An Electron Microscope Study of Bacteria in the Oocytes and Follicle Cells of *Blatta orientalis*

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With one plate (fig. 1)

SUMMARY

The bacteria present in the oocytes and follicle cells of *Blatta orientalis* Linn. were studied by means of electron microscopy. Bacteria are present in the follicle cells surrounding young oocytes, from where they invade the peripheral ooplasm. Each bacterium in an oocyte soon becomes surrounded by a system of membrane-like structures that form an outer covering. Later the bacteria congregate at the poles of the oocyte. An oval body was visible in one electron micrograph; this probably represents a stage in the life-history of the organism.

INTRODUCTION

SEVERAL of the earlier workers recorded the presence of 'bacteroid forms' in the oocytes of insects, including those of *Blatta* and *Periplaneta* (Wilson, 1928). In more recent years these organisms were seen by Nath and Mohan (1929) in the ooplasm of *Periplaneta americana* and by Gresson (1931) in the oocytes of *Blatta orientalis*. Bacteria present in the eggs of *P. americana* have been described and cultured by Glaser (1930). According to him the follicle cells become infected and from here the bacteria invade the oocytes where they 'form a closed layer around the periphery. Still later a definite congregation occurs at each pole' of an oocyte.

We observed bacteria in electron micrographs of young oocytes and follicle cells of *B. orientalis*. The following is a brief account of their distribution in these cells and of the outer covering that accompanies them. Little attempt is made to interpret their fine internal structure.

MATERIAL AND METHODS

The ovarioles of *B. orientalis* Linn. were speedily dissected and placed in 1% osmium tetroxide buffered with veronal to a pH of 7.4. They were embedded in a mixture of 85% n-butyl and 15% methyl methacrylate with 1% benzoyl peroxide as an activator. Polymerization was carried out at 60°C.

OBSERVATIONS

In electron micrographs of young oocytes of *B. orientalis* a layer of bacteria is visible in the peripheral ooplasm immediately beneath the follicle cells. The bacteria are surrounded by a system of much folded dense structures that...
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form an outer covering extending for some distance into the surrounding cytoplasm (fig. 1, A-C). The outer covering encloses all the bacteria in a layer and in section often appears to form a network or structure resembling a honeycomb. In older oocytes a concentration of bacteria was observed at each pole (fig. 1, D).

The outer covering appears to arise in the following manner. In young oocytes each bacterium becomes surrounded, wholly or in part, by a number of thin, dense, membrane-like structures that originate from a bacterium and spread out through the cytoplasm (fig. 1, C). As these structures increase in length, those originating from neighbouring bacteria come into contact with each other and give rise to the complex system described above. It would seem that the outer coverings of adjacent bacteria do not join but become associated in such a manner as to form a continuous system enclosing all, or nearly all, the bacteria in an oocyte. As an oocyte increases in size the system of outer coverings becomes more extensive and complex in nature. The exact relationship between the outer covering and a bacterium is not always obvious. In some of our electron micrographs, however, the outer covering is clearly shown to be continuous with the outer, dense, capsule-enclosing membrane of the bacterium (fig. 1, B).

The interior of the bacterial cell is often of high density; small clear spaces are, however, frequently present (fig. 1, A, B). In some of our electron micrographs the bacteria present a different appearance. In these cases dense material is present in the form of relatively large granules lying in a material of lesser density (fig. 1, C, E).

Bacteria of varying size were shown in electron micrographs of some of the follicle cells surrounding young oocytes, and small forms, devoid of outer coverings, were sometimes visible in the peripheral ooplasm (fig. 1, C, F). The latter, we assume, are stages in the invasion of oocytes from follicle cells. This invasion appears to continue for some time.

In one of our electron micrographs an oval body is present, devoid of an outer covering but closely surrounded by a membrane composed of 4 dense components separated by clear regions. It possesses a number of dense, granular structures (fig. 1, G). This, we believe, represents some phase in the life-history of the bacteria.

Fig. 1 (plate). A, part of follicle cell and young oocyte to show bacteria in peripheral ooplasm. fc, follicle cell; n, nucleus of follicle cell; oc, outer covering of bacteria.
B, five bacteria in ooplasm, at higher magnification.
C, to show layer of bacteria in peripheral ooplasm of young oocyte and smaller forms devoid of outer coverings. The boundary between the follicle cell and the oocyte is poorly defined.
sf, small forms.
D, older oocyte and part of follicle cell and its nucleus, to show concentration of bacteria at pole of oocyte.
E, bacteria in ooplasm.
F, part of young oocyte and follicle cell, to show a group of bacteria of varying sizes in follicle cell and small forms in oocyte. sf, small forms.
G, oval body in ooplasm. m, outer membrane.
FIG. 1

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DISCUSSION

The bacteria present in the oocytes of cockroaches, as far as we are aware, have not previously been studied with the aid of electron microscopy. In a recent contribution Nath, Gupta, and Lal (1958) conclude that the structures present in the oocytes of *P. americana* and described by other workers as bacteria are centres of lipid synthesis. They state that histochemical tests show that the bacteria ‘contain phospholipid mixed with free fatty acid’, and that later, when the synthesis of lipids is complete, they are negative to lipid tests. The evidence from electron microscopy shows that the bodies present at the periphery of the oocytes of *B. orientalis* are indeed bacteria, and supports Glaser’s conclusions that the bacteria migrate from the follicle cells to the peripheral ooplasm, and later congregate at the poles of an oocyte.

The bacteria shown in our electron micrographs seem to possess a capsule-enclosing membrane, a capsule space, and a cell-wall similar to those recently recorded by Chapman, Hanks, and Wallace (1959) in *Mycobacterium leprae-murium*. The oval body visible in one of our photographs appears to resemble the stable L-form of *Proteus vulgaris* described by Thorsson and Weibull (1958), except that the former is surrounded by a membrane possessing 4 dense components, while the latter is enclosed by a two-layered structure.

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