 10.0 mm. occupying a large area in highly endemic communities of Miyako Island. (Fig. 7)

Fig. 6. Showing five patterns of age distribution of the positive skin reaction in several communities

their school age.

Fig. 6 shows five patterns of age distribution of the positive skin reaction in several communities with different degree of the epidemicity. In non-endemic or obsolete endemic areas where no microfilaria carriers were demonstrated at present, the positive rate of skin reaction is very low in every age group.

However, in active endemic areas it is generally higher according to the microfilaria rates of respective areas, especially the difference is significant in the group of children below 9 years of age. (Fig. 6)

Distribution curve of the positive reaction with age shows the mode of expansion of the infection in each community. Skin reaction has stronger intensity and higher rate in the heavily infected areas. The higher the microfilaria rate in the community, the earlier and the stronger positive reaction appears. In other words, age distribution of the positive reactions and especially the rate in infants may indicate velocity of the transmission of filariasis among inhabitants in the respective community.
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In the distribution curves made of the materials from junior and senior high schools of Miyako Island, there was found a unique pattern of the curve with the second peak at intenser zone lacking the first peak. (Fig.8)

In the moderate or low endemic communities such as Sakiyama and Kurosaki of Nagasaki Prefecture, the double peak pattern could also be observed, but they differ from that of Miyako groups in some points. For example, the first peak was separated by a shallower ravine from the second peak which covers many indistinguishable reactions at the zone of 3.0 or 4.0 mm. of intensity.

The whole communities of Sakiyama and Kurosaki were, thereupon, divided into three age groups, infants (from 0 to 4 years of age), juveniles (5 to 14 years of age) and adults (over 15 years of age). The first fraction obtained from the infant group has a single peak pattern with its peak at lower intenser zone and with its skirt stretching down to 5.0 mm. This curve was found to slide along with age toward intenser zone till at last in the adult group, and to become similar to the typical double peak pattern of Miyako group. In the juvenile group, center of the second peak occupied the zone of weaker intensity of merely 3.0 to 5.0 mm. The intermediate weaker reactions were, therefore, attributed to the primary and junior high school students.

The same tendency could be observed in Kurosaki. These weak and indistinct reactions may indicate the relatively mild infection. (Fig.9)

Thus, distributions curves of intensity of the reaction in various communities vary significantly in their shape and can be utilized in analysis of the epidemiological character of filariasis in the communities.
A practical application of the skin test for preliminary survey in pre-eradication program against filariasis

Mass skin test survey was conducted on 4,310 children of junior high schools in Goto Islands, Nagasaki Prefecture. In order to see the local variation of distribution of filariasis, several student groups with their home villages were divided into further minute localities.

Frequency of the positive reaction was extremely uneven ranging from 0 to 87.3% according to the localities. Similar irregularity was found among the general inhabitants of the community where they live. (Fig. 10)

There was found a direct correlation between the rate of positive skin reaction of the whole community and those among junior high school student group in the same community. Therefore, the results obtained from junior high school students may represent the general picture of prevalence in the communities. (Fig. 11)

In 1965 and again 1966 the author visited the Republic of Tanzania to make a field survey on parasitic diseases of various origins.

With a purpose to know the incidence of filarial infection among the African residents, blood examination for microfilaria and skin test with FPT antigen were tested on 960 inhabitants of I'Agara Village and 243 students in 1965 and again 1966. The results were found as shown in Fig. 11 and Fig. 12.
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of Livingstone College at Kigoma on the east shore of Lake Tanganyika, and 210 people of Kiomboni Island, East Coast of Tanzania.

The following microfilaria rates were found for Acanthocheilonema perstans (A) and W. bancrofti (B); Ilagara 9.3% A, 0.2% B and 0.5% mixed; Livingstone college 1.2% A, 0.4% B and Kiomboni Island 4.3%B, 0%A. A. perstans infection was not demonstrated in Kiomboni Island, while it was predominant in the West Province.

The rates of positive skin reaction in each area are 31.1 to 37.0% in Ilagara Village and 31.2% of student group of Livingstone College, but it was higher of 67.6% in Kiomboni Island where bancroftian filariasis was endemic. Students of Livingstone College come from all over Tanzania. The positive rates of skin reaction among the students in each home village differ extremely each other ranging from 0 to 44.0%. This may indicate the regional difference in the epidemicity of filariasis. (Fig. 12)

Conclusion

From these results, it may be assumed that FPT antigen prepared from D. immitis can be recommended as a test antigen specific for filariasis and its positive reactions are indicative of contamination with filarial worm.

Mass skin test survey is useful to comprehend the real expansion and its dynamic aspect of the transmission in the community.

Especially the results obtained from student groups of junior high schools would be the most practical and valuable measure to find out the heavily infected areas for preliminary survey in country wide antifilarial campaign.

It is the advantage of this test that these results can be easily obtained from all school students even in the daytime and used as a measure to compare the conditions in different localities and they also indicate newer invasions of filariasis in the localities.

Acknowledgments

The author wishes to express his many thanks to co-workers, Dr. O. Yoshimura, Dr. C. Yoshida and J. Imai in the Department of Parasitology, Institute for Tropical Medicine, Nagasaki University for their co-operation throughout this study. Thanks are also extended to Dr. I. Tada and Prof. Dr. A. Sato of the Department of Medical Zoology, Faculty of Medicine, Kagoshima University for their kind advice in preparing the antigen and close collaboration in the study.

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Daisuke KATAMINE

犬糸状虫, D. immitis から分離したペプタイド抗原（FPT）によるバンクロフト糸状虫症の皮内反応

片 峰 大 助

長崎大学熱帯医学研究所寄生虫学部門（主任：片峰大助教授）

摘 要

FPT抗原を用いた糸状虫症の皮内反応については著者及び協力者の名で, 既に日本語論文としてその都度発表した。本編ではその重要な知見を総括的に紹介した。

犬糸状虫から分離したペプタイド抗原として仔虫陽性者 541名, 未感染の非流行地住民集団244名, 他
の寄生虫性疾患154名に皮内反応を実施すると同時にその一部について補体結合反応との関係を追究した。又
流行地で仔虫陰性の反応陽性者を長期に亘り経過を観察した。その結果本抗原による皮内反応は糸状虫感
染, 寄生に対して特異的で, 頻度反応や非特異的陽性反応の出現する率はきわめて低いことを知った。

この皮内反応を沖縄及び内地の流行地の集団約 18,000名に実施し, その結果を分析すると在来われてきた単
なる仔虫検査や臨床的観察だけでは知ることのできなかったバンクロフト糸状虫症の真の広がりや流行伝播の
性格など動的疫学的考慮が必要することができる。

ことに中学校集団から得られた成績はその地域を代表するもので, 流行のどこか不均等性を知るのに便
利である。従って全国的規模による糸状虫対策を行うにあたって, 対 策の対象となる濃厚流行地をさかし出す
予備調査の手段としても有効である。又皮内反応の長所は直前に, しかも学校集団では一括して実施出来る
ことである。