

II. - STUDIES ON SOIL PROTOZOA. PART II. THE FUNCTION OF MITOCHONDRIA IN SOME SOIL PROTOZOA.

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Horning (*Austr. Jour. Exp. Biol. Med. Sci.*, 1926, **3**, 89) has shown that mitochondria are cytoplasmic bodies which multiply by fission. The mitochondrial fission is irregular and is independent of the fission or conjugation accompanying the reproduction of the organism. The mitochondria probably help the intracellular digestion in the organism in view of the fact that they serve as a source of active enzymes. The object of the present investigation is to elucidate the function and behaviour of the mitochondria in some of the soil protozoa. Since the work was completed a paper by Horning (*loc. cit.*, 1927, **4**, 69) describes the cognate relationship of mitochondria to the infusorian *Nyctotherus*.

EXPERIMENTAL.

About 10 gms. of the soil was placed in a petri-dish and 20 c.c. of the culture medium added. Hay infusion (2 per cent.) containing 1.5 per cent. agar, and 1 gm. of malted milk in 100 c.c. of water were the two culture media used. Hay infusion was used at the outset and the subsequent sub-cultures were made in malted milk. The individuals were isolated from the main culture by picking them out with a capillary tube under the microscope; the isolated organism was then transferred to a hollow-ground microscope-slide containing the malted milk. In this way pure cultures of some of the different species of protozoa occurring in soils were obtained.

In order to render the mitochondria visible, Janus green, which is one of the vital stains, was first employed. Sections of the organisms were cut and subsequently stained with iron hæmatoxylin. Thick cultures of the organism were fixed in osmo-chromic mixture, the culture centrifuged, and the liquid decanted; the mass was then embedded in paraffin for cutting sections. Janus green has the disadvantage of staining also the bacteria that occur in the body of the organism, and consequently it does not differentiate the mitochondria from the bacteria. Therefore a new dye-stuff, the sodium salt of diethylsaffraninecarboxylic acid, prepared from Janus green by the hydrolysis of the nitrile, has been used. Dilute solutions of the order

of 1/5000 have the power of staining the mitochondria, which become bright pink in about 15 minutes. The substance has a selective power of staining only the mitochondria of the animal cell and not the co-existent bacteria in the body of the organism.

Sub-cultures were made every day and organisms at different stages of life were obtained. The study has been confined at present to *Amoeba*, *Oicomonas* and *Colpoda cucullus* as representatives of the three groups *Rhizopoda*, *Mastigophora* and *Ciliata*, that occur in soils.

DISCUSSION.

An examination of stained preparations reveals numerous conditions of the mitochondria which go to show that they are undergoing binary fission. The figures in plates I and II show some which are in the state of division resembling the fission of *amoeba*. An increasing constriction is formed in the rod-shaped mitochondrion which is divided finally into two, the process being complete in a few minutes. There seems to be no definite period for fission, for while some are dividing there are others which are not dividing. The mitochondria have been observed in the organisms just before division and also in the daughter cells immediately after division. Hence they ought to be inclusions of the cytoplasm. It is also found that they are arranged in definite rows in some, while in others they are scattered. It has moreover been noted that the mitochondria increase in numbers with the growth of the organisms. Taking into account their enzymatic nature, this increase can be correlated with their digestive function; for, as the organism grows old it requires more food, and so there is a greater demand on the digestive system which is met by the mitochondrial increase. This fact has received further confirmation since some mitochondria have been found in the food vacuoles (plate II, fig. 4).

SUMMARY.

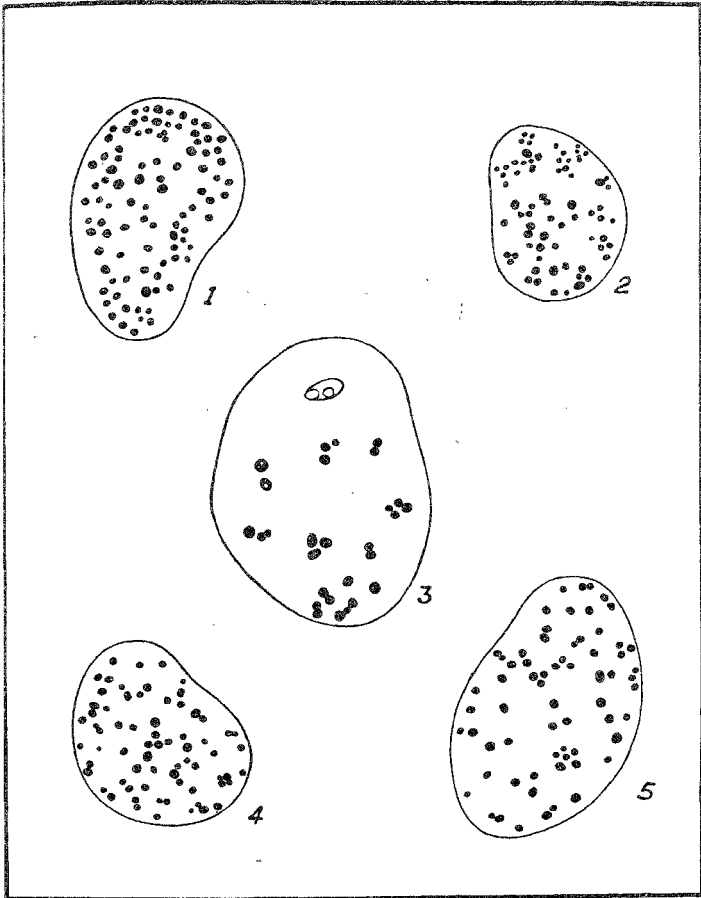
1. The sodium salt of diethylsaffraninecarboxylic acid, prepared from Janus green by the hydrolysis of the nitrile, has been used as a selective stain for mitochondria.
2. Mitochondria in organisms at different stages of life have been observed.
3. Mitochondria occur in the animal immediately before, and after division, showing that they are really cytoplasmic inclusions and not formed afresh.

4. Mitochondria have been observed in the food vacuoles and are found to increase in number with the growth of the organism.

5. These facts have been correlated to show that the mitochondria are digestive in function.

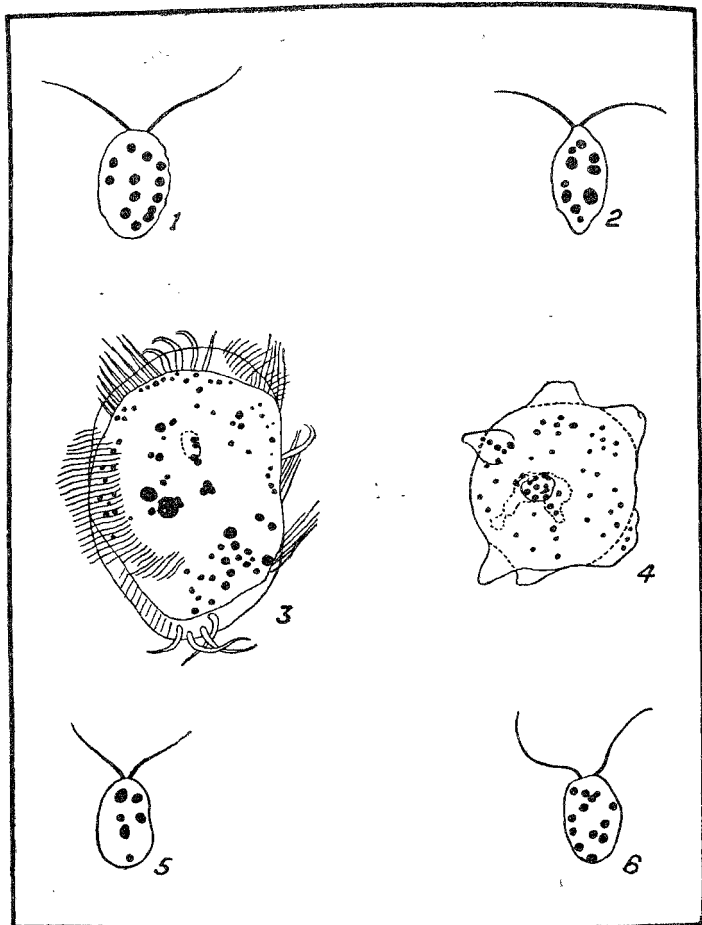
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Figures 1 to 5.

Colpoda cucullus ; showing the different stages of the organism and the enclosed Mitochondria



Figures 1, 2, 5 and 6.

Oicomonas termo ; showing the different stages of the organism and the enclosed Mitochondria.

Figure 3.

Euplotes patella ; showing the Mitochondria at different stages

Figure 4.

Amöba proteus ; the Mitochondria are seen in the food vacuole.