Experimental Studies on the Role of the House Mosquito, *Culex pipiens pallens* in the Transmission of Bancroftian Filariasis

4. Development and longevity in days of filariae in mosquitoes kept at a series of constant temperatures*

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INTRODUCTION

In the first report of the thesis, the states of development in mosquitoes kept at faborable temperatures of 25°C and 27°C were made clear. In the third report, it was proved that the filariae can not survive the winter even when the infected mosquitoes were kept in a fairly heated laboratory at Isahaya under the variable temperature conditions ranging from 15.9 to 7.3°C in daily mean. This suggests that at anywhere in Kyushu and northwards in Japan, the filariae can not survive the winter. Besides the above, a series of experiments were made to examine the development of filariae under alternating temperature conditions.

Here, it is needed to show the results concerning the development and longevity of filariae in mosquitoes kept under a series of constant temperatures, in order to facilitate the comparison (which will be made at an early date) of the results obtained under the variable or alternating temperature conditions with those under constant ones.

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METHOD OF EXPERIMENT

The procedure for obtaining infected mosquito and the constant temperature apparatus used are explained in Table 1.

The rearing of adults from the pupae collected at natural breeding places in Isahaya city, the starvation of them for several days and the feeding of them on the microfilarial carrier were made in incubation room at 25°C or 27°C. Microfilarial count given in the table is an average of those examined just before and after the feeding. The feeding technique is the same as stated in the first report. Immediately after the end of the feeding time, a

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Tab. 1 Experimental procedures for obtaining infected mosquitoes to be exposed to constant temperatures just after the feeding (results of the experiments are illustrated in Fig. 1)

Lot No.	Carrier			Date of feeding		Mean No. Mf per 20 cmm	Rearing temperature(°C)		No. mosq. dissected	Constant temperature apparatus
	Name	Sex	Year	Date	Time		before feeding	after ferding		
79.4	K. I.	♂	16	Nov., 8.'54	21.45'–22.45'	154.3	25°C	10°C	61	Thermostat
74.1	K. S.	♂	30	May, 2.'54	21.30'–22.30'	60.5	27°C	13°C	106	Thermostat
69.142	T. I.	♂	36	Nov., 28.'53	21.00'–22.00'	73.0	27°C	16°C	73	Thermostat
63.142	S. T.	♂	26	Oct., 31.'53	21.20'–22.20'	59.0	27°C	18°C	65	Incubator with ice box
69.8	T. I.	♂	36	Nov., 28.'53	21.00'–22.00'	73.0	27°C	19°C	57	Thermostat
94.1	T. S.	♂	18	Nov., 6.'55	23.00'–24.00'	30.8	25°C	22°C	56	Thermostat
69.13	T. I.	♂	36	Nov., 28.'53	21.00'–22.00'	73.0	27°C	24°C	58	Incubation room
94.4	T. S.	♂	18	Nov., 6.'55	23.00'–24.00'	30.8	25°C	25°C	36	Incubation room
71.0	T. I.	♂	36	Nov., 29.'53	21.30'–22.30'	122.5	27°C	27°C	54	Incubation room
88.1	T. S.	♂	18	Nov., 4.'55	23.00'–24.00'	38.2	25°C	30°C	41	Incubator
74.7	K. S.	♂	30	May, 2.'54	21.30'–22.30'	60.5	27°C	33°C	51	Incubator

lot of mosquitoes which took full or medial blood meals were transferred to the constant temperature apparatus.

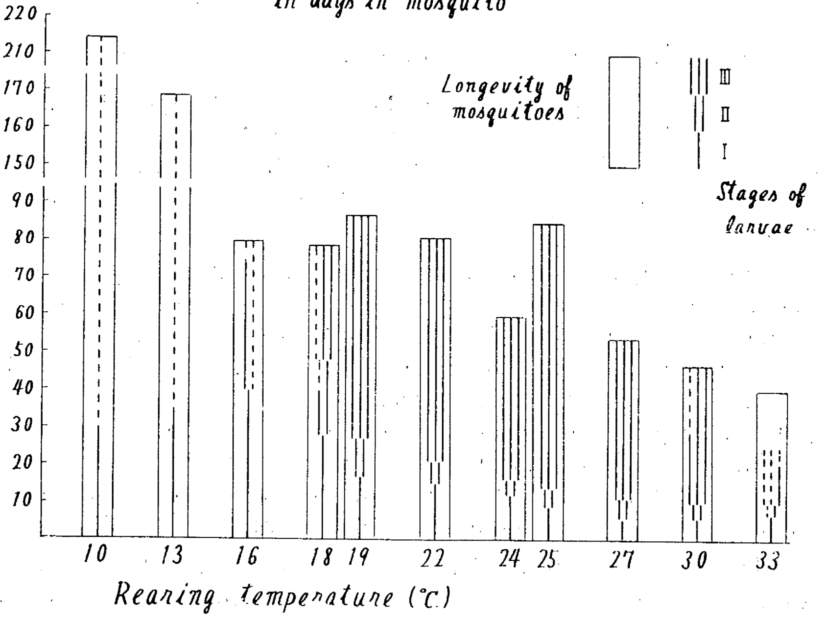
The incubation room is 180 × 180 × 180cm in space and is able to hold its temperature constant at above the room temperature with ± 1.0°C in deflection. The thermostat is 90 × 180 × 180cm in space and is able to hold its temperature constant at below the room temperature with ± 0.5°C in deflection.

In many cases the above two apparatus were used which are very useful in that they can admit a large number of rearing cages and in that the taking out of dead or living mosquitoes from the cage, the supply of sugar solution and the exchange, if necessary, of cotton pad etc. can be made by us sitting at the inside of the room or thermostat, without disturbing the temperature condition under which the mosquitoes are being kept. While in a case (Lot No. 63.1 & 2), an incubator with ice box was used which have an ice box on the top of the incubator of usual type, and can hold its temperature constant at between the prevailing room temperatures and 5 or 6 degrees below them. This incubator was used from the beginning of November to the beginning of December. Thereafter, however, the use of ice became unnecessary because of the fall in room temperature below 18°C. This incubator could hold 18°C with ± 0.8°C in deviation during the period of using the ice, while afterwards with ± 0.5°C. In the remaining cases an incubator of usual type with ± 0.5°C in deviation was used.

The most objectionable point in using the incubator is that the routine work about mosquito must be conducted at out of the incubator and consequently during the time required to do the work, mosquitoes must be exposed to the prevailing room temperature.

The relative humidity in these apparatus was not regulated. In most cases, however, excepting the case of the incubator with ice box, the humidity was rather lower standing from 60 to 80%, with extremities of 40 and 85%. To avoid the shortening in life of mosquitoes, besides the ample supply of about 2% sugar solution, the cage was lightly sprayed with water prepared on the day before in the apparatus and covered when necessary with vinyl sheet. Consequently the mosquitoes can be said as having been kept under the humidity conditions of about 75—85% with extremities of 65—95 %. While in the case of the incubator with ice box, during the period of using ice, the humidity was from 85 to nearly 100%.

Fig. 1 Development and longevity of filariae in days in mosquito



RESULTS OF EXPERIMENTS

A. Development and longevity of filariae

The results of experiments are illustrated in Fig. 1 where the solid lines and broken ones represent the living and dead filariae; a straight line, 2 and 3 parallel lines show the I, II and III or infective larvae respectively; and a combined lines with solid and broken ones show the case when the living and dead filariae are found in a female mosquito or in ones at the same time. In the case of the I stage larvae the living of them is proved by their movement when inspected under binocular microscope but it become difficult to do so in the case of long exposure to a low temperature because of their becoming quite dormant, unless the growth of them would be examined under a faborable temperature condition. In the cases of the II and III stage larvae, the living of them is easily detected by their movement and it is also the case even when they were dormant by warming them under electric light.

The rectangular pillar in the figure represents the longevity of infected mosquitoes. Mosquitoes which ere dead within 24 hours or dying or very weakened at the time of daily inspection or killed in rare case if necessary, were dissected for filariae. Therefore, the height of the pillar shows nearly the longest longevity of the mosquitoes exposed to each constant temperature.

Here, it must be noted that the developmental period of the I stage is not necessarily accurate. The reason is that the ample number of mosquitoes could not necessarily be dissected to determine the accurate period. While, the day of reaching maturity is exactly determined by dissecting adequate number of mosquitoes.

The results of the experiments are summarized as follows;

1) Filariae can complete the development at a temperature range covering from 18 to 33°C. The periods for the completion varies with rearing temperature requiring 10 days at 30°C and 33°C, increasingly longer time with fall in temperature and ultimately 48 days at 18°C. At 16°C they can not reach maturity.

2) A high temperature of 33°C is harmful to the life of filariae in that it induces deformity in many larvae in Id and II stage or actively growing stage, characterized by dwarf in shape and swelling in a part of the body especially in caudad end, making impossible to reach maturity and causing death of them in the long run; it scarcely permits others to reach normal infective larvae causing to develop into a very thin and short ones which again are killed before long; and in that it may cause the early death of infected mosquitoes (as longlived mosquitoes were found haboring no filariae as shown in the right-most rectangular pillar in Fig. 3).

The effect of high temperature appears already at 30°C in a very light degree that a very few of the II stage larvae become deformed and some infective larvae are killed on later days.

3) At low temperatures such as 13 or 10°C the I stage larvae can not grow at all and dies with the lapse of exposed time and can not survive the temperatures but about 35 or 30 days. The death of the filariae appears not to be due to the direct effect of temperature itself but to be due to the physiological function of living mosquito acting as to chitinize or to kill the filariae in the long run during their being forced to stay in mosquito in a quite dormant state by low temperatures.

The effect of low temperature in the meaning as above is slightly seen already at 16°C in that the larvae become inactive and killed little by little at time goes on. The trend is slightly seen even at 18°C.

4) It is only at a temperature range covering from 19 to 27°C (presumably to 29°C.) that all the infective larvae can live actively so far as the host insect can live. Within the range, 24−27°C seems to be the most

faborable temperatures for the life of filariae.

5) The mosquito can live longer with decrease in rearing temperature at least at a temperature range under which these experiments were carried out.

Besides the above expriments, some other experiments were made in which the Id, IIa and IIb stage larvae were suddenly exposed to 10, 13, 16, 18 and 19°C and examined for their development and duration in days. The results show that at 16°C the I stage larvae could reach only the next stage; at above 18°C the filariae reached maturity; at below 13°C they remained undeveloped; and that the Id and II stage larvae i. e. the most actively growing stage larvae were the least resistant to low temperatures. On the contrary, the infective larvae were found the most resistant to low temperatures. Therefore it seems important from the epidimiological stand point to examine more exactly the longevity of the infective larvae at various constant temperatures.

B. Ecological temperature zero point of the filariae

The periods required for filariae to reach maturity are tabulated in Table 2. In the table that obtained at 20°C is added (cf. the third report). The relation between the velocity in development of filariae and the rearing temperatures are illustrated in Fig. 2, where the centuple value of each reciprocal of the period required to reach maturity, y is indicated by a point above the corresponding x or rearing temperature. Clearly, the trend of velocity with temperature is upward and roughly linear when except the points at 30 and 33°C. Then the sample regression equation was obtained by the method of least squars. That is;

$\hat{y} = 0.703\ x - 10.291$ or

$\hat{y} = 0.703\ (x - 14.6)$ ……………… (1)

Where $\hat{y}$ is estimated velocity at a certain rearing temperature x°C.

A test of significance of the regression coefficient is given by the following;

Tab. 2 Developmentat period in days of filariae required for reaching maturity and its reciprocal

Temp. x (°C)	Develpmental period			100 × its reciprocal y
	I stage	II stage	Sum	
16	40			
18	28	20	48	2.083
19	17	10	27	3.704
20	16	10	26	3.846
22	15	6	21	4.762
24	12	4	16	6.250
25	9	5	14	7.143
27	6	5	11	9.091
30	6	4	10	
33	7	3	10	

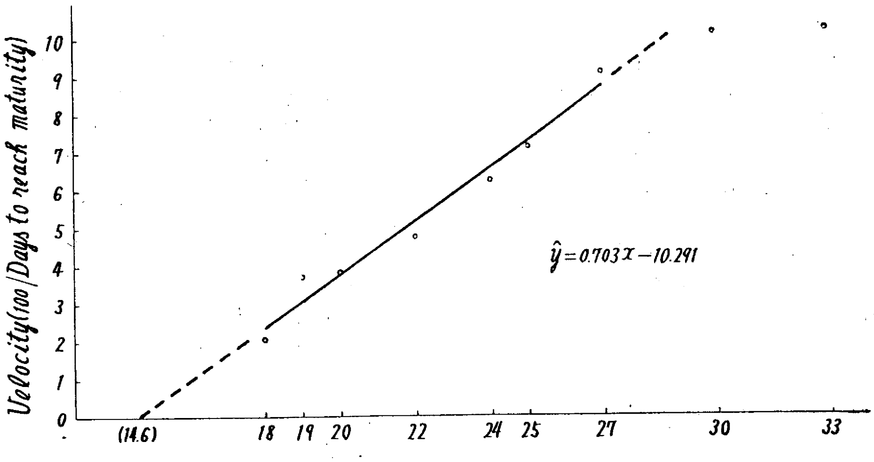
$$t_s = \frac{\text{Sample regression coefficient}}{\text{Sample standard deviation of the regression coefficient}} = \frac{0.703}{0.053} = 13.264$$

d. f. = 5 d. f. = 5

$t_s(\alpha) = 13.264 > t_0(0.001) = 6.86$ i. e. $\alpha < 0.001$

Then, we can say that the relation between the velovity in development and the rearing temperature (at a range covering from 18 to 27°C) is significantly linear.

Fig. 2 Relation between rearing temperature and velocity of development of filariae in mosquito



At above 30°C, the curve to be obtainable by connecting the corresponding points is not straight but bends somewhat downwards suggesting that these temperatures are already not suitable for the development of filariae.

At below 19°C the development of filariae is subjected to a great variation with individual mosquito and also with individual filaria larva even in the same mosquito and therefore the developmental period obtained at these relatively low temperatures may not be extremely accurate. By this reason, another regression equation is tried to make at a temperature range covering from 20 to 27°C. That is;

$$\hat{y} = 0.752\ (x - 15.3) \cdots\cdots\cdots\cdots (2)$$

The temperature at which the regression line, when prolonged, meets the temperature axis is said as developmental zero point. The point is in the case of the equation (1) at 14.6°C, and in the case of equation (2) at 15.3°C. Here, we can say with confidence that the so called developmental zero is at about 15°C. The developmental zero point obtained as above, however, is considered as "ecological temperture zero" point (Allee, 1950) and therefore the point will be slightly higher than the actual point at which the development ceases completely. At any rate, that the nematode, *Wuchereria bancrofti* is being possessed of its ecological temperature zero point in the larval stage at as high as about 15°C, appears to show that the worm is suited to distribute in the tropics and subtropics and may presumably be difficult to do so in thetemperate region unless man would play a co-operative part, though very regrettable it is, in the maintainance of the disease.

C. Lon,evity in days of infective larvae

As already mentioned, the infective larvae are the most resistant not

Tab. 3 Preparations for obtaining infective larvae to be exposed to the indicated constant temperatures (results of the experiments are illustrated in Fig. 3)

Lot No.	Carrier			Date of feeding		Mean No. Mf per 20 cmm	Rearing before exposure		Mosquitoes with infective larvae were exposed to	No. mosq. dissected	Constant temperature apparatus used
	Name	Sex	Year	Date	Time		Temp.	Days			
80.7	K. I.	♂	16	Nov., 9.'54	21.45'−22.45'	185	25°C	19	P	26	P: see blow
80.6	K. I.	♂	16	Nov., 9.'54	21.45' - 22.45'	185	25°C	19	L	32	L: see blow
79.1&2	K. I.	♂	16	Nov., 8.'54	21.45'−22.45'	154.3	25°C	14	10°C	120	Thermostat
75.5&6	K. S.	♂	30	May, 2.'54	22.50'−23.50'	65	27°C	13	13°C	81	Thermostat
84.1	Y. N.	♂	23	May, 8.'55	23.00'−24.00'	90.5	27°C	15	16°C	70	Thermostat
72.4	T. I.	♂	36	Nov., 29.'53	22.50'−24.00'	92	27°C	14	18°C	36	Incubator with ice box
72.5	T. I.	♂	36	Nov., 29.'53	22.50'−24.00'	92	27°C	14	19°C	28	Thermostat
91.2&3	T. S.	♂	18	Nov., 5.'55	23.00'−24.00'	23.3	25°C	15	22°C	74	Thermostat
70.5	T. I.	♂	36	Nov., 28.'53	22.20'−23.30'	61.5	25°C	16	24°C	30	Incubation room
94.3	T. S.	♂	18	Nov., 6.'55	23.00'−24.00'	30.8	25°C	14	25°C	36	Incubation room
74&75	K. S.	♂	30	May, 2.'54	21.30'−23.50'	62.8	27°C	11	27°C	21	Incubation room
90.1&2	T. S.	♂	18	Nov., 5.'55	21.30'−22.30'	21.8	25°C	15	30°C	103	Incubator
87.1,2&3	T. S.	♂	18	Nov., 4.'55	21.30'−22.35'	32	25°C	15	33°C	100	Incubator

Remarks. P : Winter variable temperature at the north side passage of our laboratory
L : Winter variable temperature in our laboratory

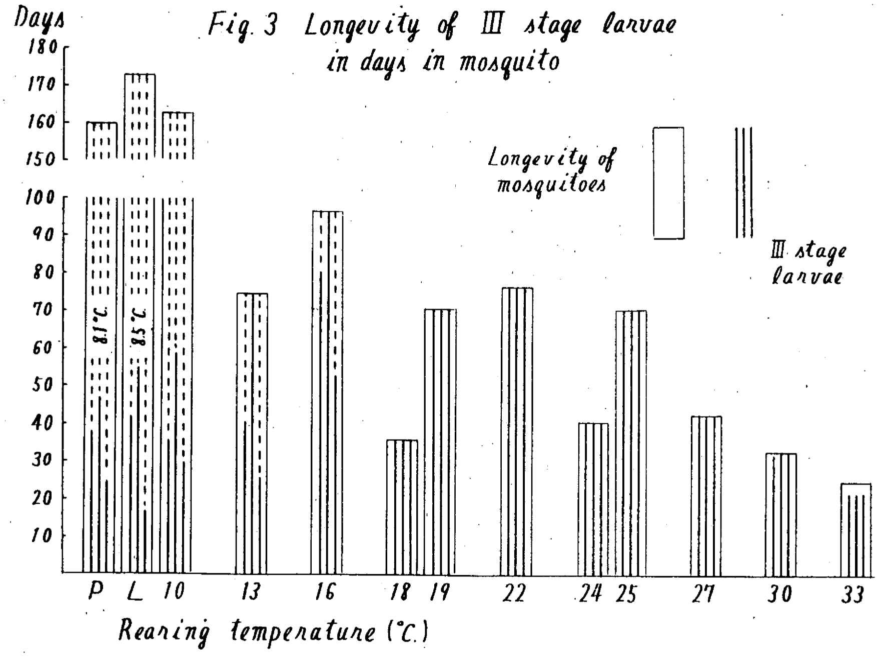


Fig. 3 Longevity of III stage larvae in days in mosquito

only to the low temperatures but also to the high temperatures. Then, experiments to examine the longevity of the larvae were projected. The infective larvae were prepared, as shown in Table 3, under the faborable temperature conditions. The periods required to reach maturity at 25°C or 27°C are 14 and 11 days respectively. The variation in days of rearing at these temperatures was merely due to the experimental convenience.

A lot of mosquitoes haboring the infective larvae were suddenly exposed to the constant temperatures. Mosquitoes were examined for filariae when they died naturally within 24 hours or were dying or very weakened at the time of daily inspection or were killed in rare case if necessary. In the routine work, the infective larvae were usually found alive in dead mosquitoes provided that they were dead within 24 hours under the high humidity. Accordingly the data shown in Fig. 3 can be said to indicate the states of living of the filariae in mosquitoes which have finished their natural span of life under each constant temperature condition.

The results of experiments are illustrated in Fig. 3 in which those obtained under the winter variable temperature conditions are added for comparison. The figure shows that;

1) The duration of mosquito was prolonged with the decrease in rearing temperature at the range under which these experiments were made.

2) At 16°C some of the larvae became very inactive and some others were found dead some days after the exposure and the number of them gradually increased with the lapse of time but fairly active larvae, though small in number, could live so far as mosquitoes could do so.

3) At above 16°C the infective larvae were very active during the whole period in which the mosquito could live excepting in the case of 30 and 33°C. However, owing to the shortening in duration of mosquito with the rise in temperature, the duration of the larvae resulted in gradually being shortened. At 30°C, a very few number of the larvae were found dead in the later days. At 33°C, in five mosquitoes which lived for from the 23 to 25th day after the exposure, no filariae were entirely found.

4) At below 16°C the duration of filariae was again gradually shortened by the reason that they were killed by the living mosquito in the long run in the course of their being forced to rest in it in a quite inactive or dormant state for a long time. The trend appeared more markedly with the decrease in rearing temperature.

5) The harmful effect of low temperature was found more remarkably during exposure to the winter variable temperatures in our laboratory or at the north side passage of the laboratory in 1954–55. Mean temperatures during from the day of exposure to the last day on which the filariae were proved yet alive were 8.5 and 8.1°C respectively.

In short, at about 16°C (presumably at a little higher) the infective larvae can live for the longest time becoming, however, short-lived the more remarkably towards the extremities of temperatures under which these experiments were carried out, owing to the effect of temperature which act as to shorten the longevity of mosquitoes in the higher side and to the effect which act as to make filariae inactive or dormant in the lower side.

SUMMARY AND CONCLUSION

This is the fourth report of the thesis and deals with the results of experiments made to examine (A) the development and longevity of filariae in mosquitoes exposed to a series of constant temperatures just after their feeding on the microfilarial carrier, (B) the relation between the velocity in development or the reciprocal of developmental days to reach maturity and the rearing temperature, and (C) the longest longevity of infective larvae in mosquitoes kept at the constant temperatures.

(A) Filariae can complete its development at a temperature range covering from 18 to 33°C. However, 30°C seems slightly harmful and 33°C is notoriously so to the development. At 18°C they require 48 days to reach maturity. At 16°C they can not do so. At below 13°C they can not grow at all though they can live for about 35 and 30 days at 13°C and 10°C respectively.

The filariae which reached maturity can remain alive and very active so far as the host insect can live at a temperature range covering from 19 to 27°C.

The most faborable temperatures for the development seem to lie at 24--27°C.

(B) Between the velocity in development of filariae and rearing temperature under which infected mosquitoes are kept, a linear relation holds very well over the range of temperatures 18--27°C. The regression equation obtained at this range is;

$\hat{y} = 0.703\ (x - 14.6)$

and another one obtained at temperaturs range of 20--27°C is;

$\hat{y} = 0.752\ (x - 15.3)$

Now, we can say that the ecological temperature zero in the larval stage of this worm lies at as high as about 15°C. This suggests that the nematode, *Wuchereria bancrofti* is suited to distribute in the tropics and subtropics and may presumably be difficult to do so in the temperate region unless man would play a co-operative part, though very regrettable it is, in the maintainance of the disease.

(C) The longevity in days of infective larvae which is the most resistant to low as well as to high temperature were examined over the range of temperature from 10 to 33°C. The result shows that at about 16°C, the infective larvae can live for the longest time, becoming, however, increasingly short-lived towards the extremities of the range owing to the effect of temperature which act as to shorten the longevity of mosquitoes in the higher side and to the effect which act as to make filariae inactive or dormant in the lower side.

LITERATURES

1) ALLEE, W. C. et al. : Principles of animal ecology. Philadelphia and London, 1950.

2) **BASU, B. C. and RAO, S. S.** : Studies of filariasis transmission. Ind. J. Med. Res. 21 (1) : 233−249, 1939. 3) **HU, S. M. K.** : Preliminary observations on the longevity of infective larvae of *Wuchereria bancrofti* Cobbold in *Culex pipiens* var. *pallens* Coquillett. Chin. Med. J. 49 : 529−536, 1935. 4) —— : Observations on the development of filarial larvae during the winter season in Shanghai region. Amer. J. Hyg. 29 (2), Sec. D, : 67−74, 1939. 5) **OMORI, N.** : Experimental studies on the role of the house mosquito, *Culex pipiens pallens* in the transmission of bancroftian filariasis 1. Development, distribution and longevity of filariae in mosquitoes kept at 27°C. and 25°C. Nagasaki Med. J. 32 (11) : 1434−1445, 1957. (In Japanese with English summary) 6) —— : —— 2. On the pattern of spatial distribution of microfilaria in the peripheral blood stream of the carrier. Nagasaki Med. J. 33 (8) : 1045−1053, 1958 (In Japanese with English summary) 7) —— : —— 3. Duration of filaria larvae in mosquitoes exposed to winter temperatures. Yokohama Med. Bull. 9 (6) : 32−40, 1958. 8) **SNEDECOR, G. W.** : Statistical methods applied to experiments in agriculture and biology. The Iowa State College Press, Ames, Iowa. 1956. 9) **TOYAMA, H.** : Oecological studies on the larvae of *Wuchereria bancrofti* in the mosquito (*Culex pipiens*). J. Yonago Med. Ass. 8 (3) : 355−408, 1957 (In Japanese) 10) **UTIDA, S.** : Developmental zero temperature in insect. Jap. J. of Appl. Ent. and Zool. 1 (1) : 46−53, 1957 (In Japanese with English summary) 11) **WIGGLESWORTH, V. B.** : The principles of insect physiology. London. 1950.

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