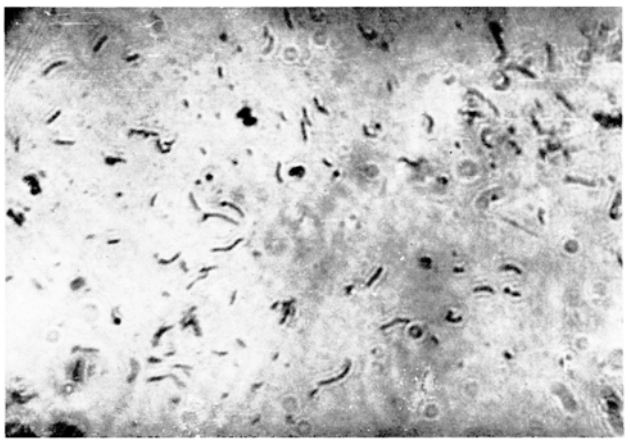
On the Functions of Intracellular Symbiotes of *Sitophilus oryzae* Linn.

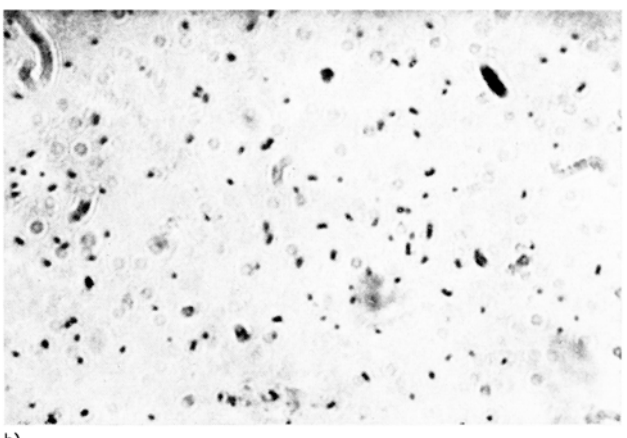
Sitophilus oryzae Linn. contains intracytoplasmic symbiotes of unknown function which are transmitted from one generation to another by ovarial infection[1]. Experiments on nutritional requirements with this insect revealed that *Sitophilus* can develop on a diet deficient in vitamins of B group including nicotinic acid and riboflavin. Individual deficiency of other members of B group in the diet did not create any adverse effect, as is the case with many other insects.

Investigations were, therefore, further extended to find out the role and nature of the symbiotes which have now been shown probably to possess a trophic function somewhat similar to those shown in *Lasioderma* and *Stegobium*[2]. The microorganisms derived from egg, ovary and mycetome of *Sitophilus* have been successfully cultured in nutrient agar medium.

Symbiotes (Figure). In culture the microorganism is rod ashaped (1.4–1.9 × 0.5–1.2 μm), motile spore forming gram-negative bacterium occurring in chains. It shows profuse moist growth of yellowish creamy colour on potato, utilizes citrates and different sugars, except lactose, without production of gas; hydrolyzes starch; reduces nitrates and produces hydrogen sulphide. It cannot liquify gelatin nor produce indole. Methyl red- and Voges Proskauer-tests are negative. In situ the bacterium is a little longer and curved.



a)



b)

Photomicrographs showing a) symbiotes in the smear of mycetomic tissue; b) cultured symbiotes.

The bacterium was found to resemble very closely *Bacillus circulans*. Serological tests confirmed that the cultured bacterium was similar to that found in the insect tissues.

The symbiote is capable of fixing small quantities of nitrogen from air in vitro and also synthesizes nicotinic acid, pantothenic acid and riboflavin. Tests with other vitamins have not yet been carried out. This agrees well with the nutritional findings and explains why *Sitophilus oryzae* could probably tolerate the deficiencies of these vitamins in artificial diets offered in the form of pallets.

Although there is experimental evidence for the nitrogen fixation property of symbiotes of *Sitophilus oryzae* yet the possibility of this function of insect symbiotes has been a debatable issue in the literature. CLEVELAND[3] suggested such a possibility on the grounds that mycetomes are always provided with an abundant supply of tracheae. This is also the case with *Sitophilus*. TÓTH[4] has claimed that cultures of symbiotes of aphids enrich the medium, probably by fixing atmospheric nitrogen. The method of nitrogen estimation and the purity of culture have been questioned by BUCHNER[5]. KOHLER and SCHWARTZ[6] were able to demonstrate at least a slow growth of symbiotes of *Pseudococcus citri* in liquid medium lacking nitrogen compound.

Attempts to make insects aposymbiotic by oral administration of antibiotics (like aureomycin, hostacyclin, penicillin, polymixin, sulfadiazine and sulfanalimide) by subjecting them to γ-radiation at the rate of 2.25 kr per min, to sub-lethal doses of methyl bromide upto 4 generations, to high temperature of 31 °C up to 4 generations and to centrifugation for 10 min at a speed of 10,000 g/min did not prove successful. Micro-quantities of antibiotics have also been injected into the adult insects, but the rate of mortality was too high to make this technique of any practical use in these investigations. However, in surviving insects the number of symbiotes in the mycetome was very much reduced.

Full details of these investigations will be published elsewhere[7].

Résumé. *Sitophilus oryzae* peut se developper après une diète artificielle présentée sous forme de palettes. L'insecte peut se développer avec plus ou moins de succès en l'absence de facteurs essentiels tirés du complexe de la vitamine B y-compris l'acide nicotinique. Il est apparu que les symbiotes de l'insecte peuvent synthétiser au moins 3 vitamines et peuvent fixer l'azoté de l'air in vitro. Les essais de rendre les insectes aposymbiotiques n'ont pas eu de succès jusqu'à présent.

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