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*S. Mahdihassan:***BACTERIAL SYMBIOSIS IN APHIS RUMICIS.**

With regard to symbiosis in Eriococcidae, STEINHAUS (1) summarises as follows: „The symbiotes of the citrus mealy-bug, *Pseudococcus citri*, are considered by many to be yeasts or yeast-like organisms. BUCHNER, in 1930, finally came to the conclusion that the symbiotes of *P. citri* were bacteria and not yeasts. The symbiotes may be spherical, oval, or sausage-shaped and usually have blunt rounded ends. The protoplasm contains some granules, but apparently no distinct nucleus.“ This would hardly support a description of a bacterium typical of its kind and it would be shown on another occasion that it does not apply to the real symbiote. Thus, when doubts exist whether the symbiotic germ, is an yeast or a bacterium it shows the scanty knowledge we still possess on the subject.

The above remark applies with equal force to symbiosis among aphids. STEINHAUS (1) writes, „There has been considerable controversy among European investigators as to the true nature of some of the intracellular symbiotes in aphids or plant-lice. Although early workers believed them to be yeasts or yeast-like organisms, the present trend is to consider them very pleomorphic, intracellular bacteria. PAILLOT is one of the leaders who is convinced of their bacterial nature, and he has presented much evidence supporting his views.“ My own work fully confirms PAILLOT's conclusion, but he seems to have believed the symbiote of aphids to be bacteria in order to explain polymorphism, for yeasts exhibit it only slightly, while bacteria may do so very extraordinarily. Thus STEINHAUS says, „According to PAILLOT, when the various forms occur together in the same insect specimen, they frequently appear as different species of micro-organisms; (different) forms presumably arise from the one simple bacterial form“. In his Fig. 87, STEINHAUS reproduces from PAILLOT, the illustration of symbiotes of *Drepanosiphum platanoides* showing short bacillary cells and large pleomorphic forms. In the text STEINHAUS writes, explaining them as follows: „The symbiotes of *D. platanoides* occur in two greatly contrasting morphologic forms. One is a typical bipolar bicillus frequently considerably elongated. The second form is a large, very polymorphic, elongated cell... These forms resemble fungi, but originate from the smaller bacterial forms... Other species of aphids, having similar contrasting forms, include *Macrosiphum tenaceti* and *Aphis rumicis* etc.“ Finally STEINHAUS adds, „Attempts to cultivate the symbiotes of aphids have generally been unsuccessful. PIERANTONI, in 1910 and PEKLO in 1912 and 1916, have reported success

in some cases but their work (still) remains to be confirmed". I may state immediately in this connection that I have been also unable to do so.

STEINHAUS does not mention the work of SCHOEL (5) nor does his name appear any where in the index. I remember from my correspondence with Dr. SCHOEL that he worked in the Botanical Institute, Königsberg, and he has favoured me with a reprint, perhaps of his thesis, which unfortunately, does not give in print the name of the Journal where it has appeared. My indications are those found in pencil, probably by himself. His paper has a summary, in English, from which the following is extracted, „After it had been established that (some aphids) are monosymbiotic ... the isolation (of the symbiotes) was undertaken. The culture showed that in the symbiotes it is a question of typical rod bacteria, the peculiar ball-shape of which inside the host animals is probably caused by special conditions ruling in this case. In the state of isolation the bacteria show a complicated pleomorphism in the course of which the rod and the ballshapes are appearing alternatively.“ This he also illustrates in Fig. 1, p. 162.

I have studied several aphids but I have never observed any polymorphism of their symbiotes, either in tissue or in cultures. The germ that Dr. SCHOEL describes was apparently an external associate. At least he admits that the coccus-type, as seen in tissue, becomes a rod-type in cultures. I should fear the germ cultivated was not the symbiote.

ŠULC, in 1910, also studied the symbiotes of aphids and believed them to be yeasts. BUCHNER (2) in 1912 followed this view and reproduced Šulc's illustration and his designation, *Schizosaccharomyces aphidis* and further created another name *Schizosaccharomyces drepanosiphii*, being a new species of yeast in symbiosis with an aphid. To PAILLOT, therefore goes the credit of being the first to believe that the symbiotes of aphids are bacteria and not yeasts. However I am not sure whether the polymorphic forms he illustrates are to be understood as bacteria, or even as germs of any kind. My own observation has forced me to doubt the living nature of such objects which are assumed to be very polymorphic.

*Macrosiphum tenaceti* is one such aphid where two contrast forms of bacteria are present and I have been able to study the insect very satisfactorily. I never observed such an abnormal stage of polymorphism as to mistake it for that of a fungus. Smears showed a coccus type of germ and another of typical bacterium type. On culturing them the coccus type grew a little larger in size, but otherwise showed no polymorphism or deviation from the shape observed in the insect tissue. The longer, bacterium-type, was almost identical with the shape seen in tissue smears and likewise exhibited no polymorphism worthy of mention. However, there arose a very serious difficulty. In 1924, UICHANCO found only one symbiote in *M. tenaceti* which was the coccus type invariably found in all aphids, but in 1927, KLEVENHUSEN (3) found two symbiotes in the same aphid species, the additional symbiote, being the typical long bacterium mentioned above. It is easier to overlook the smaller coccus type than the larger bacterium. At any rate smears easily show both these forms. However, late in summer, at Breslau I found *M. tenaceti* to contain only the coccus type and it was apparently free from the larger bacterium. It would thus appear that UICHANCO had

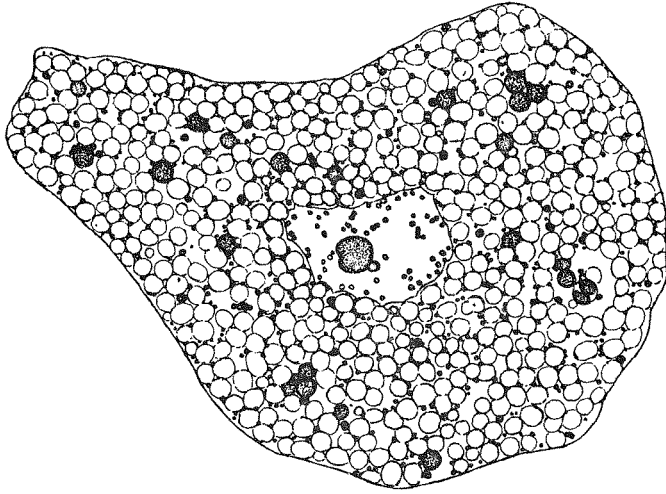


Fig. 1. The major symbiote of *Aphis rumicis*, a coccus type of bacterium, as illustrated by A. KOCH.

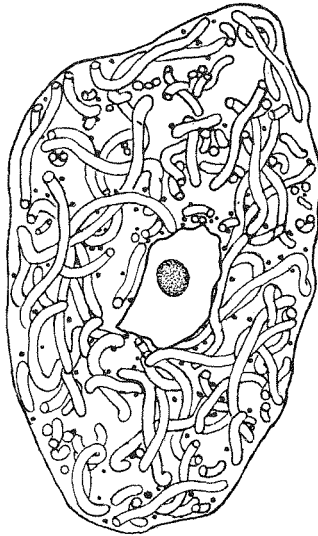


Fig. 2. The minor symbiote of *Aphis rumicis*, a bacterium type of symbiote, as illustrated by A. KOCH. It never shows polymorphism.

perhaps collected similar material. The question arose at the time, whether the insect was properly identified. Prof. BÖRNER of Naumburg was the best specialist in Germany and he also confirmed the species of aphid as *M. tenaceti*; I still possess Börner's letter to this effect. In as much as I could confirm both UICHANCO and KLEVENHUSEN I felt it proper to select another aphid for an intensive study.

*Aphis rumicis* as mentioned before is disymbiotic and Prof. BUCHNER was anxious to know whether in such cases it is the same germ with two morphological forms or entirely two different germs. Like *M. tenaceti* the aphid, *A. rumicis* also shows two distinct bacteria. I could observe no polymorphism, nor intermediary forms which would give rise to such a suspicion. The coccus type is far more predominant than the larger bacterium type. Although the symbiotes have been illustrated by others, A. KOCH (4) gives the best enlargement, fig. 1 copied from him shows the coccus type, typical of aphids, while fig. 2 gives the larger bacterium type, found specifically in *A. rumicis*. I do not mean that fig. 1 represents a germ found in all aphids but merely that in shape they appear so. Even in appearance they do not resemble yeasts or any species of *Schizosaccharomyces*. On culturing the two germs were separate and showed no sign of intermediary forms or polymorphism. The coccus type grew somewhat larger in size otherwise there was no deviation from the findings of tissue smears.

Since the point was controversial I wanted to confirm the result serologically and continued my work in the laboratory of Dr. G. MEISSNER at Breslau as she had done similar work on the symbiotes of luminescent bacteria in cephalopods. The work was repeated by cultivating all possible associates of the insect. The aphid was artificially propagated on as many weeds as were conveniently found but only of *Rumex* species. At the beginning I had mixed *A. rumicis* with *Aphis sambuci*, hence I preferred to confine myself only to the insects I had myself infected or found as pure colonies on *Rumex* weeds.

The germs externally associated with *A. rumicis* did not prove so numerous as was feared. There was only one species of a small yeast very minute in size and which gave a faint rose pigment like *Torula rosaceae*. There were three coccus type of germs and eight different bacteria. They thus represented the microflora outside the body besides the intercellular symbiotes. Thus there were in all two genuine symbiotes and 11 external associates.

Four rabbits were taken and every fourth day through their veins in the ear, an emulsion of macerated insect tissue was injected. Freshly collected insects were weighed to give about half a gram of weight. They were macerated thoroughly with water and ground in a glass mortar and pestle. The emulsion was centrifuged and the residue treated with water and centrifuged for the second time. For half a gram weight of insects only five cubic centimeters of water were used in all. This emulsion of bacteria was injected to promote specific antibodies. The emulsion contained large numbers of coccus type of the germ, and less of the large bacterium type, which even in the insect body was relatively insignificant in numbers.

After six weeks from the first day of experiment, when, in all, each rabbit had been injected with 2 grams of insect, blood was taken from the heart. After coagulation the blood gave a clear serum. This was centrifuged and used as standard. It should contain antibodies against the two symbiotes, shown in figs. 1 and 2 here. Upto dilution 1/250 the coccus type, fig. 1 reacted showing that the coccus was a real symbiote. In dilution 1/500 it was both plus and minus so that the previous reading of dilution 1/250 alone was taken as correct. The large bacterium type of symbiote, showed self agglutination, when it was kindly tested by Dr. MEISSNER herself by the method of complement fixation. She found the longer germ did not react to the serum. It was feared that even 20 cc. of the emulsion of insects did not contain enough bacterial protein to give rise to sufficient antibodies. It was the one germ out of the thirteen that were cultivated which was very easy to compare with the smears from the tumour showing the bacteria to be the same as seen in fig. 2 here. They approached the germ that produces Butyric acid; they also produced a similar odour but not so unpleasant. There was no spore formation. The colonies were somewhat rough resembling those of the *Mesentericum* type.

### Summary.

Symbiotes of aphids were supposed to be yeasts but PAILLOT has shown them to be bacteria which have not been cultivated. In *Aphis rumicis* there is the common coccus-type of preponderating symbiote; in cultures it grows a little larger in size than in tissue. It has been shown to form antibodies, when 2 grams of fresh insects have been used for that purpose. The serum containing specific antibodies in dilution 1/250 reacted against this major symbiote. Such serum could not confirm the isolation of the minor symbiote, present in smaller numbers and as such did not form antibodies sufficient for the purpose. The serum did not react against any of the other eleven germs externally associated with the aphid. The minor symbiote morphologically is a very easy object to study. In smears it is large enough and can be easily compared with smears from isolated cultures. The morphological characters however could confirm the isolation. No evidence was found to show that the symbiotes of aphids are two forms of the same bacterium. My thanks are due Dr. MEISSNER for her kind help and to Prof. BUCHNER for the suggestion.

### References.

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