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STUDIES ON THE SYMBIOSIS OF THE BODY LOUSE

I. ELIMINATION OF THE SYMBIONTS BY CENTRIFUGALISATION OF THE EGGS

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(With Plate XII)

IN a very great number of arthropods there are regularly found certain micro-organisms which, owing to their intracellular life, closely resemble parasites. The invasion differs, however, from a true parasitic one in that the micro-organisms are regularly transmitted to the offspring of the host and are present in fairly constant numbers in all members of the same species.

Buchner and his co-workers, who have made the most extensive and detailed study of this subject, concluded from the morphological analysis of the life cycles of a very great number of these micro-organisms that this type of relationship cannot be classified as either parasitism or commensalism. The regularity of appearance and the intimacy of the mutual adaptation find, according to Buchner, a satisfactory explanation only if we suppose that there exists a relationship between the arthropod and the micro-organisms which is mutually beneficial, or in other words is a true symbiosis. According to this view it is essential that both parties to the relation must benefit. On the one hand the micro-organisms by their presence provide some necessary requirement of the arthropod while they, on the other hand, are themselves enabled to multiply. Facilities for the multiplication of the micro-organisms and for their transmission to the progeny of the arthropod are provided by the development of a special organ called the mycetome.

The fundamental difference between this relationship and that of parasitism lies in the lack of mutual benefit in the latter case where one party lives entirely at the expense of the other.

Buchner's view has not been generally accepted. Doubts have been expressed as to whether a symbiotic relationship can be definitely postulated on the basis of morphological findings alone, and the suggestion has been made that some other explanation exists.

It is still believed, especially among parasitologists, that all relations between host and inhabiting micro-organisms can be expressed as parasitism. According to this view, the so-called symbionts are more or less harmless commensals, while the bacterial organs, the mycetomes, are considered as formations analogous to the plant gall produced by the host as a response to the irritation caused by the foreign inhabitant.

The problem to be solved is whether the association of the two unequal partners is a mere accident, or whether it serves a useful purpose. This can only be decided by experiment. If an experimental separation of the micro-organism from its host causes injury to the latter, and if it can be shown that the only cause of this injury is the absence of the micro-organism, then the existence of a true symbiosis between the host and its micro-organisms, as claimed by Buchner, would be definitely proven.

The object of this paper is to describe a simple method of eliminating the symbionts from the louse, a typical symbiont host, and the effect of this elimination on the host.

The symbiosis in the louse has been observed independently by Sikora (1919) and Buchner (1920). The life cycle of the symbiont has been worked out and described in detail by Ries (1932). From his description, which in the course of our work we were able to confirm in all essential particulars, several facts may be cited which are necessary in order to understand the principle of our method.

In the larva of the body louse, all symbionts are concentrated in a small organ, which is called *Magenscheibe* (stomach disc) by the German authors. The organ is round or oval in shape and is fixed to the outer ventral mid-gut, where it always lies exactly in the mid-ventral line of the body, slightly nearer the anal region than the head (Fig. 1). This organ is composed of cells derived from the mid-gut, it is covered with an irregular outer layer of mesodermal tissue, and encloses a more or less spherical cavity. This cavity is formed during the embryonic development of the louse as part of the stomach lumen, but after the larva is hatched it has no open connection with the lumen of the gut. It is divided radially into 12–14 chambers enclosing the symbionts (Fig. 7).

The whole organ has an opaque, yellowish colour, produced by certain granules of high refraction index, enclosed in the inner cells. Because of its colour, which stands out sharply against the dark red background of the blood-filled stomach, it can easily be recognised in the living animal even with an ordinary hand lens.

Prior to the formation of the stomach disc in the embryo the symbionts are enclosed in a group of cells which float freely in the yolk inside the lumen of the mid-gut. This group of cells, together with the enclosed symbionts, is called the primary mycetome. The symbionts are transmitted from the primary mycetome inside the mid-gut to the secondary mycetome, the stomach disc, in the following manner. The primary mycetome migrates to the place on the ventral mid-gut wall where later the stomach disc is formed. Here the wall of the mid-gut is pushed out in a hernia-like fashion so that a pocket formation results which includes the primary mycetome with its symbionts. This pocket is closed later in such a way that the symbionts are retained in the newly formed cavity and the cells of the primary mycetome are pushed back into the yolk where they degenerate and are finally dissolved. The newly formed cavity containing the symbionts is then divided by radial septa into chambers and thus the previously described stomach disc results. In the male host the symbionts remain in the stomach disc from the larva stage until its death, while in the female host a few days before the imago stage all the symbionts emigrate from the stomach disc and infect a certain region in the oviducts. This region later develops into the so-called ovarian ampules.

In the present work the effect of centrifugalisation upon the developing eggs of lice was studied. It was found that eggs which had been exposed to centrifugalisation between the second and the fifth day of development (1500–2000 revolutions per minute, radius 20 cm., for about 8 hours daily) yielded

5–10 per cent. of larvae with displaced stomach discs[1]. In some of them the mycetome was on the dorsal side of the stomach instead of in the usual position on the ventral mid-gut. In others this organ was definitely displaced towards the anal region or towards the lateral parts of the body (Figs. 2–6).

Sometimes the mycetome was actually divided into two parts. In such a case one part was always situated in the normal place, while the position of the other was very variable; in some, both parts were on the ventral side, in others one was on the ventral, the other on the dorsal side. More than two parts were never observed.

No other change than the displacement of the mycetome could be detected outwardly; histological examination, however, showed that all the displaced mycetomes, and only those, were entirely free from symbionts. The structure of the displaced mycetomes differed from normal in that the regular division into chambers was replaced by an irregular, more or less compact, massing of cells. The mycetome itself, as in normal organs, consisted of cells derived from the mid-gut, which were covered by an irregular layer of mesodermal elements.

Occasionally irregular spaces were observed inside these organs, filled with a granular mass, which consisted, most probably, of the degenerated symbionts; but intact micro-organisms were never found in these animals, either in the mycetomes or elsewhere. In other words the larvae with the displaced mycetomes were absolutely free of symbionts while the other larvae in which the stomach disc was not displaced had their normal symbiont population.

In former papers (Aschner, 1932; Aschner and Ries, 1933) we were able to show that extirpation of the stomach disc at the time when it was filled with symbionts caused grave deficiency symptoms, while the same operation performed after migration of the symbionts to the oviduct had no influence at all on the well-being of the louse. From these results it was concluded that it was not the operation, nor the lack of the mycetome, but rather the absence of symbionts that caused the observed deficiency symptoms.

The larvae which had been freed from their symbionts by centrifugalisation of the egg offered an opportunity to test the above conclusion. In the previous experiments we studied lice lacking both mycetome and symbionts, and lice without mycetome but with symbionts; in the present experiment it was possible to follow the fate of lice in which the mycetome was present and the symbionts absent.

The centrifugalised larvae, normal, as well as those free of symbionts, were kept together constantly in the same breeding cage, under absolutely identical conditions. At the beginning no difference was observed among the two groups. All larvae fed and developed normally until the fifth or sixth day. Then the symbiont-free larvae died suddenly. At this time first-stage larvae usually

[1] As no laboratory centrifuge which could be used for so long a time without interruption was available, the eggs were fixed in various positions to the inner side of the transmission wheel of a dynamo which worked that number of hours daily. The eggs did not suffer from this treatment and a large percentage hatched even when fixed to the wheel for the whole period of development.

moult into second-stage larvae, but there does not seem to be any connection between the moulting and the sudden death of the symbiont-free larvae. It is true that most of them died immediately before or during the moulting, but some of them succeeded in passing through this process, only to exhibit the same symptoms shortly afterwards. It would seem, therefore, that the death of the symbiont-free lice is related to a time interval rather than to the process of moulting.

These observations are convincing confirmation of the previous results obtained by an entirely different method. There, too, we demonstrated that after the elimination of the symbionts the *Pediculus* larvae can live normally for only about six days and die more or less suddenly after that time.

In cases where the mycetome was divided by centrifugalisation into two parts, the part situated in its normal place had its share of symbionts while the displaced part was free of them. In these larvae only a part of the symbionts was eliminated, and the condition created was the same as that obtained by partial extirpation of the mycetome. The quantity of symbionts in this case depends only upon the relative size of the two parts, the smaller the displaced part of the mycetome the smaller the number of symbionts eliminated. In cases of partially displaced mycetomes, the lice lived longer than those which were entirely sterile but never as long as normal lice. The results are, therefore, in complete accord with those obtained with larvae with a partially extirpated mycetome.

The reason for the sudden death of the symbiont-free larvae will be dealt with in another paper. Here we must deal with the problem of how the centrifugal force is able to displace the mycetome and why the symbionts disappear.

At first it was supposed that centrifugalisation forced the primary mycetome, while still floating in the yolk, inside the mid-gut, away from its normal place on the inner mid-gut wall. But histological examination of the displaced mycetomes did not support this assumption. In most cases there was no trace of a connection between the epithelium of the stomach and the corresponding cells of the mycetome; it seems, therefore, very improbable that the mycetome developed from the mid-gut epithelium at these places. Furthermore, this theory does not explain why the same centrifugal force should push one part of the mycetome to the dorsal and the other to the ventral side of the stomach. Nor does this assumption explain the fact that in cases of division of the stomach disc one part always remained in its normal place.

In order to explain these different facts it must be assumed that the centrifugal force affects the stomach disc and not the primary mycetome. In all cases the primary mycetome reached its normal destination on the inner ventral side of the mid-gut, where the stomach disc developed in the usual way. It is only after the formation of this organ that the centrifugal force is effective in totally or partially uprooting and displacing it. The final shape and location of this organ depends on the position of the egg during the centrifugalisation and the duration of the deforming effect of this process. As soon as the latter

ceases, the organ remains unchanged, is covered in the usual way with mesodermal cells and becomes fixed wherever it happens to be.

This explanation anticipates that the stomach disc in the embryo is of a quasi-plastic consistency. This probably does not hold true for the organ in the larval stage, but it is quite possible that in the process of formation its condition is different from that in the final stage. Indeed in examining a large number of centrifugalised larvae the "dripping" of the stomach disc can be seen in various intermediate stages. The typical drop-shaped mycetome, shown in Fig. 4, illustrates this process especially well. If the centrifugal force in this case had acted a little longer two rounded mycetomes would have resulted as shown in Fig. 5.

If the centrifugal force, due to the position of the egg, acted simultaneously in a lateral as well as in a dorsal direction, then the mycetome was shifted to its new position by floating around outside the wall and, in cases where only a part of the stomach disc was pushed away, larvae resulted with one mycetome on the dorsal side and another on the ventral side of the mid-gut (Fig. 2).

The peculiar fact that the centrifugal force affects the stomach disc only, while all the other organs, including the primary mycetome, are not disturbed in the slightest way, is most probably due to the granules already described which develop only in the cells of this organ. These granules, from their behaviour towards different acids, consist most probably of calcium oxalates. There is no doubt that the specific gravity of the cells harbouring these granules is greater than that of all the other tissues, and consequently these cells are more affected by the centrifugal force than the rest of the embryo.

The reason for the disappearance of the symbionts in the displaced mycetome is still unknown. We can only surmise that the uprooting of the mycetome from its original place causes certain changes and that the symbionts are unable to survive even for a short time under these changed conditions.

This clearly shows that the formation of the mycetome in the louse cannot be compared with the encapsulation of a focus of parasitic infection, as observed in higher animals. In the latter a disturbance of the existing equilibrium would as a rule cause a spreading of the parasites to the non-infected parts of the host. In the louse the mycetome seems to be rather a protecting pocket for the micro-organisms which are unable to live in any other part of the host.

That the mycetome could not be explained as a gall formation was shown by the former experiments on the extirpation of the stomach disc. We were able to demonstrate that the different mycetomes of the louse, the primary, the stomach disc, and, in the female, the ovarial ampules, were formed in the usual way without the possible irritating effect of the symbionts present (Figs. 8 and 9).

SUMMARY AND CONCLUSIONS

A simple method is described for eliminating the symbionts from the body louse during the egg stage, and thus producing symbiont-free larvae. The

behaviour of lice freed of their symbionts by this method is described and compared with that of lice freed of their symbionts by extirpation of the larval mycetome.

The complete accord of the results obtained by the different methods warrants the conclusion that the symbionts play an essential rôle in the life of the louse, so much so that without them death of the larvae results. It is, therefore, justifiable to consider this relationship true symbiosis.

REFERENCES

Aschner, M. (1932). Experimentelle Untersuchungen über die Symbiose der Kleiderlaus. *Naturwiss.* **20**, 501.

Aschner, M. and Ries, E. (1933). Das Verhalten der Kleiderlaus bei Ausschaltung ihrer Symbionten. *Z. f. Morph. u. Oekol. d. Tiere*, **26**, 529.

Buchner, P. (1920). Zur Kenntnis der Symbiose niederer pflanzlicher Mikro-Organismen mit Pediculiden. *Biol. Zbl.* **39**.

—— (1930). *Tier und Pflanze in Symbiose*. Berlin.

Ries, E. (1932). Die Symbiose der Läuse und Federlinge. *Z. f. Morph. u. Oekol. d. Tiere*, **20**, 233.

Sikora, H. (1919). Vorläufige Mitteilung über Myzetome bei Pediculiden. *Biol. Zbl.* **39**.

EXPLANATION OF PLATE XII

Fig. 1. Ventral view of a normal *Pediculus* larva showing the position and shape of the stomach disc.

Fig. 2. Ventral and dorsal view of a louse with a partially displaced stomach disc. Only a small portion of the mycetome remained on the ventral side (*a*), while the larger part is situated on the dorsal side (*b*).

Figs. 3–5. Different stages of a partial displacement of the stomach disc in three different lice. Ventral views.

Fig. 3. Beginning of the deformation of the stomach disc.

Fig. 4. Drop-shaped stomach disc. The displaced part is still connected with the remaining part of the mycetome.

Fig. 5. The displaced part is shown definitely separated from the remaining part.

Fig. 6. The whole stomach disc displaced toward the anal region.

Fig. 7. Sagittal section through the stomach disc of a normal louse embryo. The formation of the chambers with the symbionts clearly visible inside. In the yolk are visible the empty cells of the primary mycetome with the first signs of degeneration.

Fig. 8. Sagittal section through the stomach disc of a symbiont-free louse embryo where mother was freed of its symbionts in the third larval stage by extirpation of the stomach disc. Formation of the chambers is the same as in the normal embryo, but inside the chambers fragments of the primary mycetome and single yolk clumps are present instead of the symbionts.

Fig. 9. Section through the ovarian ampule of a *Pediculus* female freed of its symbionts in the third larval stage. In a normal female the symbionts would be found in the large group of cells where the ovarioles join together.

Fig. 10. Section through a displaced stomach disc (on the dorsal side of a larva). The irregular compact massing of cells instead of the regular chamber as in Fig. 7 is shown. There are no symbionts present.

Magnification: Figs. 1–6 ×25; Figs. 7–9 ×250; Fig. 10 ×300.

Preparations are stained with iron-haematoxylin.

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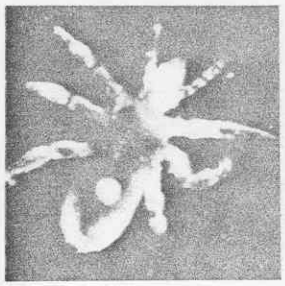

Fig. 1

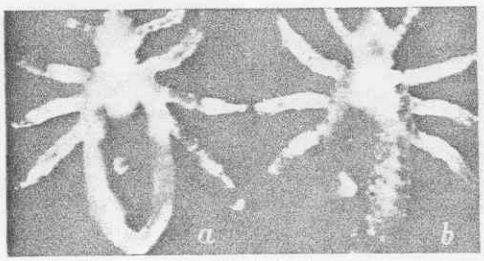

Fig. 2

Fig. 3

Fig. 4

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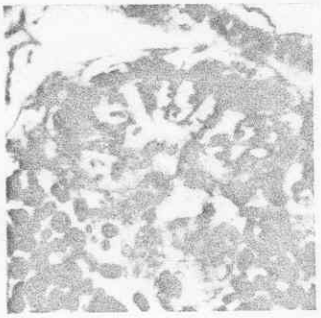

Fig. 7

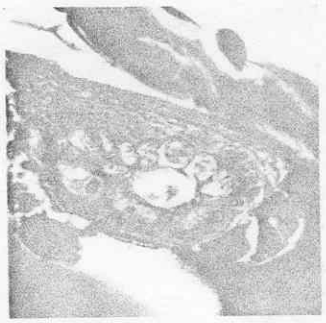

Fig. 8

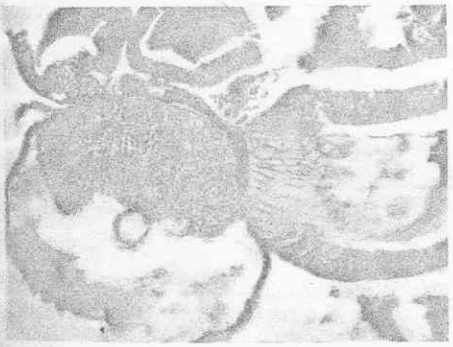

Fig. 9

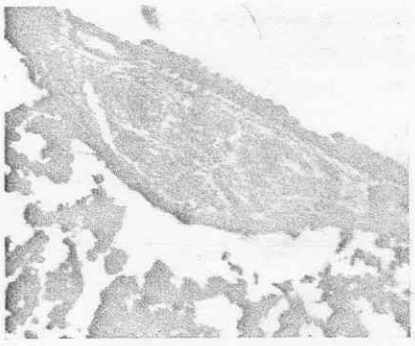

Fig. 10