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Studies on the Intestinal Protozoa of Termites

IV. Glycogen in the Body of *Trichonympha agilis* var. *japonica* under Experimental Conditions.

By

Masatake YAMASAKI

(Zoological Laboratory, Kyoto Imperial University)

With 7 Text-figures

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A considerable amount of glycogen is found in the body of *Trichonympha agilis* var. *japonica* which is a parasite on termites, *Leucotermes* (*Reticulitermes*) *speratus*. Glycogen, as has been reported in my previous paper (III. Report), is deposited in three regions of the body of this protozoa: on the inner lining of the wall of the bell, in the corbule and in the endoplasmic region of the posterior part of the body. This glycogen is thought to be derived from the wood that has been taken into the body of the protozoa as food, but its fate during the further metabolic processes of the organism is not yet clear. The quantity of glycogen and the manner of its deposit are very variable even under normal conditions. Such variations are more striking, if the protozoa are subjected to abnormal or artificial conditions. Now, if the reaction of glycogen varied definitely following the variation of some factors given in the experiments, and if there were some parallelism between the reaction and the experimental factors, it might be possible to deduce the part played by glycogen in the vital process of the organism.

In this paper it is proposed to inquire as to the fate of glycogen in five series of experiments: starvation, incubation, abrupt rise of temperature, oxygenation at a moderate temperature and oxygenation at a low temperature.

In all experiments the protozoa are fixed with 90% alcohol and stained by EHRLICH'S hæmatoxylin and BEST'S carmin for the measurement of the deposit of glycogen in their bodies.

Experiments

A large amount of glycogen is deposited in the body of *Trichonympha agilis* var. *japonica*. The glycogenic reaction is detected in three regions in the body of the organism. First on the inner surface of the wall of the bell portion, second in the corbule, and third in the endoplasmic part of the posterior body region. The reactions in these three regions are distinctly differentiated, for *Trichonympha* has a body that is structurally separated into divisions. Though the quantity is scarce, the glycogen deposit is distinctly observable on the inner surface of the bell-wall, but often the reaction fades away. Glycogen deposit is always found both in the corbule and the body part. In the corbule, glycogen is deposited over the nucleus which is situated at the bottom of this little basket-like structure. In the body region glycogen is detected in all parts except the space occupied by the food vacuoles. As explained in my previous paper (1937), the deposit in these two portions are remarkably variable in quantity and in the manner of the deposit. Ordinarily, when the deposits in the two portions are not the same, that of the corbule is greater than that of the posterior part of the body, and the inverse case is very rare (Fig. A, 1, 2, 3 and 4). Rarely some exceptional features of the glycogen deposit are observable in the *Trichonympha* taken from termites in a normal or wild state. It is to be expected, therefore, that when the termites are placed under abnormal or experimental conditions, glycogen in the body will show various changes. In fact we can see in the following experiments varying abnormal ways in which glycogen is deposited or diminished.

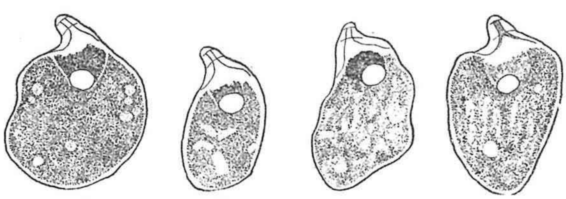

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Fig. A. 1—4. Out-line figures showing the various manners of the glycogenic deposit in the body of *Trichonympha agilis* var. *japonica* in normal conditions. The dots represent the granules of glycogen.

STARVATION: After being cleaned of all food particles, the termites are placed in two petri dishes. Necessary moisture is supplied to the vessel by inserting a sheet of wet filter paper between the dishes. This vessel is put in the incubator and the temperature is kept at 25°C. The protozoa are examined every six hours for their glycogen content.

In this case glycogen in the body of the *Trichonympha* disappears in two different ways after 24-30 hours. First, an abnormality appears on the corbule which is compressed laterally while the nucleus is compressed along its longitudinal axis. Moreover, a vacuole is produced in the region surrounding the corbule which has been occupied by the corbule prior to the compression. Resulting from this distortion of the corbule, its glycogen is remarkably condensed and gives a very intense and distinct reaction. Glycogen in the corbule is thus condensed, while the same in the body region becomes scarce. As a result the contrast between the densities of glycogen in these two regions becomes more distinct than in the usual case. In the *Trichonympha*, however, such a deposit of glycogen is often observable in the normal condition. This manner of deposit, therefore, does not result from the pathological effects of the experiment, but from malnutrition. If this undernourishment continues, the organisms are driven into a pathological state, and the compression of the corbule becomes more and more intense, at which time its glycogen becomes greater in density, though it diminishes in quantity. Glycogen in the body region is also reduced so much that in some individuals reaction in the body region is completely lacking. This process continues until all the glycogen in the body completely disappears. Shortly afterwards the organism itself perishes (Fig. B. 1—4).

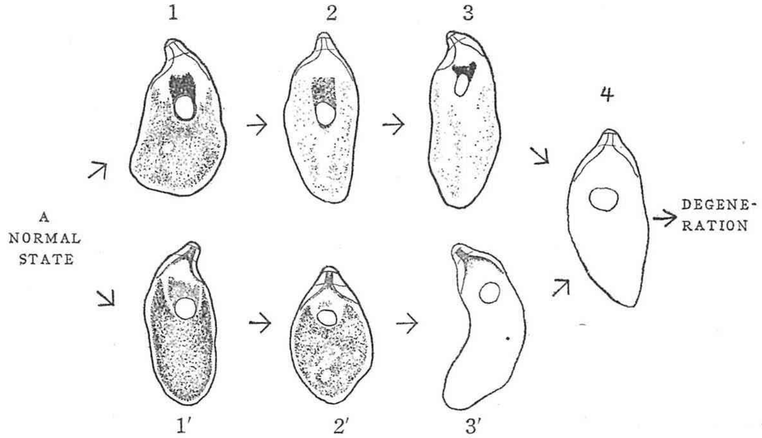

Fig. B. Glycogen deposit in the body of *Trichonympha agilis* var. *japonica* in a starvation experiment. 1—4: Manner of the diminution of glycogen observed in the first way of the experiment. 1′—3′ and 4: Those observed in the second way.

Secondly, there is no morphological change to be seen in the body. A diminution of the glycogen appears first in the corbule. In this case the glycogen is changed to very fine granules and the reaction becomes very weak. The quantity of glycogen must then be unusually scarce. The reduction of the body region takes place more slowly than in the corbule, so that in this case the glycogenic reaction in these two portions is very different, although the relation between them is inverse to that of the above described case. That is, the reaction of this corbule is less than that of the body region. In this stage another change in the deposit of glycogen is detected on the inner surface of the bell-wall. As the deposit appears on the inner surface of the bell-wall, the glycogen in the corbule begins to diminish. This deposit is scarce in quantity and is rare under normal conditions (Fig. A. 4). But in the experiment, this deposit is found whenever glycogen in the corbule decreases sooner than that of the body region. In the next stage, glycogen in the corbule disappears completely while that of the body remains, though remarkably reduced in quantity. Then following this stage, the reaction in the body also fades away leaving some deposit on the inner surface of the bell-wall. At last when all the deposit has completely vanished the organism itself degenerates and perishes (Fig. B. 1′—3′ and 4). Such a course in the depositing of glycogen is observed only under experimental conditions.

INCUBATION: Termites are placed in the same vessel used in the former experiment with food, kept at a temperature of 33°C., and examined every six hours.

In this experiment the diminution of glycogen takes place more rapidly than in the previous case. The process of diminution is similar to that described in the formerly mentioned second case of starvation, namely, that while no remarkable morphological distortion is brought out in the body of the protozoa, the glycogen itself rapidly diminishes first from the corbule. The contents of glycogen in the corbule are, as shown in the normal organism, usually greater than that of the body region, and the two portions can easily be discriminated. In this experiment, nevertheless, the differentiation between these two portions becomes temporarily obscure owing to the reduction of the quantity of glycogen in the corbule. But passing through this stage the reduction goes on rapidly in the corbule which soon becomes empty while the body is still preserving a moderate quantity of glycogen, and again two portions become distinctly

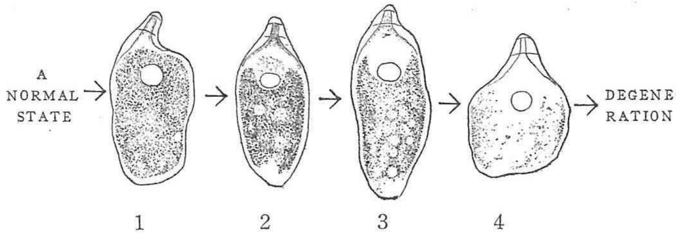

Fig. C. 1—4. The diminution of glycogen in the body of *Trichonympha agilis* var. *japonica* in the incubation experiment. Dots show the granules of glycogen.

distinguishable. In this instance, as in the case of the previous experiment, glycogen begins to deposit on the inner surface of the bell-wall, although this deposit is later decreased when the deposit of the body region is also diminished. Thus all the deposit of glycogen disappear from the body and the organism itself perishes (Fig. C. 1—4).

This process is essentially similar to the second way of starvation. The organism is driven to an overmetabolism by an abnormally high temperature, the consumption surpasses the supply of nutrition and a malnutritional state results.

OXYGENATION AT MODERATE TEMPERATURE: Termites with their food are placed in a thick test tube 2.5 cm. in diameter and 20 cm. in depth. Into this tube is introduced oxygen gas, the pressure of which is carefully maintained at room level. This vessel is dipped into a thermostat at a temperature of 25°C. The protozoa are tested every three hours.

In this case the glycogen of the body part first begins to diminish. Then the corbule begins to be compressed sidewise and its glycogen is condensed. This phenomenon is not so remarkable as was observed in the starvation experiment. Later, the compression of the corbule and the diminution of the glycogen in the body part become more and more unusual. In this experiment, however, the glycogen of both parts does not disappear completely until the moment when the organism itself begins to degenerate. The degeneration begins at the nipple. This is indicated by the fact that the nipple structure becomes obscure. Shortly afterwards the organism perishes. These phenomena are more or less similar to those of the first starvation experiment. But the compression of the corbule and the condensation of its glycogen are not so remarkable in this experiment as in the other. Furthermore, the degeneration of the body appears at an early stage when the glycogenic deposit still exists. It is inferred from these facts that the organism is toxically affected by the oxygen used in the experiment. Oxygen may excessively promote the metabolic function producing toxic effects, from which the organism becomes ill. (Fig. D. 1—3).

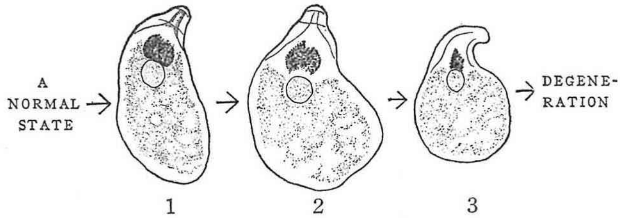

Fig. D. Changes of the deposit of glycogen in the body of *Trichonympha agilis* var. *japonica* during oxygenation at a moderate temperature. Dots show the granules of glycogen.

OXYGENATION AT LOW TEMPERATURE : This experiment has been performed in the same way as the previous one except that the temperature has been kept at 7°C., and the intestinal protozoa were examined every one hour. The termites used had been formerly exposed to the same temperature as those in the above experiment for more than two weeks.

At a temperature lower than 10°C. the termites become sluggish or fall into a cold torpor. In such a condition the intestinal protozoa are affected by oxygenation differently than at the moderate temperatures.

The diminution of the glycogen took place in three ways during this series of experiments. The first was alike that of the first way of the starvation experiment. The corbule is compressed laterally, its glycogenic deposit is condensed, and then the glycogen of the body region is diminished. The compression of the corbule becomes more and more unusual and the deposit in the body region is reduced extremely. The reactions in both parts, however, do not disappear completely until the organism itself degenerates (Fig. E. 1—2). The second way in which the glycogen diminishes is similar to that of the second way of the starvation or that of incubation. The corbule is not compressed, the glycogen in it disappears, and the deposit appears on the inner surface of the bell-wall. The diminution of glycogen in the body region is very slight or almost invisible. The organism perishes when the corbule becomes empty although a large quantity of glycogen is still reserved in the body region (Fig. E. 1′—3′). The third way is inactive. The body of the organism shrinks to a spherical shape and a large quantity of glycogen is preserved in it. In such individuals the differentiation between the corbule and the body region is invisible. Such organisms are often found among the termites that had been kept at the lower temperature. They perish showing no visible changes due to the reaction of glycogen. This seems to be the organism's way of resisting low temperatures. (Fig. E. 1″).

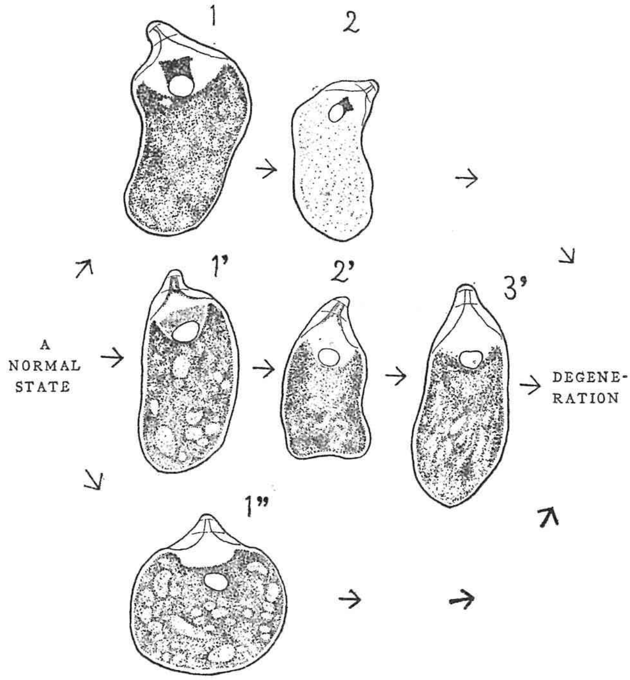

Fig. E. Glycogenic reaction in the body of *Trichonympha agilis* var. *japonica* during the oxygenation at a low temperature. 1—2: The first way of diminution. 1′—3′: The second way of diminution. 1″: The deposition in the third way of the experiment. The reaction of glycogen is represented by the dots.

At a certain low degree of temperature at which termites are driven to hibernate, the intestinal protozoa also become inactive and all their vital functions are lowered. If an organism in such a condition is brought into a pure oxygen atmosphere the organism may awaken in an abnormal manner. It may be disturbed in its sleep by the action of oxygen but the temperature is still low enough to keep the organism in a state of cold torpor. Under such conditions it is imaginable that the organism partially awakens, or in other words, that only certain vital processes are awakened by the action of the oxygen, while the other functions which are comparatively indifferent to the action of oxygen remain arrested. This unbalanced state may compel the organism to an undernutritional or an overnutritional pathological state.

The most interesting and remarkable phenomenon observed during this experiment is that which is presumptively called "Predatism among the intestinal protozoa of termites". The intestinal protozoa of the termites are, as a rule, hervivorous animals. Although some of them are said to be parasitic, nothing is known about their carnivorous activities and a few species known to capture its fellows in rare cases (CLEVELAND, 1925 d.). In my experiment, however, after three hours of oxygenation one or more minute forms of *Dinenympha* were found in the bodies of many *Trichonympha*. As the bodies of swallowed organisms are somewhat destroyed, their identification was not an easy matter, yet they were easily recognized as species of *Dinenympha*. These victims were generally found in the body region and rarely in the corbule of the *Trichonympha*.

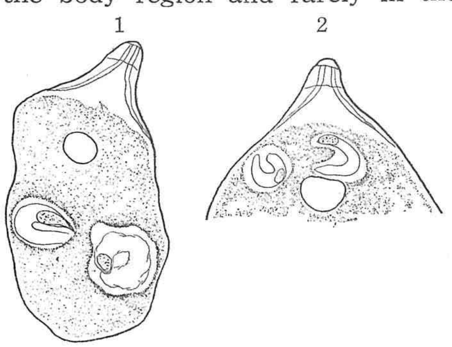

Fig. F. "Predatism" in the body of *Trichonympha agilis* var. *japonica*. 1: The forms of *Dinenympha* are found in the body region. 2: The forms of *Dinenympha* are included in the corbule.

Under normal conditions, *Trichonympha* takes wood particles into its body region but not in the corbule. The fact that *Dinenympha* are found in the corbule suggests the possibility that the *Dinenympha* invade the body of the *Trichonympha* to escape its corrupted environment. Yet, from the fact that the *Dinenympha* found in the body of the *Trichonympha* are always in a state of decomposition, and that the "predatism" is always found only in the body of the *Trichonympha*, it is also probable that the *Trichonympha* takes the *Dinenympha* as a food. This problem, however, can not be correctly answered yet. It can safely be said at this point that oxygen stimulates somewhat the hibernating organism (Fig. F, 1—2).

ABRUPT RISE OF TEMPERATURE: The termites were first kept at a temperature lower than 10°C. for more than two weeks and then were placed abruptly in a thermostat regulated at 25°C. Thus the temperature is raised from 7°C. to 25°C. in a short time. Examinations were made every 24 hours.

In this experiment the termites are awakened from their cold torpor by means of the abrupt change of temperature. The temperature is raised from about 7°C. to 25°C. and thus the organisms are subjected to a very strong metabolic disturbance. Of course such a sudden rise of temperature does not occur in the natural environment of the termites.

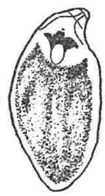

Fig. G. Change of the deposit of glycogen of *Trichonympha agilis* var. *japonica* in the experiment of an abrupt rise of temperature.

In this case, first the corbule is compressed laterally and the glycogen in it is condensed. During this process the glycogen content in the body region shows a tendency to reduce, a phenomenon similar to that in the beginning of the first way of the starvation experiment. In the present case, however, the diminution of glycogen is restored after 24–48 hours to the normal condition. A reasonable explanation for this is as follows : in general, when the animal awaken from their hibernation they soon consume their reserve body nutrition, become slowly active, and begin to take new food from the outside, due to the gradual rise of the temperature. But in this experimental case, the temperature is quickly raised to 25°C., the organisms are activated so abruptly that the reserve glycogen is consumed very vigorously, and the protozoa temporarily fall into an undernutritional state. This method of decreasing the glycogen is a physiological one rather than pathological, as was the case in the experiment in which the animals suffered ill effects (Fig. G.).

A General Consideration

As the results of five series of experiments : starvation, incubation, oxygenation in moderate temperatute, oxygenation in low temperature and abrupt rise of temperature, the writer has ascertained two main processes whereby the glycogen in the body of *Trichonympha agilis* var. *japonica* is diminished.

One process was observed in the first case of starvation, in the abrupt rise of temperature, in the first way of oxygenation in low temperature and in the oxygenation in moderate temperature experiments. In these cases, the corbule is always compressed laterally and its glycogenic deposit is condensed, while the glycogen in the body region is quickly reduced. The other manner whereby the glycogen was reduced was observed in the following experiments: the second way of starvation, the incubation and the second way of the oxygenation in low temperature. In these cases glycogen in the corbule first diminishes and at the same time a deposit appears on the inner surface of the bell-wall, while that of the body region remains unchanged.

It may be supposed that the glycogen deposit in the corbule and on the inner surface of the bell-wall is the source of energy for the nucleus and the flagella, while the body region is the factory where the glycogen is made and at the same time its warehouse. In other words, the anterior part of the body is the main region of consumption and the posterior part the region of supply.

Under experimental conditions, the organism is affected mainly either in the anterior half or in the posterior half of the body according to the nature of the conditions of the experiment. Especially in the oxygenation experiments the organism is affected by the action of oxygen in different ways according to the change of temperatures. This throws light on the problems in connection with the defaunation of the intestinal protozoa by means of oxygenation. By the defaunation experiments on *Leucotermes* (*Reticulitermes*) *speratus*, the writer (1931) found that the intestinal protozoa were killed in various ways and at times not proportional to the change of temperature from 0 to 36°C. Furthermore, CLEVELAND (1934) reported in the defaunation experiments on the wood-feeding roach, *Cryptocercus*, that when oxygen was given under one atmospheric pressure the protozoa were killed in shorter time at 4 to 5°C. than at 23 to 25°C., while in the cases in which four atmospheres of oxygen were used the time required for the defaunation was longer at 4 to 5°C. than at 23 to 25°C.. These results cannot be sufficiently explained only from the view point of the physical nature of oxygen. This confusion of results shows that the metabolic processes of the protozoa play an important role in determining the nature of the results of the experiments.

So far as *Trichonympha agilis* var. *japonica* is concerned, it seems most probable that the body is differentiated into several parts morphologically and physiologically. The part affected is unequal according to the nature of given factors, and, hence, such diverse results from oxygenation are obtained as described above.

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