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

6. Effects of short-term exposure to low temperatures on the survival of filariae in mosquito*

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バンクロフト糸状虫症の伝搬に関わるアカイエカの役割に関する実験的研究．第6報　短期低温接触の影響．
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Experiments were conducted to determine the effect of short-term exposure to low temperatures upon the survival of filariae in mosquito, in November, 1954.

It seems convenient to classify the developmental stages more precisely, in case of determining the exact phase in development in which filariae show greater tolerance to an environmental stimulus than in the other phases. The proposed substages (Omori, 1957) are as shown in Table 1.

Method of Experiment

Female mosquitoes, *Culex pipiens pallens*, emerged from wild caught pupae, and starved for several days under the room temperature conditions, were allowed to feed on a filarial carrier in an incubation room at 27°C for one hour during 10-12 p.m. Mosquitoes were kept subsequently at 27°C till the day on which the filariae in them reached a certain substage. Each lot of mosquitoes prepared as above was exposed to low temperatures in a refrigeration room for a certain period; the period is shown in solid thin line in Fig. 1, 2, and 3. After removal from the refrigeration room mosquitoes were kept at laboratory for from one to three days; this period is shown in broken line in Figs. Finally they transferred to the incubation room at 27°C (this is shown in thick solid line) where the number of infective stage larvae was counted after their reaching maturity. The infective larvae subjected to low temperatures were examined merely for vitality after their being transferred to the incubation room at 27°C.

Microfilarial counts in 20mm^3 blood of a carrier were made just before and after the time of mosquitoes being allowed to feed on him. The expected number of microfilariae to be taken up by a female mosquito in a full meal is taken to be a 30% of the average number of microfilariae contained in 20mm^3 of carrier's blood, as a female was engorged with 6mm^3 of blood in an average, on those days of these experiments being conducted.

The number of infective stage larvae which survived low temperatures or which reached the stage after having survived as younger stage larvae,

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Table 1 Diagnosis of developmental stages and substages of filariae in *Culex pipiens pallens* by the body length

Stage	Substage	Body length (mm)	Remarks
I	a	0.238-0.306	Microfilariae in the stomach of mosquito for several hours after being taken up.
	Exsheath		
	b	0.304-0.172	Larvae develop in the thoracic muscles of mosquito.
	c	0.170-0.136-0.170	
	d	0.172-0.338	
	Ist ecdysis		
II	a	0.340-0.680	〃
	b	0.682-1.020	
	c	1.020-1.190	
	2nd ecdysis		
III	a	1.190-1.360	Move about in the body cavity of mosquito.
	b	1.190-1.870	Move about and also penetrate into interstices or pits of tissues or organs in mosquito.

was compared with the expected initial number of microfilariae in a mosquito. On the propriety of this procedure a discussion will be made later.

Results of Experiments

Three experiments were conducted. In Experiment I, the Ist stage larvae of different substages were exposed to low temperatures with the results shown in Table 2 and Fig. 1. In Experiment II, mainly the growing larvae belonging to various substages were subjected to low temperatures. The results are shown in Table 3 and Fig. 2. In Experiment III, earlier substage larvae of the first stage, and the third stage larvae were subjected to lower temperatures for longer period with the result shown in Table 4 and Fig. 3.

Experiment I

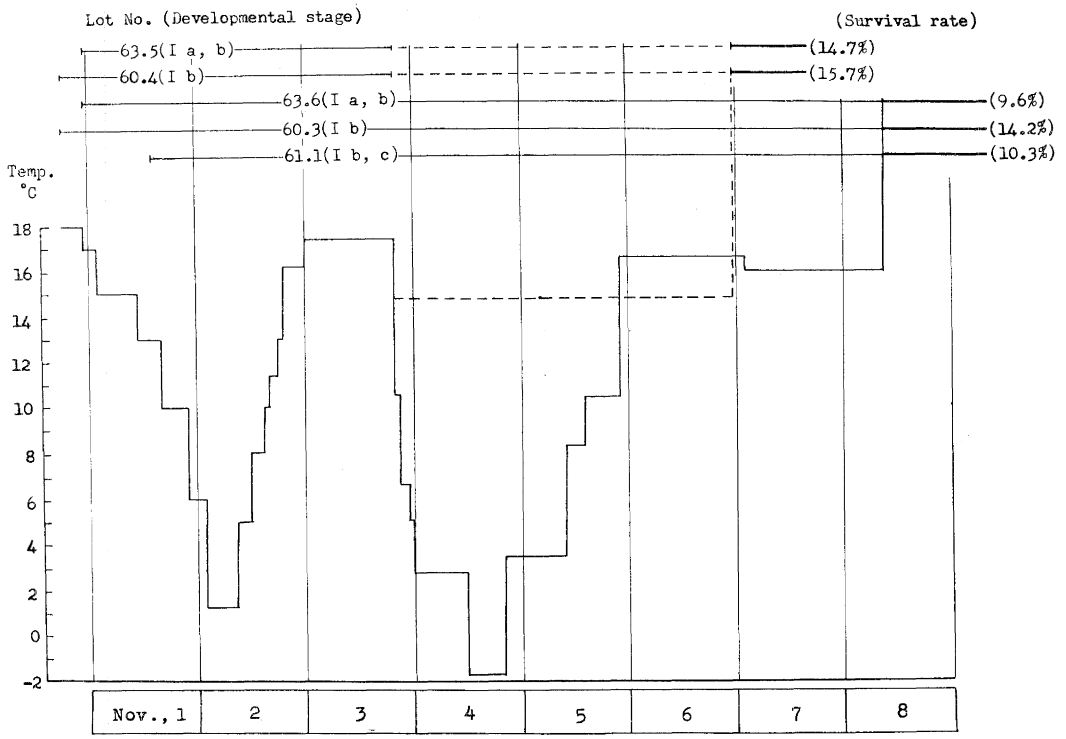
Lot No. 63.5 and 63.6 were transferred to the refrigeration room one hour after the feeding, the temperature of which were lowering stepwise, for 3 hours at 17°C, 9 hours at 15°C and so on. During exposure to 15-17°C for about a half day, inspite of fairly low temperatures, many filariae seemed to have got to thoracic muscles of the host insects. Accordingly, in mosquitoes of these lots, some filariae were still in the stomach as microfilarial substage (Ia) and many were in the thoracic muscles as Ib substage larvae.

In the case of lot No. 63.5, after 69 hours' subjection to a course of low temperatures having a minimum of 1.2°C for 7 hours, it was removed and kept under room temperature of 14.8°C in average for 75 hours and thereafter 27°C (Table 2 and Fig. 1). While, in the case of lot No. 63.6, it was subjected to two courses of low temperatures having respective minima of 1.2°C for 7 hours and -1.8°C for 8 hours and then transferred directly to the room at 27°C. In the 27°C room, the mosquitoes of the lots were reared till the filariae reached maturity and dissected with the results that 2.6 and 1.7 filariae per female or 14.7% and 9.6% of the expected initial numbers of microfilariae could respectively survive the exposure and reach maturity thereafter.

Table 2 Result of Experiment I, showing the effect of short-term exposure to low temperatures (cf. Fig.1) on the survival of the Ist stage larvae in mosquito

Lot No.	No. mosquito exposed	Stage of filariae before exposure	Period and temperature °C of exposure					Expected No. microfilariae per ♀ per meal	No. filariae survived & reached infective stage per ♀	Survival rate to expected No. microfilariae
			Total hours	Mean temp.	Highest temp.	Lowest temp.	Hours kept below 5°C			
63.5	14	Ia, b	144	13.7	17.0	1.2	10	17.7	2.6	14.7%
60.4	26	Ib	149	13.8	18.0	1.2	10	32.4	5.1	15.7%
63.6	22	Ia, b	178	11.4	18.0	-1.8	10+35	17.7	1.7	9.6%
60.3	27	Ib	183	11.5	18.0	-1.8	10+35	32.4	4.6	14.2%
61.1	16	Ib, c	163	11.0	16.6	-1.8	10+35	34.1	3.5	10.3%

Fig. 1 Effect of short-term exposure to low temperatures on the survival of the Ist stage larvae in mosquito



Remarks. ——— : Period of exposure of infected mosquitoes to the low temperatures.

--------- : Period kept under the room temperature.

——(%) : Rearing at 27°C. Figures in parentheses show the percentage survival found as infective stage larvae per female mosquito, against the expected initial number of microfilariae in a full blood meal.

No. 60.4 and No. 60.3 were reared at 27°C for 36 hours after the infective meal allowing filariae to reach Ib substage, and treated quite similarly as the above two lots. The filariae survived the low temperatures and reached maturity in 15.7% and 14.2% respectively. In No. 61.1, the filariae of substage Ib and c were exposed to two courses of low temperatures. They reached maturity in 10.3%.

It is revealed from Table 2 and Fig. 1 that the filariae of early substages can survive these low temperatures and that Ib larvae are slightly more resistant than Ic and Ia, although the survival rates of these substages are as low as 10-16%. The low rate of survival seems to be mainly due to the high mortality during exposure but partly to the death of some survived filariae during the subsequent rearing at 27°C which may be weakened by the exposure. Ia substage filariae which were in the stomach of the mosquitoes as microfilariae, appear to be slightly less tolerable to low temperatures than Ib and Ic larvae.

Experiment II

Infected mosquitoes were kept at 27°C till the harboring filariae reached the substages indicated in Fig. 2 and Table 3. The first four lots were subjected to a course of low temperatures with a minimum of -0.6°C for 3 hours. Among these, the first two, No. 60.2 and No. 61.2, were removed after 27 hours, to the laboratory for 39 hours and then to the 27°C room, while No. 64.3 and No. 62.1 were removed after 37 hours' exposure, to the laboratory for 29 hours and then to the 27°C room.

After removal to the 27°C room, the filariae of No. 60.2 reached infective stage in 14.5%. In No. 61.2, the advanced substage larvae (IIb) were nearly all killed during subjection to the low temperatures with only a survival rate of 2.6%. In No. 64.3 and No. 62.1, all these developing larvae could not survive the low temperatures.

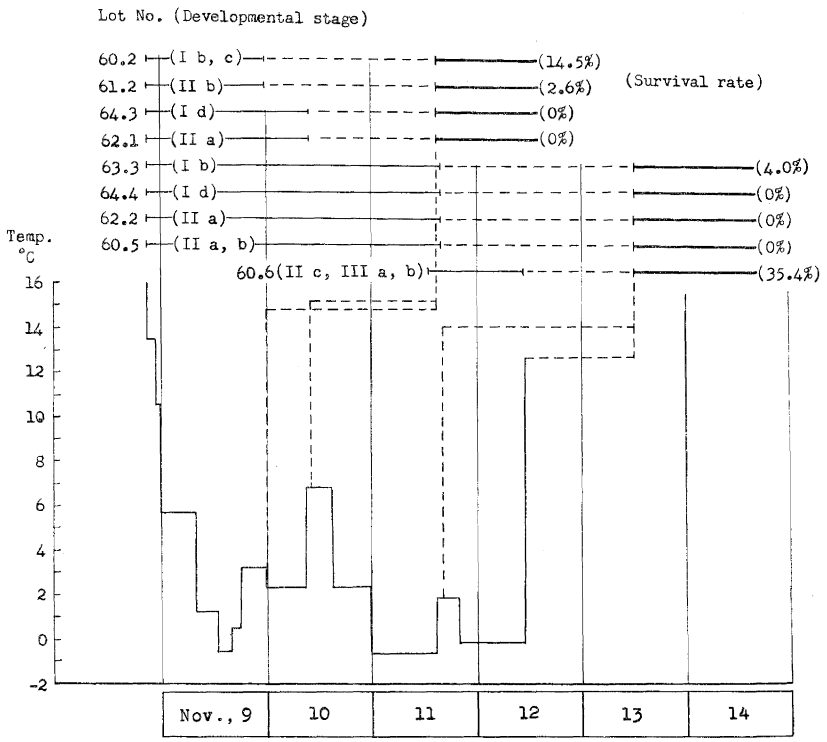
Four lots, No. 63.3, 64.4, 62.2, and 60.5 were subjected to two courses of low temperatures with the minima of -0.6°C for 3 hours and -0.7°C for 15 hours, for 67 hours ; and then transferred to laboratory for 44 hours and subsequently to the 27°C room, where the Ib substage larvae (No. 63.3) only could survive and reach infective stage in a very low rate (4.0%).

The last lot, No. 60.6 were kept before the exposure,

Table 3 Result of Experiment II, showing the effect of short-term exposure to low temperatures (cf. Fig. 2) on the survival of growing filariae in mosquito

Lot No.	No. mosquito exposed	Stage of filariae before exposure	Period and temperature °C of exposure					Expected No. micro-filariae per ♀ per meal	No. filariae survived & reached infective stage per ♀	Survival rate to expected No. micro-filariae
			Total hours	Mean temp.	Highest temp.	Lowest temp.	Hours kept below 5°C			
60.2	22	Ib, c	66	10.9	16.0	-0.6	16	32.4	4.7	14.5%
61.2	26	IIb	66	10.9	16.0	-0.6	16	34.1	0.9	2.6%
64.3	18	Id	66	8.9	16.0	-0.6	25	28.5	0	0%
62.1	19	IIa	66	8.9	16.0	-0.6	25	19.7	0	0%
63.3	27	Ib	111	7.2	16.0	-0.7	25+25	17.7	0.7	4.0%
64.4	10	Id	111	7.2	16.0	-0.7	25+25	28.5	0	0%
62.2	26	IIa	111	7.2	16.0	-0.7	25+25	19.7	0	0%
60.5	35	IIa, b	111	7.2	16.0	-0.7	25+25	32.4	0	0%
60.6	11	IIc, IIIa, b	44	6.5	12.7	-0.7	22	32.4	11.5	35.4%

Fig. 2 Effect of short-term exposure to low temperatures on the survival of growing filariae in mosquito



Remarks. (1) In lot No. 60.6, the larvae which reached the 3rd stage i.e. III a and b before the exposure, nearly all survived the low temperatures.

(2) For the explanation of the Fig., refer to the remarks in Fig. 1.

at 27°C for 13 days where many of filariae reached IIIa substage and some number of them did IIIb, while considerable number of them remained in IIc and even in IIb and a few in IIa. Such a great variation in development in the simultaneously infected mosquitoes are often observed in cases of mosquitoes being allowed to feed on a heavily infected carrier and having taken up by chance a great number of microfilariae. The lot was suddenly exposed to temperatures of about 0°C for 22 hours and then removed to room temperature of about 12.7°C for 22 hours. The second stage larvae are killed mostly during the exposure and some are killed after being transferred to laboratory, while IIIa and b substage larvae nearly all survived these temperatures. The survival rate of alive infective larvae to the expected initial number of microfilariae was 35.4%.

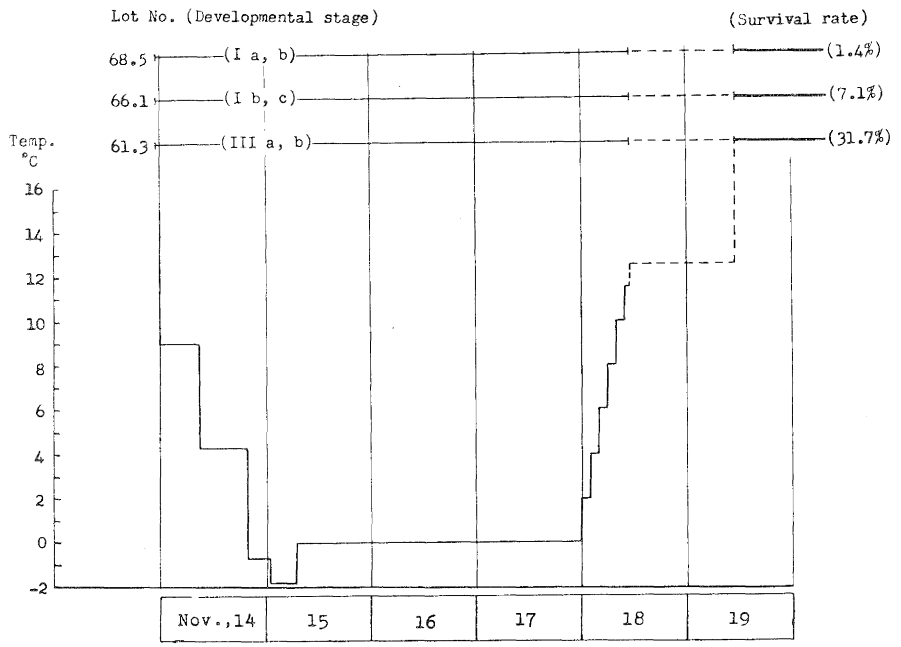
Experiment III

The filariae of earlier substages of the 1st stage and 3rd stage larvae were found more resistant to low temperatures and hence in this experiment the mosquitoes having filariae of these substages were subjected to lower temperatures for longer period than in the previous experiments, as shown in Table 4 and Fig. 3. They were then kept under laboratory temperatures of about 12.5°C for 24 hours, and after this transferred to 27°C room.

Table 4 Result of Experiment III, showing the effect of short-term exposure to low temperatures (cf. Fig. 3) on the survival of the earliest and the full grown filariae in mosquito

Lot No.	No. mosquito exposed	Stage of filariae before exposure	Period and temperature °C of exposure					Expected No. micro-filariae per ♀ per meal	No. filariae survived & reached infective stage per ♀	Survival rate to expected No. micro-filariae
			Total hours	Mean temp.	Highest temp.	Lowest temp.	Hours kept below 5°C			
68.5	20	Ia, b	132	3.8	15.0	-1.9	91	7.2	0.1	1.4%
66.1	17	Ib, c	132	3.8	15.0	-1.9	91	9.9	0.7	7.1%
61.3	16	IIIa, b	132	3.8	15.0	-1.9	91	34.1	10.8	31.7%

Fig. 3 Effect of short-term exposure to low temperatures on the survival of the earliest and the full grown filariae in mosquito



Remarks. (1) In lot No. 61.3, the filariae (most of them were the third stage, III a and b), almost all survived the exposure to the low temperatures.

(2) For the explanation of the Fig., refer to the remarks in Fig. 1.

Lot No. 68.5 was exceptionally kept under outdoor temperatrues of 14.8°C for 2 days, when about a half of filariae appeared to have got to thoracic muscles, while the others were still in the stomach of mosquitoes. Lot No. 66.1 was kept at 27°C for 3 days and some of filariae just reached the shortest or Ic substage. The survival rate was only 1.4% in the former lot, while it was 7.1% in the latter suggesting that among Ia, b, and c substages, Ib is most resistant, Ic comes next, and Ia is least resistant to low temperatures.

Mosquitoes of lot No. 61.3 were reared at 27°C

for 15 days and most of the filariae reached already IIIb substage but some were in IIIa and a few in IIc. Most of IIc larvae were found killed during the exposure, while some of them and a few of IIIa larvae were found weakened or dying. Most of IIIa and all of IIIb larvae could survive the exposure and became very active after being transferred to 27°C room.

Considerations on the Propriety of the Survival Rate Used to Evaluate the Effect of Low Temperatures on the Survival of Filariae in Mosquito.

As reported by the author (1958), the distribution pattern of microfilariae in the peripheral blood stream of a carrier is usually of Pólya-Eggenberger type i. e. of aggregate type, and therefore the number of microfilariae to be taken up by a female mosquito should be subjected such a great variation that the mean of the numbers of microfilariae taken up by females engorged simultaneously with about 6mm³ blood of a carrier, does not approach to the number of microfilariae in the same quantity of the carrier's blood unless a great many mosquitoes, over several hundreds, are taken, in taking the average. This seems to be the reason why the mean number of microfilariae for a batch of mosquitoes often varies greatly with batches, in spite of being fed simultaneously on the same carrier. Next, some number of microfilariae are excreted in the droppings for about a half day from just after feeding, and some filariae are killed during their development even under a favorable condition, apparently more often in case of heavier infection. The loss of filariae due to the above cases may vary greatly with the changes in environmental temperatures.

By the reasons as above, it may be understandable that the number of infective larvae should be considerably smaller than the initial number of microfilariae taken up by a female mosquito, and moreover that the number of filariae in mosquito is not necessarily proportional to the expected number of microfilariae to be taken up by a mosquito even in an average of several tens of mosquitoes, and therefore that it is not adequate procedure to compare the final number of infective larvae with the expected initial number of microfilariae. With all the reasons, the author adopted the above procedure in evaluating the effects of low temperatures upon the different stages of filariae because there are apparently no adequate means of measuring the effects.

Table 5 Comparison of the survival rates (shown in bold letters) of filariae in two lots No. 60.6 in Experiment II and No. 61.3 in Experiment III

Lot No. (1)	Expected No. Mf per ♀ (2)	Substages before exposure (3)	No. filariae per ♀ found after removal to 27°C (% against the expected initial No. Mf)				Total
			II stage		III stage		
			alive	dead	alive	dead	
60.6	32.4	mostly IIIa, some IIc, some IIIb, rarely IIb	0 (0%)	7.2 (22.2%)	11.5 (**35.5%**)	0.5 (1.5%)	19.2 (59.3%)
61.3	34.1	mostly IIIb, some IIIa	0 (0%)	3.1 (8.2%)	10.8 (**28.6%**)	0 (0%)	13.9 (36.8%)

(1) : As to the temperatures and periods of the exposure, cf. Tables 3, 4, Figs. 2, 3, and also see text.

(2) : Expected number of microfilariae to be taken up by a female mosquito in a full infected meal.

(3) : Substages of filariae in mosquitoes just before the exposure to low temperatures.

Now, an example will be shown in Table 5. As mentioned above, the III stage larvae are most resistant, while the growing larvae are most susceptible to low temperatures. Table 5 shows that the total number of filariae found on dissection after transferred to 27°C room are 19.2 and 13.9 per ♀ respectively or 59.3% and 36.8% against the expected initial number of microfilariae. The number in the former is larger than in the latter, in spite of the smaller expected initial number of microfilariae. In the former, II stage larvae and a few III stage, perhaps IIIa substage larvae, were killed and 11.5 per ♀ or 35.5% infective larvae survived vigorously. While, in the latter, II stage larvae having been remained in that stage were only killed and 10.8 per ♀ or 28.6% infective larvae survived vividly. Survived numbers of infective larvae are nearly equal in the two lots but the survival rate is considerably higher in the former, here again, because of the smaller initial number of microfilariae. In fact, in the latter, so far as the infective larvae are concerned they could survive the lower temperatures for longer period apparently in a 100% survival rate.

The mosquitoes of the lots No. 60.6 and No. 61.3 were fed on the same carrier. The former allowed to feed on him at from 9.10'-10.10' p.m. and the latter at 10.30'-11.30' p.m. The microfilarial counts at 9.00', 10.20', and 11.40' were 89, 127, and 100 per 20mm^3 respectively. The average counts during the feeding times for the lots were 108 and 113.5 per 20mm^3 or 32.4 and 34.1 per 6mm^3 respectively.

Thus, it is not an adequate procedure to compare the final number of infective larvae with the expected initial number of microfilariae because of the two numbers being not necessarily proportional with each other. This inconsistency seems to be due mainly to the distribution pattern of microfilariae in the peripheral blood stream of the carrier being not of random type.

Summary

Experiments were conducted to examine the effect of short-term exposure to low temperatures upon the survival of various substages of filariae in mosquito with the following results : The third stage especially the infective larvae (IIIb) are highest in resistance nearly all surviving a course of low temperatures of about 2°C in an average with a minimum of -1.9°C for about 4.5 days ; the earlier substage (Ib) of the first stage comes next surviving the same course of low temperatures only in a very low percentage. The growing filariae from Id to IIc substages are very susceptible to low temperatures, failing to survive a course of low temperatures of 3.8°C in an average with a minimum of -0.7°C for only 37 hours.

References

1) **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).

2) **Omori, N.** : Experimental studies on the role of the house mosquito, *Culex pipiens pallens* in the transmission of bancroftian filariasis. 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).

3) **Omori, N.** : Experimental studies on the role of the house mosquito, *Culex pipiens pallens* in the transmission of bancroftian filariasis. 3. Duration of life of filariae in mosquitoes exposed to winter temperatures. Yokohama Med. Bull., **9**(6) : 382-390, **1958**.

4) **Omori, N.** : 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.

Nagasaki Med. J., **33**(11), Suppl.: 61-70, **1958**.

5) **Omori, N.:** Experimental studies on the role of the house mosquito, *Culex pipiens pallens* in the transmission of bancroftian filariasis. 5. On the distribtuion of infective larvae in mosquito and the effect of parasitism of filariae upon the host insect. Nagasaki Med. J., **33**(11), Suppl.: 143-155, **1958**.

総　　括

蚊体内のフイラリア幼虫の生存に及ぼす短期低温の影響を調べるために，一定発育期の幼虫を持った蚊を低温に接触させ，その後，室温に1，2日放置後これを27°C温室内で飼育して，生き残り幼虫が感染幼虫になるのを待ってその数を調べた．感染幼虫を低温に接触させた場合には27°C温室へ移してから蚊体内でのその生存数を調べた．実験の結果，次の事が判明した．第Ⅲ期幼虫特に感染幼虫は低温に最も強く，平均2°C，最低-1.9°Cの温度に4.5日接触させて殆んど全部生き残り得る．第Ⅰ期の発育初期（Ⅰb）のものは次に強く，上記の低温に接触させると，生き残って感染幼虫となる率は極めて低いが若干個体はこの低温に耐え得る．然し発育中の幼虫（Ⅰd—Ⅱc）は低温に極めて弱く，平均，3.8°C，最低-0.7°Cに37時間接触させると悉く接触中，或るいは少数のものは接触後死亡して，感染幼虫となるものは全く見られない．

Received for publication February 9, 1962.