Oöcyst-Like Bodies on the Midgut of *Stilbometopa impressa* (Bigot) (Diptera: Hippoboscidae)

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DURING the past several years investigative work has been in progress on the fly-bite transmission of *Haemoproteus lophortyx* (O'Roke) by the quail louse fly, *Stilbometopa impressa* (Bigot). Although *S. impressa* has been found to be a vector of *H. lophortyx* by fly-bite transmission (*1*), an exhaustive search for the oöcysts on the midguts of the flies has proved futile. The presence, however, of peculiar, oöcyst-like nodules on the midguts of *S. impressa* has been revealed (Figs. 1–3).

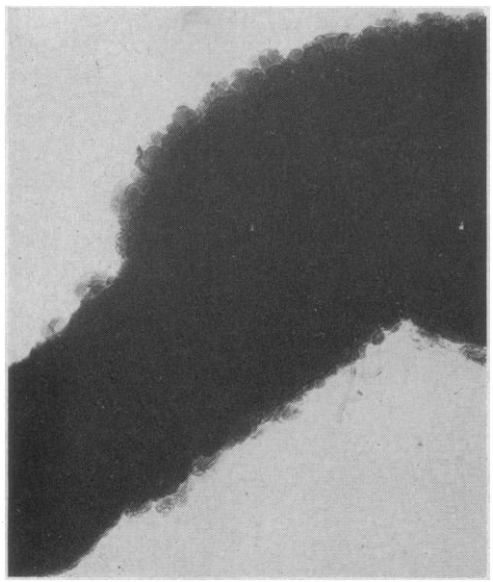

FIG. 1. The midgut of an unfed *S. impressa* (Bigot) showing nodules. × 50.

The oöcyst-like nodules are so similar in appearance to the oöcysts of the Plasmodiidae that one might be tempted to regard them as oöcysts of *H. lophortyx* were it not for the fact that they appeared in more than 85% of the dissected flies, regardless of the age, sex, and other conditions of the flies. This, plus additional evidence presented below, makes it seem more reasonable to interpret these nodules as a new type of mycetome containing symbiotes.

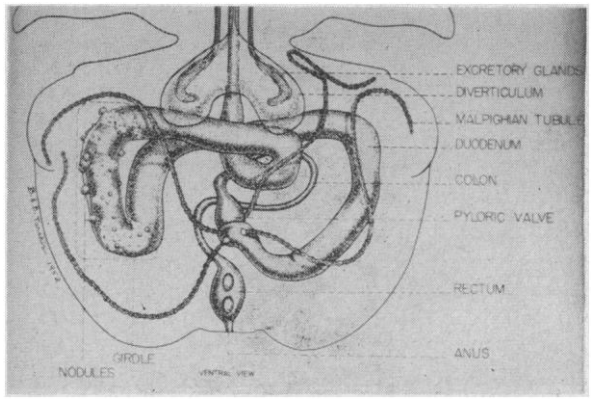

FIG. 2. Drawing of the midgut of *S. impressa* (Bigot) showing location of nodules and opaque white patches.

The oöcyst-like nodules were first discovered about 2 yr ago on the midguts of flies fed on *Haemoproteus*-positive Valley California Quail, *Lophortyx californica californica* (Shaw and Nodder). Subsequent search for these nodules revealed that flies newly emerged from their pupal cases and flies fed on *Haemoproteus*-negative quail also have nodules on the midguts. Newly emerged flies that have not had a blood meal generally have many more nodules than flies that have engorged several times.

Closer examination shows that the nodules are not directly attached to the normal midgut wall, but to heavy, opaque white, irregular patches which girdle the widened, sausage-shaped portion of the midgut (Figs. 2, 3). The location of these opaque white patches and the nodules is unvarying. The nodules are actually outcroppings of the patches and often appear as buds projecting from them (Fig. 4). As the gut is rolled over successive groups of various sized nodules can be observed.

A total of 193 freshly killed flies of both sexes of *S. impressa* were dissected and though 19 midguts were found devoid of all nodular bodies the opaque white patches were present in all 193 flies. The midguts without the nodules were found in flies taken from trapped quail and from flies that had lived on laboratory quail for several months. The remaining

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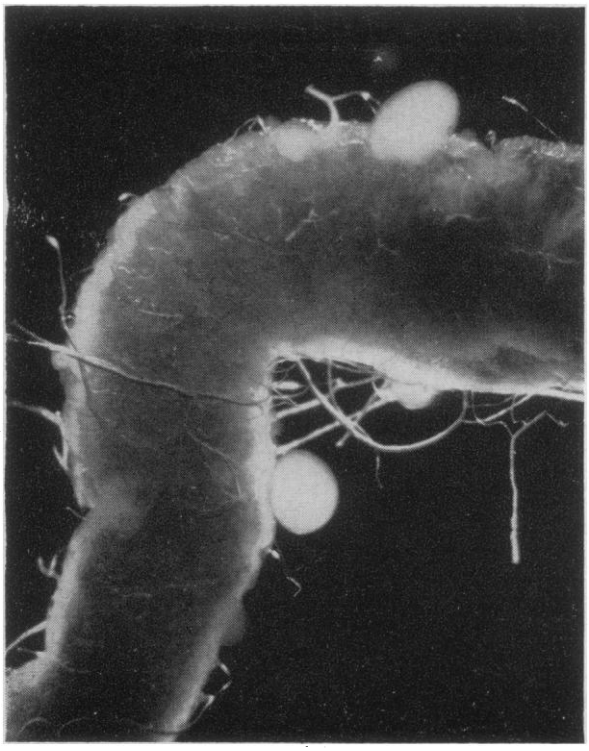

FIG. 3. The midgut of a fed *S. impressa* (Bigot) showing nodules of varying sizes. × 55.

174 midguts examined showed nodules of varying numbers and dimensions. These latter midguts were found in flies taken from trapped quail, laboratory reared flies fed on both infected and noninfected quail, and newly emerged unfed flies (Table 1).

The nodules are subglobular to spherical in shape and vary in size from 3.28 to 217.6 microns at the greatest diameter. The nodules occur singly and in groups of two or more. They are opaque white with sharply defined outlines and their contents are densely granular. The unbroken nodules appear to be filled

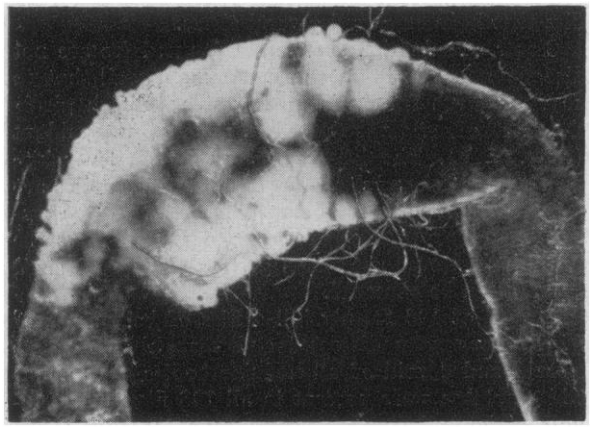

FIG. 4. The midgut of an unfed *S. impressa* (Bigot) showing opaque white, irregular patches. Note nodules projecting from patches. × 55.

TABLE 1

TABULATION OF *Stilbometopa impressa* (BIGOT) DISSECTED AND FOUND TO HAVE NODULES ON THE MIDGUTS

Number of fed flies found to have nodules on midguts				Number of flies taken from trapped quail found to have nodules on midguts		Number of newly emerged flies, not having had a blood meal, found to have nodules on midguts	
Fed flies from infected quail		Fed flies from non-infected quail		From infected and non-infected quail			
♂	♀	♂	♀	♂	♀	♂	♀
36	30	13	12	14	16	25	28

with roundish particles. When the nodules are broken, fine sandlike masses can be seen flowing from them

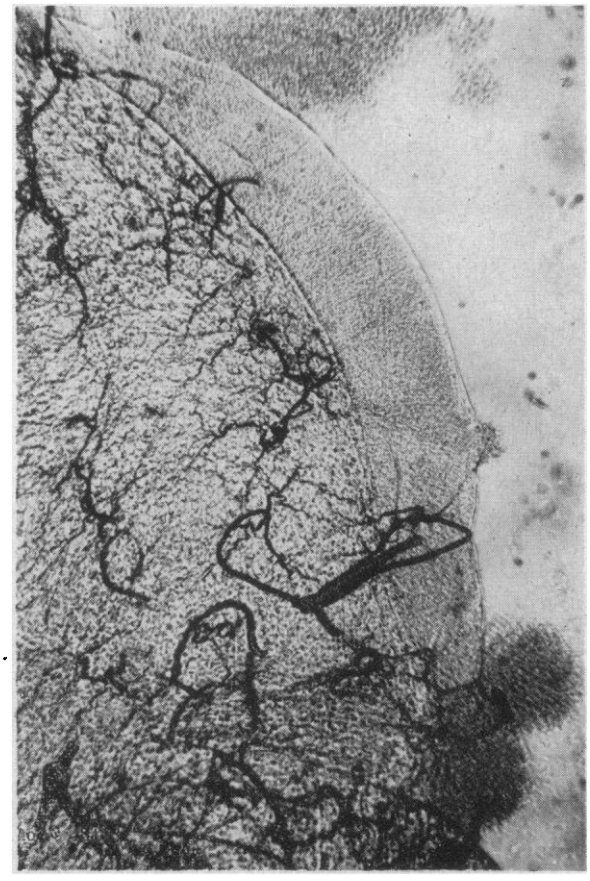

FIG. 5. An enlarged portion of the midgut of *S. impressa* (Bigot) showing a portion of a large opaque patch (the narrow granular band) attached to the midgut. Note the two nodules (bottom, center) attached to the opaque patch. The granular mass (top, center) is a large group of microorganisms escaping from the perforated edge of the patch. × 90.

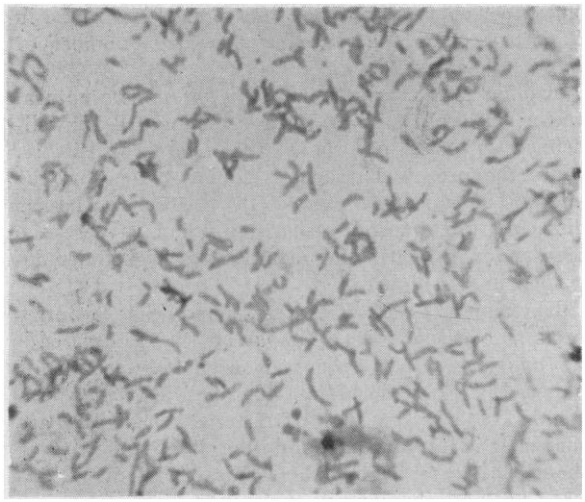

FIG. 6. A mass of stained microorganisms obtained from a nodule on the midgut of an unfed *S. impressa* (Bigot). × 1200.

(Fig. 5). This can be observed under the low and high dry objectives of the compound microscope.

The granular content of the nodules is revealed as a multitude of individual rod- or sausage-shaped structures which appear to be analogous to certain Gram-negative, bacteriumlike microorganisms found in some species of *Glossina* and in such Pupipara as *Lipoptena, Hippobosca, Ornithomyia,* and *Nycteribia* (*1–4, 14*). The microorganisms of *S. impressa* have a length of from 3.49 to 4.62 microns and a width of from 0.66 to 0.99 micron. The rod- or sausage-shaped microorganisms occur singly, in pairs, and in chains (Figs. 6–8). In the chained arrangement they sometimes form an S or Y.

All the microorganisms from within the nodules and the opaque white patches have been identical in appearance, size, and shape. The age, sex, and other conditions of the fly seem to cause no variation in the microorganisms. They are the same whether taken from the midguts of newly emerged flies that have not had a blood meal, from flies having fed for a period of several weeks to months on *Haemoproteus*-positive quail, or from flies fed for varying time periods on *Haemoproteus*-negative quail.

The microorganisms within the nodules and opaque white patches on the midguts of *S. impressa* can be stained with either Giemsa's or Gram's stain and are Gram-negative.

Attempts to grow these organisms on Locke-semisolid, Noeller-blood agar, and peptone-gelatin blood media were unsuccessful.

Serial sections of the midguts of *S. impressa* containing the nodular masses are being prepared for study at a later date.

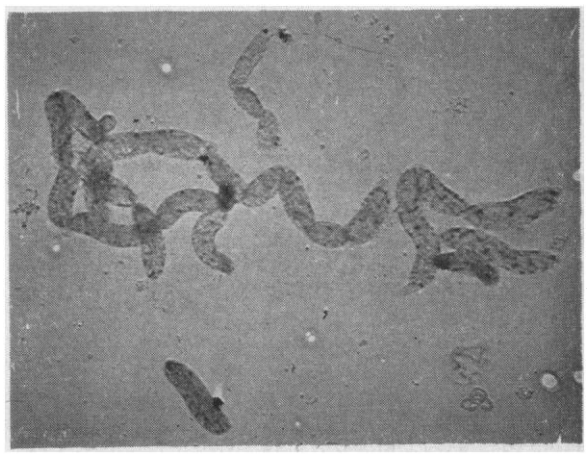

FIG. 7. Electron micrograph of microorganisms taken from nodules on midgut of an unfed *S. impressa* (Bigot). × 5320.

It has been reported in the literature that symbiotes of other Pupipara were found on the anterior portions of the midguts where the epithelium had become so developed as to form definite whitish collars or rings

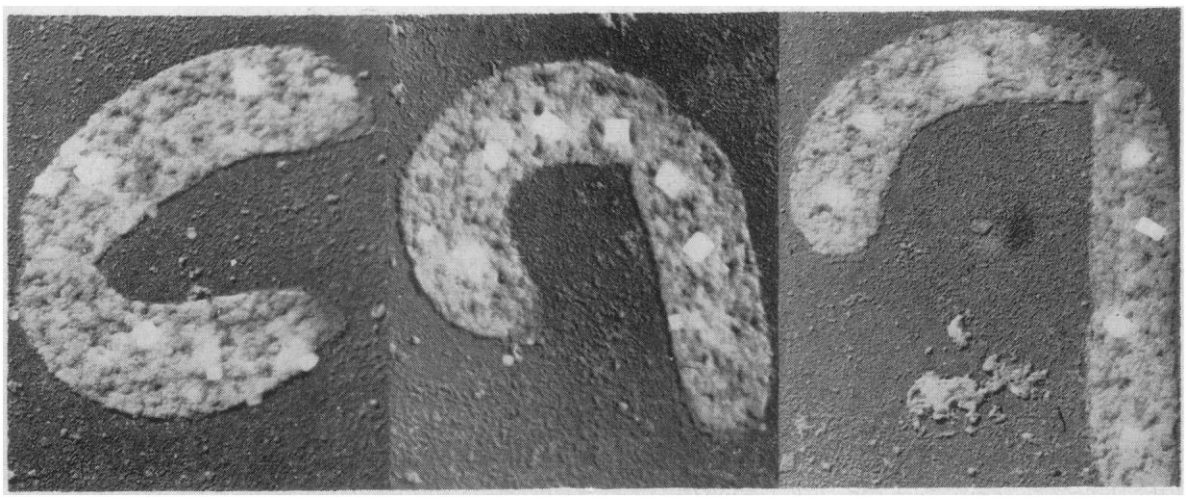

FIG. 8. Electron micrographs of individual microorganisms taken from nodule on the midgut of an unfed *S. impressa* (Bigot). The white spots are saline crystals. × 15,500.

(the mycetomes). There seems to be enough similarity between the opaque white patches found on the midguts of *S. impressa* and the ringlike mycetomes found on the midguts of other Pupipara to conclude that they are essentially the same. The writer, however, has not been able to find descriptions in the literature of any symbiote-filled nodular bodies such as he has been able to find budding off the opaque white patches on the midguts of *S. impressa* as shown in Figs. 3 and 4.

Recent investigative work on the biology of *Ornithomyia fringillina* (Curtis) and *Ornithoica vicina* (Walker), Hippoboscidae of the white crowned sparrow (*5*), has revealed nodular bodies on the midguts of these flies which appear to be similar to those of *S. impressa*. The nodules have also been found to contain microorganisms analogous to those found in the nodules of *S. impressa*.

The Sergents (*6*) demonstrated experimentally the transmission of *Haemoproteus columbae* Kruse of the domestic pigeon by the bite of *Pseudolynchia canariensis* (Macquart). Adie (*7, 8*) later described as oöcysts of *H. columbae* nodular projections which she found on the midgut of *P. canariensis* on the 4th day after the fly had an infective blood meal. The young cysts measured from 7.2 to 8.2 microns. She further stated that the mature cysts were approximately 36 microns in diameter and that they were filled with pigment. She described the pigment as consisting of "roundish (not rod-shaped), particles." As stated above, the microorganisms seen within the nodules on the midguts of *S. impressa* also appear roundish in the intact nodules. However, once they escape from the broken or ruptured nodules they assume the rod- or sausage-shaped form as shown in Fig. 6.

O'Roke (*9*) incriminated *Lynchia hirsuta* (Ferris) as a vector of *H. lophortyx* by finding what he regarded as oöcysts on the midguts of flies taken from infected quail. The present author has been unable to find either oöcysts or nodules, such as he has found in *S. impressa*, on the midguts of a large number of *L. hirsuta* dissected.

Kadner (*10*) incriminated *S. impressa* as a natural vector of quail malaria when he found 10–15 well-defined supposed oöcysts on the midgut of one fly. Since the present author has found nodules on the midguts of more than 85% of the *S. impressa* he has dissected, the question comes to mind, "Were the oöcysts found by Kadner identical to the nodules described in this paper?"

Kartman (*11*) states that he found what he interprets as oöcysts of *H. columbae* on the midguts of 9 *P. canariensis* taken from pigeons in Hawaii. In his paper, Fig. 3 on the bottom of page 131, two photographs of oöcysts taken from *P. canariensis* are shown. It is interesting to note the great similarity of these oöcysts to the nodular bodies found in *S. impressa* as described and pictured in this paper.

Lastra-Galler (*12*) and Coatney (*13*), while working with *P. canariensis* as a vector of *H. columbae*, were unable to find any oöcysts on the dissected and sectioned midguts of these flies.

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Microorganisms or Mitochondria?

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UNDER THE TITLE "Observations on the Supposed Symbiotic Microorganisms of Aphids" (Science, **115**, 459 [1952]), U. N. Lanham has expressed the curious opinion that the particles contained in the mycetomes of aphids are not symbiotic bacteria but cell particulates. His paper was criticized by Trager (Science, **116**, 332 [1952]) in a communication entitled "Mitochondria or Microorganisms?" to which Lanham replied in the same number of Science. He pointed out with respect to the particles in question, ". . . the hypothesis that they are symbiotic microorganisms seems to be a more unlikely, difficult, and complex one than the hypothesis that they are intracellular particulates of the nature of mitochondria." And Lanham added: "The aphid particles are said to have been grown *in vitro*. All such claims need verification. Some reportedly successful experiments involve very simple techniques and can easily be repeated. My own attempts to cultivate them, including the use of hanging drop tech-