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Studies on the Intestinal Protozoa of Termites

II. Oxygenation Experiments under the Influence of Temperature

By

Masatake YAMASAKI

With 1 Table in Text

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The hind-gut of the xylophagous termite is usually infected by many, at least more than four, species of flagellates. In studying the physiology of these protozoa it is most desirable to prepare a host that carries only one or two species of the parasites, and this was achieved by CLEVELAND (1924, 1925, 1926) by means of several methods such as incubation, starvation, oxygenation or certain combinations of these. By incubation for 24 hours at 36°C., he (1924) succeeded for the first time in getting *Reticulitermes flavipes* free from intestinal protozoa. By oxygenation, starvation or a combination of the two he (1925d) obtained at will *Termopsis* entirely devoid of its protozoa or harbouring only a few species. He (1925e) showed also that the oxygen had the effect of removing the intestinal protozoa equally from four other termites and from many other animals such as cockroaches, earthworms, frogs, gold-fish, and salamanders. Thus oxygen is known to have the excellent effect of removing the parasitic protozoa from the intestinal tract of the host. Similar oxygenation experiments were carried out by the writer with the Japanese termite, *Leucotermes (Reticulitermes) speratus* and it was found that the temperature is a very important factor in the effect, as is briefly reported in this paper.

Material and Method

Workers of *Leucotermes (Reticulitermes) speratus* collected in Kyoto were the chief materials. This termite is, as was noted in my previous paper (1930), the commonest species in Japan proper and harbours in its hind-intestine eleven forms of flagellates. In order of size they are: *Teratonympha mirabilis*, *Trichonympha agilis* var. *japonica*, *Pyrsonympha grandis*, *P. modesta*, *Dinenympha rugosa*, *D. exilis*, *D. porteri*, *D. leidyi*, *D. nobilis*, *Holomastigotes elongatum* and *D. parva*. All the termites were first separated from the wood in which they had lived, then fed with wet absorbent cotton for at least ten days till used in the experiments. The idea was to put all the insects on a practically equal footing with regard to nutrition.

From fifteen to thirty termites were placed with food in a glass test-tube, about 3 cms. in diameter and 18 cms. in depth. The rubber stopper of this test-tube was pierced by two thin glass tubes which connected the test-tube with an other test-tube prepared in the same manner. Several such chambers were arranged one after the other and the air inside them was driven away by oxygen gas introduced from a commercial bomb. After the air had been completely replaced by the oxygen the tension of this gas in the chamber was so adjusted as to be practically at one atmospheric pressure. This apparatus was dipped in a thermostat and the temperature was kept constant at 36°, 33°, 30°, 25°, 20°, 15°, 10° and 0°C. respectively.

By taking out any test-tube independently without disturbing the others, the termite within it could be inspected for intestinal protozoa. In this manner, ten individual termites from each tube were examined and it was determined by the mobility of the protozoa, whether they were dead or not. The time during which the termites were exposed to any degree of temperature was changed according to necessity, and the same experiment was repeated more than three times. In this way the length of time required to kill the various protozoa in all the hosts was determined. Control experiments were run conducted with animals kept under exactly the same conditions except that they were not oxygenated.

Important Results of Experiments

The experiments being chiefly concerned with the effect of changes in temperature, they can conveniently be divided into three groups: I. high temperature, above 30°C., at which the termites themselves were affected by the temperature; II. moderate temperature, between 30° and 15°C., at which the termites remained healthy and showed no visible influence of temperature, and III. low temperature, below 15°C., at which the insects became sluggish or lost their activity and at last fell into dormancy.

I. High Temperature, above 30°C.

In this condition even the termites in the control experiments suffered ill effect from the temperature, which is the result obtained by CLEVELAND in his incubation experiment.

I. a) *Incubation experiments without oxygenation (control)*

36°C. At this temperature all the termites were freed from their protozoa very quickly. All the protozoa were killed at almost the same time i. e. after three hours of incubation, except that a few *Teratonympha* were still able to show very feeble movement of the flagella. After 4 hours no protozoa were observed alive. The hosts survived more than 24 hours.

33°C. In this case, the death of the protozoa was fairly slow. The examinations were made every six hours. *Holomastigotes* and *P. modesta* were the first victims, disappearing in all the termites at between 18 and 30 hours. *D. leidyi* and *D. nobilis* were the next, being killed at between 30 and 36 hours, and then *D. exilis, P. grandis, D. porteri, D. parva* and *D. rugosa*, died at between 36 and 48 hours, so that after 48 hours there were only *Teratonympha* and *Trichonympha* left. The last mentioned, however, was found in greater abundance and survived longer than *Teratonympha*. The longest living individual of *Teratonympha* was seen at the end of 84 hours and that of *Trichonympha* at 138 hours. The host died at between 120–140 hours. Accordingly some individuals of *Trichonympha* can be said to die at the same time as their host, or rather, they have to die because the host does not survive.

30°C. At this temperature the protozoa died still more slowly. The results were checked every 24 hours. The chronological order of disappearance of protozoa was in general similar to that in the previous case; namely, *Holomastigotes* and *P. modesta* in 6–15 days, *P. grandis, D. leidyi, D. nobilis, D. parva*, and *D. porteri* in 13–19 days, *D. exilis* and *D. rugosa* in 14–30 days. In this case too, *Teratonympha* and *Trichonympha* were the last two to remain, the former surviving for 15–29, the latter for 16–36 days. Some *Trichonympha* survived as long as their hosts.

It is clear from these results that the lower the temperature, the longer the life of the protozoa, the order of the disappearance of the protozoa being always the same, i. e. *Holomastigotes* and *P. modesta* first and *Teratonympha* and *Trichonympha* last.

I. b) *Incubation experiments with oxygenation.*

36°C. At this temperature the protozoa disappeared very quickly. After 3 hours oxygenation all the protozoa were gone from all the termite while the host died shortly before 24 hours. As in the control experiments mentioned above, *Teratonympha* remained longest.

33°C. In this case also the life of the protozoa was markedly shortened and they disappeared in about the same order as in the control, namely, *Holomastigotes* after 18 hours, *P. modesta* after 24 hours and *D. nobilis*, *P. grandis*, *D. leidyi*, *D. porteri*, *D. exilis*, *D. rugosa* and *P. modesta* after 30 hours. *Trichonympha* and *Teratonympha*, though they diminished both in number and in activity, remained more 12–16 hours. Some *Trichonympha* seemed not to perish until their host died after 54 hours.

30°C. At this temperature the protozoa disappeared more slowly than in the previous case. Inspections were carried out every 24 hours and the result was different from that in any of the previous cases, whether with O_2 or without O_2. After 24 hours of oxygenation the protozoa were still visible and there was no distinct decrease in number. After 48 hours, various grades of the effect of oxygenation were observed, namely, 9 out of 30 individual termites still harboured all the species of protozoa, 4 had already lost the protozoa completely, while the other 17 retained one, two or more species of surviving protozoa. Diminution was most marked in *Teratonympha*, *Holomastigotes*, *P. modesta* and *Trichonympha*. After 72 hours the hosts themselves began to die. Meanwhile the number of protozoa, especially that of the four forms just named, was decreasing with great rapidity, although all the individual termites, when killed after 96 hours, were not absolutely free from the protozoa. Since it is quite reasonable to assume that the protozoa found in most abundance in the last surviving termites are the most resistant, I take *Holomastigotes* and *P. modesta* to be the shortest lived, *Trichonympha* and *Teratonympha* to be the next shortest and *P. grandis* to be the longest lived.

In this case, it is remarkable that *Trichonympha* and *Teratonympha* showed a tendency to disappear early, not so late as in the foregoing cases in which they always remained to the last; and that *P. grandis*, which had disappeared comparatively early in the previous cases, was found most abundant until late.

II. Moderate Temperature, between 25° and 15°C.

At this moderate temperature, no ill effects were observed in the non-oxygenated group of termites. In the oxygenated group the protozoa were removed so slowly that the inspection was made every 24 hours.

25°C. *Teratonympha* began to disappear in some individuals at the end of the first day of oxygenation and completely disappeared from all the termites between the 6th and the 8th day. *D. nobilis* next disappeared between the 7th and the 9th day. *Trichonympha* remained rather abundant until the end of the sixth day, then it diminished suddenly and was exhausted after 9 days of oxygenation. Other forms of protozoa began to suffer very ill effects after 8 days of oxygenation and were exterminated between the 9th and the 10th day when their hosts also died at the same time.

20°C. At this temperature the protozoa disappeared more slowly than in the foregoing cases and some individual hosts survived for more than a fortnight. In this case too, *Teratonympha* was the first to perish, and disappeared from all the termites within 7–12 days. Following to this, between the 8th and the 14th day both *Trichonympha* and *D. nobilis* were affected, and between the 8th and the 15th day both *D. leidyi* and *P. modesta* were killed completely. The other forms of protozoa though decreased in number as well as in activity, lasted until their hosts died. At this temperature the duration of life of the host was somewhat variable, some individuals from one colony dying as early as on the 9th day while those from the other colony survived as late as the 16th day. But the order of disappearance of the protozoa was always the same.

At the moderate temperature, all the protozoa except three, *Trichonympha*, *Teratonympha* and *D. nobilis*, remained alive throughout their host's lifetime so that the complete removal of protozoa was impossible. Here it is noticeable that the tendency seen in the oxygenation experiment at 30°C. became more distinct, namely, *Teratonympha* and *Trichonympha* were removed at first and *P. grandis* remained to the last and most abundant.

III. Low Temperature, below 15°C.

Although low temperature makes the termites lazy and sluggish, their intestinal protozoa do not seem to be affected and remain in an apparently healthy condition for a long time.

15°C. In the experiments at this temperature, two different kinds of results were obtained in the two groups of termites, that is, the disappearance of the protozoa occurred in two very different orders: (A) in the termites from one colony the protozoa died in a manner similar to the case at 20°C. though with some prolongation in time, (B) contrary to this in the termites from the other colony, the protozoa disappeared in a manner quite different from all the foregoing cases. In the latter case, all the termites were freed of their protozoa very quickly and examination every 6 hours was necessary. Between the 6th and the 12th hour *P. modesta* and *D. nobilis* disappeared and between the 12th and the 18th hour *D. leidyi* and *Holomastigotes* were eliminated completely. 30 hours of oxygenation was sufficient to remove all the protozoa from all the termites, though some hosts survived for more than three weeks.

Thus 15°C. seems to be a critical temperature at which the termites began to enter an inactive state.

10°C. At this temperature termites became very inactive. The effect of oxygenation was the same as in (B) of the last case. At the end of 24 hours of oxygenation many individual termites lost their intestinal protozoa entirely. But, it took as long as 144 hours to remove all the protozoa from all the individual termites. *Holomastigotes* and *P. modesta* died between the 24th and the 48th hour and *D. nobilis* between the 24th and the 120th hour. The other eight forms of protozoa began to disappear after 48 hours and were completely exterminated after 144 hours. The host remained apparently intact throughout the time in which all the protozoans disappeared.

0°C. In this case the termite fell into dormancy, so that the results were quite different from those in all the foregoing cases. With no exception, the termites were freed from their parasites in 24–30 hours. *Trichonympha* became absent in many termites even at the end of the 6th hour and from all the individual termites at the end of the 18th hour. After 18 hours of oxygenation there remained only *Teratonympha*, *P. grandis* and *P. modesta*, although in quite exceptional cases a few individuals of *D. leidyi* and *D. nobilis* also were seen.

At low temperatures it was possible to remove the parasites completely without injuring the hosts, with the exception of case (A) in the experiments at 15°C. In this case *Trichonympha* and *Teratonympha* were the last to disappear as in the case of high temperatures, except one case at 0°C. *P. grandis*, which was removed rather early at the higher temperatures remained in this case to the last in considerable abundance, accompanied by *Trichonympha* and *Teratonympha*, so that in this case it must be stated that the largest of the protozoa lived longest.

It is interesting to note that at 0°C *Trichonympha* and *Teratonympha*, which in the other cases disappeared in immediate succession were eliminated at widely different times.

Order of disappearance of protozoa

36°C		33°C		30°C	
Incubation	Oxygenation	Incubation	Oxygenation	Incubation	Oxygenation
		Holomastigotes	P. modesta	Holomastigotes	Holomastigotes
		P. modesta	Holomastigotes	P. modesta	P. modesta
		D. nobilis	D. nobilis	P. grandis	Teratonympha
		D. leidyi	P. grandis	D. leidyi	Trichonympha
		D. exilis	D. leidyi	D. nobilis	D. exilis
		P. grandis	D. porteri	D. parva	D. nobilis
		D. porteri	D. exilis	D. porteri	D. porteri
		D. parva	D. rugosa	D. exilis	D. leidyi
		D. rugosa	D. parva	D. rugosa	D. parva
		Teratonympha	Teratonympha	Teratonympha	D. rugosa
Teratonympha	Teratonympha	Trichonympha	Trichonympha	Trichonympha	P. grandis

25°C	20°C	15°C		10°C	0°C
Oxygenation	Oxygenation	Oxygenation A	Oxygenation B	Oxygenation	Oxygenation
Teratonympha	Teratonympha	Trichonympha	P. modesta	P. modesta	Trichonympha
D. nobilis	Trichonympha	D. nobilis	D. nobilis	Holomastigotes	D. exilis
Trichonympha	D. nobilis	Teratonympha	D. leidyi	D. nobilis	Holomastigotes
D. leidyi	D. leidyi	D. leidyi	Holomastigotes	D. parva	D. rugosa
P. modesta	P. modesta	D. porteri	D. exilis	D. leidyi	D. parva
D. exilis	D. exilis	P. modesta	D. rugosa	D. porteri	D. porteri
Holomastigotes	Holomastigotes	Holomastigotes	D. parva	D. exilis	D. nobilis
D. porteri	D. parva	D. exilis	Teratonympha	D. rugosa	D. leidyi
D. parva	D. porteri	D. rugosa	D. porteri	P. grandis	Teratonympha
D. rugosa	D. rugosa	D. parva	Trichonympha	Trichonympha	P. modesta
P. grandis	P. grandis	P. grandis	P. grandis	Teratonympha	P. grandis

The results of all these experiments are summarized in the accompanying table, in which eleven protozoa are arranged according to the length of time they survived, the shorter lived ones coming first in each list. The following points are readily seen:

(1) The order is dissimilar at different temperatures. (2) The difference is most evident at two critical points, namely, 30°C. and 15°C. At 30°C. the order of disappearance is different according to

whether the termites are oxygenated or not; if they are exposed to the mere effect of temperature the result at 30°C is similar to that obtained at higher temperatures, while if the effect of oxygenation is added the result falls into a type usually seen at moderate temperatures. At 15°C. two very different results are obtained according to the colony of the host, in spite of the treatment given to each colony being entirely the same: in the one case the order of disappearance is the same as that at moderate temperatures, while in the other case the order is what we see at 10°C. Furthermore, the order obtained at 0°C is different from all the others and is quite peculiar to this degree of temperature.

(3) Five species of protozoa exhibit the highest abnormality in the duration of life. The ones which die earliest are *Holomastigotes*, *P. modesta*, *Trichonympha* or *Teratonympha*, whereas the species that survive longest are *P. grandis*, *Trichonympha* and *Teratonympha*. The variation in the resistance of *Trichonympha* and *Teratonympha* is especially striking, being weakest at moderate temperatures and strongest at either high or low temperatures.

A General Consideration

In a previous paper (1930) the writer recognized the fact that in starvation experiments the duration of the life of parasites was influenced by the temperature to which the host was exposed during the experiments. Now in these oxygenation experiments, it was ascertained that the temperature interfered in a peculiar way and very interesting, not only with the duration of life of the protozoa but also with the order of their disappearance.

At high temperatures, the protozoa were removed in the same order both in the incubation (control) and in the oxygenation experiments, the only difference being that the process took place somewhat quicker in the latter case. In this case, therefore, the effect of oxygen was overwhelmed by that of temperature and oxygen has presumably merely an accelerating effect. While *Reticulitermes flavipes* on which CLEVELAND (1924) carried out his experiments, became free from its intestinal protozoa in 24 hours of incubation at 36°C, the termite in my experiments was destitute of them even after 4 hours at the same degree of temperature. Now, to take the effect of oxygenation into consideration, while CLEVELAND (1925d) waited 72 hours before all the protozoa were removed from his termite, *Termopsis*, at one atmospheric pressure, I had to wait more than ten days until my termite reached the same state under the same pressure and at a moderate temperature. From these facts, it is clear that my termite can reasonably be considered very weakly resistant to incubation but pretty strongly resistant to oxygenation. Accordingly, it may be inferred that at high temperatures and under one atmospheric pressure, the effect of the oxygen is too weak to overcome the effect of temperature and bring about a specific result.

At moderate temperatures, all the protozoa except *Trichonympha*, *Teratonympha* and *D. nobilis*, survived throughout the life-time of the host and died at the same time. In this case, therefore, it is not certain whether the disappearance of the protozoa is started by the toxic action of oxygen or whether it is caused indirectly by an abnormal condition that arises in the host. At low temperatures, the protozoa were removed completely, for the first time without injuring the host. It is most probable that in this case the protozoa were killed by the direct effect of oxygen. At such temperatures too the protozoa of my termite disappeared generally in the same way as those in CLEVELAND's (1925d) termite, *Termopsis*, in which the smaller forms died first and the largest ones remained to the last. At low temperatures, termites become inactive and their ability of adjusting themselves to their environment must be diminished, and therefore, the oxygen, solubility of which is increased by the fall in temperature, might show its toxic effect on the intestinal protozoa more distinctly.

At 0°C the protozoa disappeared in a peculiar way, quite different from all the other cases. The reason why the protozoa were removed in such a manner is not clear at present. Perhaps one of the important factors is that the host falls into dormancy at such a low temperature.

At high and moderate temperatures the oxygen at one atmospheric pressure seems to be insufficient to kill the protozoa without injuring the hosts. What pressure of oxygen is nesessary for this purpose or in what manner the protozoa will be removed if sufficient pressure of oxygen is applied are questions that remain for future study. As has been described above, a striking disagreement of results is visible at 15°C, according to the colony from which the termite is selected. Why this is so, I am not able to explain at present, although the fact seems to suggest that this temperature may be one of the critical points at which some important change in the physiological process in the termite's body takes place.

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