(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?

Jaroslav Peklo

Prague XII, Chodska 3II, Czechoslovakia

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-

niques where individual particles could be observed, were not successful." In this incident, Lanham sees another proof of his idea that mycetome inhabitants of aphids are mitochondria. In the following article I accept Lanham's imperative appeal for the verification and ask for permission again to report my own results on the same matter, published in 1916 (*1*) in the Czech language (*2, 3*).

Plate I, 3 represents a cross section through a part of the body of *Phylloxera vastatrix* Pl. (Chermesini), which lives on the root nodules of grapevines. Around the eggs (one of them has fallen out) there is a wreath of mycetocytes, to the number of 5 or 9 respectively, containing Lanham's particles (300 ×). They were fixed according to Juel and are to a large extent poorly stained (iron-alum 24 hr, Heidenhain 24 hr; counterstained with aniline-safranin) because their protein contents had already been for the most part consumed and exhausted (by the eggs?). On the other hand, in the adjacent egg, the yeast *Torula* (*Torulopsis, Cryptococcus,* or *Sulcia*) can be seen. I suggest the name *Sulcia* owing to many cases of the identical, but very variable organisms (in my opinion not belonging to Saccharomyces) described by Šulc in his papers on Homoptera (*Ptyelus,* [*4*]; *Oliarius,* Fulgoridae; *Margarodes,* [*5*], Coccidae, [*6*]), and which I have described and isolated in numerous samples from the fat body in the larvae of different insects (*7–11*). Tóth (*12,* Fig. 12) has also pictured a ring of mycetocytes, probably not exhausted, around one egg of *Pemphigus* showing deeply stained organisms (*Sulcia*). By contrast, in the same figure, the mycetocyte particles are only faintly stained. Nevertheless, they fill the whole space of the mycetocytes and clearly reveal their bacterial form. In the above-mentioned preparation of the mycetocytes of *Phylloxera* there are, apart from remaining particles, bacterial cells with protein contents, and numerous more or less empty hypertrophied cells reminiscent of the bacteroids of the Leguminosae; nevertheless, they are by no means so clearly differentiated as the former. The normal particles measure 1.8 μ. On the other hand, in the mycetocytes (Plate II, 4) and embryos (Plate II, 5) of *Schizoneura lanigera* H. the inclusions are much larger, 2.7–3.6 μ, and they entirely fill the cell. Also, Plate I, 1, representing an embryo of *Schizoneura lanigera,* and Plate I, 2, which shows the mass of symbiotes squeezed out from a young insect should be noted. Were these mitochondria, their size would surely be astonishing! They also were fixed according to Juel, strongly stained with Heidenhain's safranin and, in the same egg, were slowly but abundantly dissolved and digested. Likewise, in samples of *Lecanium persicae* from peaches and plums, I found very small mycetomes filled with extremely fine particles (bacteria), very weakly stained in contrast to numerous black sulciae round about, and in other places black, long-shaped, yeast-like sybiotes. Paillot (*13*) stained, after Giemsa, "microorganismes symbiotiques," prepared from the aphid bodies ("de frottis de Puceron dilacere"), including the mycetomes of *Schizon. lanigera,* of *Tetraneura ulmi, Macrosiphum tanaceti,* the black aphid of plantain, *Aphis atriplicis, Macrosiphum jaceae, Aphis rumicis, Chaetophorus aceris,*

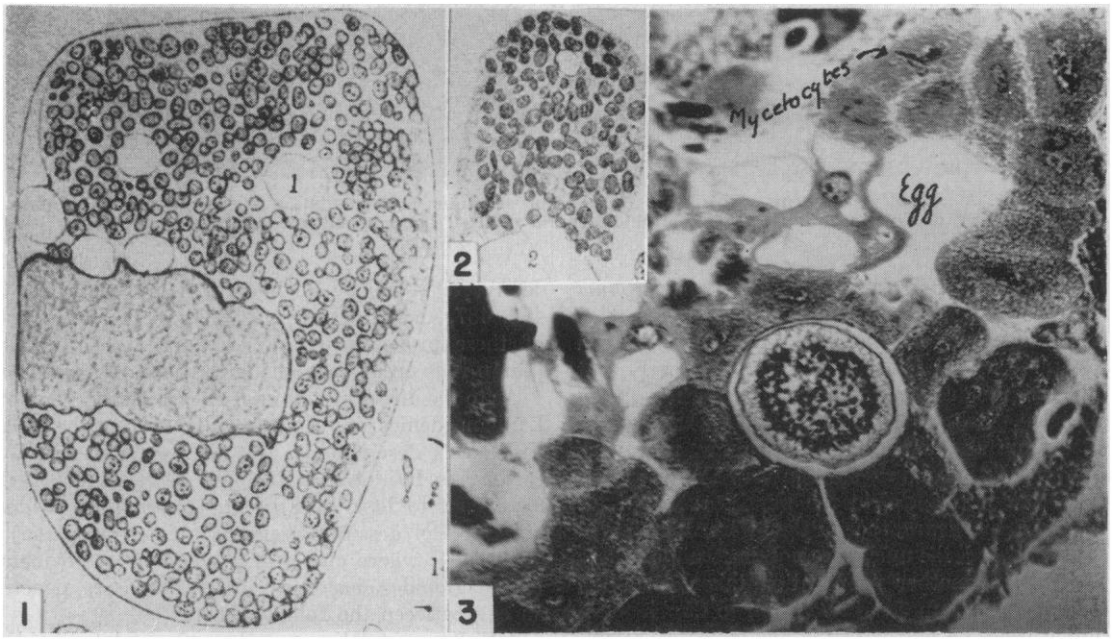

PLATE I. 1. An embryo of *Schizoneura lanigera* H. sketched *in vivo*; filled with large bacterial symbiotes (400 ×). 2. A mass of symbiotes squeezed out from a young *Schizoneura* (400 ×). 3. A cross section through *Phylloxera vastatrix* Pl. living on a root nodule of grapevine (300 ×). Around the eggs are the mycetocytes. An egg has fallen out during the preparation; another one contains numerous torulae (sulciae), stained deep black (Heidenhain). The mycetocytes are almost colorless.

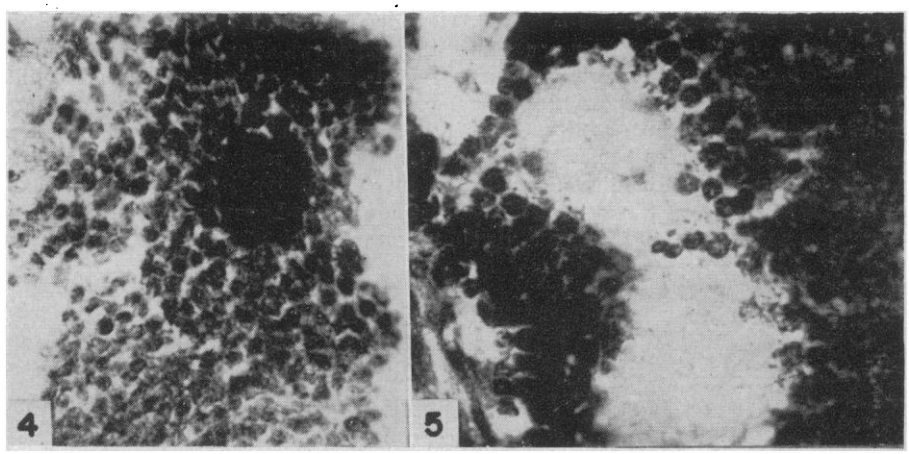

PLATE II. 4. A mycetocyte of *Schizoneura* Heidenhain-aniline-safranin; in the middle a nucleus (830 ×). 5. A cross section through an egg of *Schizoneura*. Heidenhain-aniline-safranin (830 ×). Large symbiotes, giant cocci; in the middle, they are digested.

C. lyropictus, Aphis forbesi, and *Rhopalosiphum ribis*. In general, the form of the bodies was that of our well-known globular particles. Consequently, when Lanham asserts: "It should be noted, however, that when techniques are available which bear critically on the problem, such as the acid-Giemsa technique, the evidence from staining is, at least so far, negative," Paillot's results show just the contrary. With regard to the question to nuclei in the particles: in my inclusions of *Schizoneura,* though they were well-stained and differentiated, the nuclei are not discernible (Plate II, 5). But in another preparation taken from the same insect there appears, in addition to the bacterial symbiotic nests, an egg with the symbiotes of a similar form, also well-stained, but digested in the form of large plasma masses in which some cells of the endophyte remain, showing red nuclei: the fungus *Sulcia,* in contrast to the previously mentioned bacteria. A glance at the pictures by Paillot (*13*), pp. 336, 358, 361, 362, 364, 365, 366, 373, 374—"coupes de myceto"—and by Buchner (*14*), pp. 460, 475, 481, 483, 487, and others, reveals that in the mycetocytes of these insects more or less large cocci or short, plump rods occur, much as in the case of *Azotobacter.*

As to the direct infection of eggs with the symbiotes, Lanham denies it. And in this respect he recalls Uichanco's descriptions. But these are incorrect, according to Paillot and Buchner. Nevertheless, it suffices, e.g., to look at Fig. 11 (*Pemphigus filaginis*; hinterer Pol des Wintereis, Symbioten Invasion) of Tóth (*15*), where one clearly sees how the symbiotes (small globules, bacteria) in great masses penetrate an egg between the follicle cells.

Also ". . . jumbling together of unrelated evidence from diverse groups of insects," disregarded by Lanham, can be worth while. Thus in *Doryphora decemlineata* L., I have distinctly stained, after Heidenhain, the masses of small sulciae occupying young cells of the fat body. Later on, in the older ones, they are larger, swollen, faintly stained, and digested. Bac-

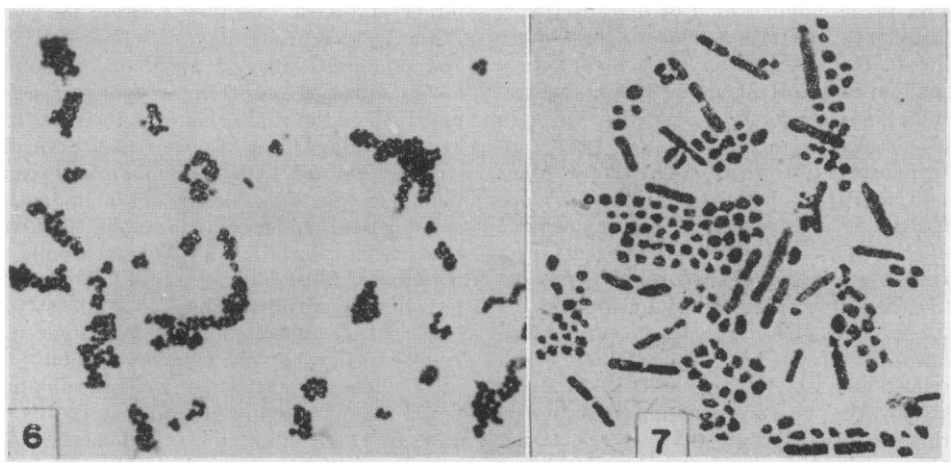

PLATE III. Isolates from *Schizoneura,* the same strain. 6. *Phaseolus* + sucrose-agar, pure culture 14 days old. Karbolfuchsin-Král 20 min. The cocci are very small (800 ×). 7. *Phaseolus* + sucrose solution, 24 hr old. Löhnis-Victoria blue, Gram. Cocci and rods (100 ×).

terial masses in the fat body of young larvae of *Apis mellifica* L. were stained with Heidenhain's tanninstrong aniline-safranin. After digestion, their remnants appear blue with Giemsa. In the gut of the same larvae there are localized great masses of large bacteria, stained a pink rose with Giemsa, and in digested masses. In the nuclei of caterpillars of *Lymantria monacha* L. I am able to differentiate with Giemsa the chromatin (red) from the very small sulciae (blue), on their way to digestion, and so on. The Feulgen reaction is convenient for the investigation of more important phenomena than are Lanham's mycetome particles. To sum up: Lanham's assumption, that on his inability to stain mycetome particles in some stages of development disproves their bacterial nature, is not worth considering.

In 1914, I started my cultivation experiments with an aphid that lives on the leaves of *Acer platanoides*. The following culture media were used: bouillon or white bean decoction + 6% sucrose (this disaccharide is produced in great quantities in the leaves of maples), mostly diluted + $CaCO_3$; tap water + sucrose + tricalcium phosphate; tap water + glycogen; asparagine. Turbidities soon appeared in the liquid media; on agar there were films white, yellow, pink, dry, slimy, tough, scabby pellicles. There were isolated 50 strains from the same species of the aphid. The trials, of course, took time. Also, Tóth (*16*) speaks of the "mühevolle Arbeit mit der Isolation der Aphidensymbionten." Unfortunately, he gives only superficial descriptions of his cultures, and without any bacterial morphology. My own pure cultures consisted of very small cocci. Very often they showed great variability. Thus, there appeared among the cocci short rods passing into cocci; mostly in young cultures, which by repeated plating, were revealed to be entirely pure. In other cases there were found more or less regular sarcinae (better termed: morulae). All combinations of these forms occurred. For example, in glycogen + sucrose + water a coccus produced small, oval, monociliate, motile cells, and, later on, sarcine clusters. Occurrences of short or longer rods in coccus cultures were regular features in the pure cultures. By careful examination, strains consisting both of cocci-rods and sarcine-like clusters were repeatedly discovered; on agar, the latter produced even large—I might say, giant—cocci, in big, slimy packets—as they are described in the *Azotobacteriaceae*. *Phylloxera* (30° C), tap water + sucrose + glycogen, bouillon, offered similar isolates. When young larvae of *Lecanium persicae*, especially in bouillon + 1% amygdalin, were used, there appeared a small coccus, with later discovered minute mycetomes. Since these studies were tiresome, they were later on replaced with large, pregnant bodies of *Schizoneura lanigera*. After sterilization of the insects with alcohol and flame, the bodies were sucked out with very fine capillaries; or by means of extremely careful pricking and subsequent squeezing, the isolation mass was limited to the smallest possible area and crushed between splinters of cover glasses in the solutions or placed on agar dabbed with distilled

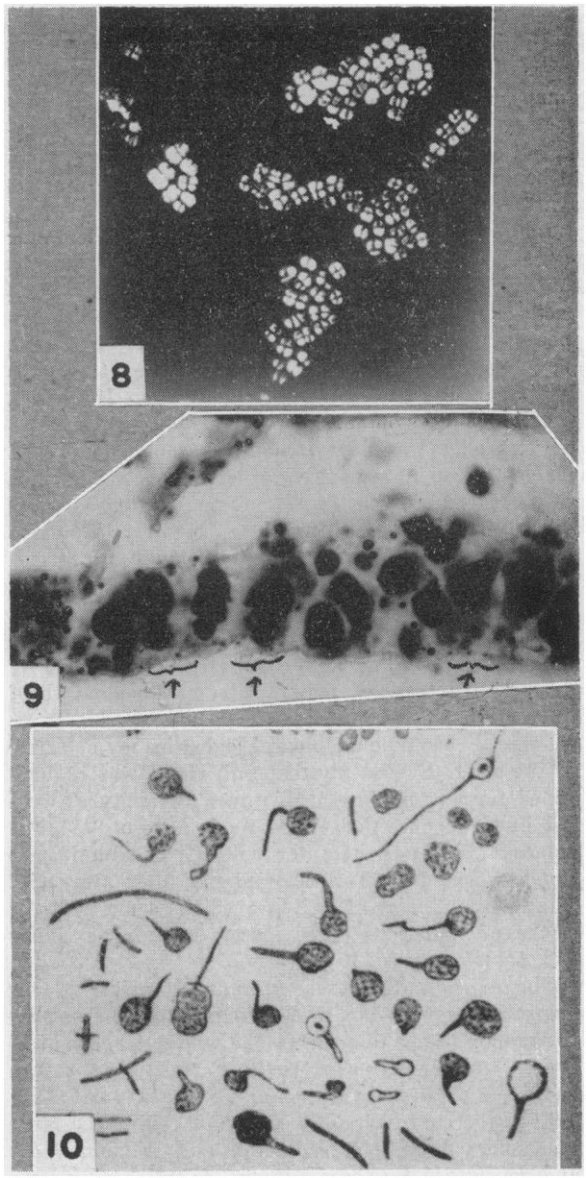

PLATE IV. 8. Same as 6 and 7. Bouillon + sucrose agar. Photo *in vivo* by means of ultraviolet light. Negative from a diapositive. Sarcinae-clusters (500 ×). 9. A longitudinal section through mycetome from a very young caterpillar of *Lymantria monacha* L. Heidenhain. Large hypertrophies of the symbiotic *Sulcia* and very small dot-like ones, suggestive of chondriosomes (400 ×). 10. Germinating giant cocci from an egg of *Schizoneura* in a hanging drop. Different media, *in vivo* (1000 ×).

water. There again appeared cocci the relation of which to the egg (Plate I, 1 and 2) or mycetome (Plate II, 4) inhabitants was proved especially by their sometimes larger size and by their schizosaccharomyces-like fission. Apart from this, and still more convincing, are the cycles consisting of all three forms in one and the same strain: cocci (originally small), Plate III, 6; cocci + rods, Plate III, 7; sarcinae, Plate IV, 8. Finally, very large, truly giant

cocci are formed, e.g., on the ends of agar slopes, and in no way different from the big inclusions in the eggs. Plate IV, 10 demonstrates a hanging drop with giant cocci prepared from a single egg, sprouting into bacterial rods. By means of phloridzin, a bark glycoside of apple trees, a rod bacterium was isolated which in bouillon produced a red color very similar to that synthesized in the body of the woolly louse. Probably, it did not belong to the chief flora of the mycetomes or eggs. From these surprising similarities in form of the *Azotobacter chroococcum,* I do not doubt that the bacterial symbiotes of *Schizoneura lanigera* as well as those of other aphids belong to this genus. Of course, they must not be mistaken for *Sulcia* when it appears in aphids. There were isolated more than 10 strains of *Azotobacter* symbiotes from *Schizoneura.* The pure cultures of the aphid symbiotes grew adequately on media poor in nitrogen. Mixed cultures consisting of the cocci secured from *Phylloxera* or *Schizoneura* grew still better, even quite well; the same slimy bacterium alone did not grow prolifically, but in the mixed cultures with the symbiotes there appeared characteristic, even vigorous, membranes resembling mixed cultures of *Radiobacter-Azotobacter autorum.* A bacterium reminding one of *Radiobacter* and already described by Krassiltchick was isolated from the chylus-stomach of an aphid.

I was at that time unable to kjeldahlize my cultures, having no H_2SO_4 at my disposal, for it was requisitioned for war purposes. Moreover, the cultures were lost in those unsettled times. As is known (*16*), the numerous analyses with the cultures of aphid symbiotes executed by Tóth showed that they are really able to fix nitrogen. And I proved the same with the larvae of numerous other insects containing *Sulcia* and *Azotobacter* (*9, 10*).

The question of "mitochondria as organisms" is now more than ever in the air. With regard to the latest literature referring to some "isolates" from the mouse-ascites tumors supposed to be mitochondria, I can quote *Naturwissenschaften* (*17*) : Seyfarth *et al.,* 192; but, on the contrary, see Lettré, *ibid.,* 267. The large sarcosomes figured by Watanabe and Williams (*18*) and considered by these authors, as well as by Lanham, to be mitochondria, may, in my opinion, rather be *Sulcia,* owing, especially, to the fact that they propagate by budding. Also, their forms and size correspond to my cultures of those organisms from the above-mentioned insects. For a long time I have suspected that the symbiotic fungus *Sulcia* studied by me and isolated from the caterpillars of *Liparis monacha* L. (unpublished) can produce, in addition to the great hypertrophy changing it into very complicated propagation structures, granules—particles—so very minute that they closely resemble chondriosomes. With the aid of streptomycin one also can evoke them in great numbers in cultures. I have published, in 1950 (*10*) and in 1951 (*11*), a picture of *Sulcia* growing in the fat body of *Drosophila.* A photograph of the mycetome (Plate IV, 9) from a very young caterpillar of *Lymantria monocha* shows many micro-sulciae, some of which are of such extremely fine size that they might be taken for mitochondria. Should this be proven—which so far is only the music of the future—then it would represent the first instance of a definite microorganism producing mitochondria. In this respect one should also consult Tóth (*19*) regarding the main cells (Hauptzellen) of the salivary gland of *Stomaphis graffii-platanus* that very likely contain numerous micro-sulciae. The inclusions of rat liver cells described by Lagersted (*20,* Fig. 13) and others possibly also belong here.

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