uct was filtered and then washed, first with 10-percent sodium bicarbonate solution and then with water. After crystallization from a 50–50 mixture of ethanol and trichloroethylene, followed by recrystallization from ethanol, a 140-g yield (19-percent theory) was obtained; melting point, 179.8° to 180.6°C (corrected). Analytic calculated for $C_{14}H_7Br_7$: Br, 76.15 percent; found: Br 76.87 percent.

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References and Notes

1. This paper represents part of a thesis submitted by A. O. Geiszler in partial fulfillment of the requirements for the M.S. degree at the North Dakota Agricultural College.
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Intracellular Symbiosis in Cockroaches: II. Mitotic Division of Mycetocytes

In part I of this series, we describe the histology, slow growth, and poor reproductive performance of aposymbiotic German cockroach nymphs (*Blattella germanica* L.) (*1*). These were produced by continuously feeding their parents Aureomycin, an antibiotic that prevents the ovarial transmission of the symbiotes (bacteroids). In connection with that work, stained serial sections of a few normal control roaches showed various stages of mitosis among the mycetocytes (the symbiote-containing cells of the fat body, Fig. 1.) Figures 4 and 5 are photomicrographs of representative dividing mycetocytes. Such cells arrested our attention (*2*), because the failure of earlier authors to find mitosis had suggested that the mycetocytes increase by amitosis during nymphal growth (*3*).

The division of the mycetocytes was synchronized so that numerous mitotic figures were distributed throughout the entire abdomen of all nymphs showing mitoses. In most nymphs, no divisions were seen. Bursts of mitotic activity are known in other organisms, so we made a series of preparations of nymphs of known ages to see whether the bursts were correlated with the molting cycle.

Under our rearing conditions, nymphs molt approximately every 10 or 11 days, passing through six molts and maturing at about 60 to 65 days. A total of 80 nymphs, ranging from newly hatched to 40 days, most of them in the second instar, were sectioned and stained. All of the sections of the abdomens were inspected. Counts were made of nymphs with the most easily recognizable stages —that is, metaphase and anaphase. A total of 14 nymphs showed mitotic mycetocytes on the 9th, 16th, 18th, 20th, 30th, and 40th days. In the second instar, of 14 nymphs between the ages of 11 to 14 days, inclusively, there were no mitoses; while of 24 nymphs between the ages of 16 to 20 days, inclusively, there were 10 with mitoses.

It was clear that mitosis occurs in bursts in the latter half of the instar. Frequently mitosis in the mycetocytes was accompanied by mitosis in the mid-gut epithelium and in other cells of the fat body, either urate- or fat-cells (indistinguishable in young nymphs). Divisions were not noticed in the epidermis. Undoubtedly mitosis has escaped previous detection because it occurs in nymphs of an age not usually studied.

Mitotic processes were also seen in mycetocytes and other fat-body cells in nymphs of the wood roach, *Parcoblatta pennsylvanica* (DeGeer), which had been collected while they were hibernating in February and kept active in the laboratory until they were sacrificed in June.

Mycetocytes differentiate in aposymbiotic embryos—that is, in embryos developed free of bacteroids. These "empty mycetocytes" are present as clusters of cells with large nuclei and small amounts of fibrous-appearing cytoplasm devoid of bacteroids (Fig. 2). There are 10 clusters, one in each lateral half of abdominal segments 2 through 6. The positions of the empty cells are the same as the positions of the mycetocytes, which in normal embryos are formed in anticipation of their infection by the bacteroids (*3*). The empty mycetocytes were never seen dividing. Seemingly, without the pressure of a growing population of bacteroids, the mycetocytes lack the stimulus to divide and separate; thus they remain in clusters and at their original number.

An interesting contrast to the empty mycetocytes was the behavior of certain bacteroid-containing mycetocytes that grew to gigantic proportions in other experimental roaches. Serial sections of various developmental stages following short Aureomycin treatment (*1*) showed that some individuals were deficient only in bacteroids; that is, they were semi-aposymbiotic. Only a small fraction of the normal complement of bacteroids had been transmitted to the nymphs; these bacteroids were present as subnormal populations in the cytoplasm of just a few mycetocytes. The few mycetocytes eventually grew, by the end of 9 wk, to about 200 times the normal volume instead of dividing (Fig. 3). Perhaps this

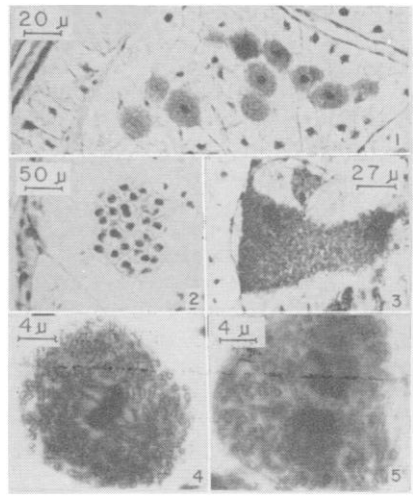

Fig. 1. Part of a cross section of a 2-wk old normal nymph showing distribution and size of mycetocytes. Fig. 2. Part of a cross section of a 2-wk old aposymbiotic nymph showing one cluster of empty mycetocytes. Fig. 3. Section through a giant mycetocyte in a 9-wk old retarded nymph. Figs. 4 and 5. Photomicrographs of sections through mycetocytes whose nuclei are undergoing mitosis. The bacteroids are so numerous that the spindle fibers are almost obscured. The bacteroids look hollow as a result of the fixing and straining procedure. All material was fixed in Carnoy's fluid, sectioned at 10 μ, and stained with Delafield's hematoxylin, counterstained with erythrosin in clove oil.

phenomenon may be accounted for by a postulated lack of a dividing stimulus, correlated with an inadequate bacteroid population when the cells were young and ordinarily would have undergone division. There seemed to be no check on the multiplication of the bacteroids.

We conclude that mitosis is the normal means of division in mycetocytes and that it occurs in bursts near the end of each instar. If only a few bacteroids are present originally, the bacteroids increase but the mycetocytes do not divide, whereas if bacteroids are absent the mycetocytes remain small, in clusters, and do not divide.

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