Mitochondrial Behaviour during the Life-cycle of a Sporozoon (Monocystis).

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With Plate 9.

Introduction.

At the present time the behaviour and function of mitochondria within the animal and plant cell is a matter of controversy among cytologists, and, in order to help to elucidate the nature of these bodies, observations were made upon these cytoplasmic inclusions within a common sporozoon (Monocystis). The changes which mitochondria undergo during the life-cycle of this organism, together with the role they appear to play in cellular metabolism, is also discussed.

Life-Cycle of Monocystis.

If the sperm-sacs of the common Australian (European) earthworm are examined, it will be noticed that they are occasionally infected with Monocystis. The life-cycle of the parasite is well known. When reproduction is about to take place the two trophozoites or adult individuals come together, become rounded off, and finally secrete a cyst, in which two gametocytes become enclosed. The two nuclei of the adult gametocytes divide repeatedly until a large number of nuclei are formed. Later, each individual nucleus becomes surrounded by a small quantity of protoplasm. From this stage small minute cells (gametes) are finally formed. These minute gametes subsequently combine in pairs, and from these the zygotes or spores arise, so that the cyst at this phase of the life-cycle contains many such bodies. The nucleus of each zygote
undergoes fission giving rise to a number of fusiform sporozoites, generally eight in number. The spore coat finally ruptures and these young fusiform sporozoites are freed, and then make their way to the developing clumps of sperm, eventually becoming surrounded by sperm-cells. Finally, they grow into the adult organism.

**METHODS OF INVESTIGATION.**

In order to observe the behaviour of mitochondria during the life-cycle of this Sporozoon, the sperm-sacs of the common European earthworm, which occasionally swarm with *Monocystis*, were fixed in osmo-chromic fluid or else in a Champy solution. The fixed material was then washed in running water for a period of twenty-four hours, brought up through the alcohols and eventually embedded in paraffin, and sectioned. The sections were then cut 2–5 µ in thickness and stained with Heidenhain's iron haematoxylin, and occasionally counterstained with eosin.

An examination of these sections with high magnifications shows that the cytoplasm of the trophozoite or adult individual contains numerous small, bent, rod-shaped bodies, together with a smaller number of spherical bodies scattered irregularly throughout the medullary region of the organism (see fig. 1, Pl. 9). These cytoplasmic inclusions certainly present a bacterial appearance, but their subsequent reaction to certain stains and fixing reagents shows quite clearly that they cannot be foreign organisms. Also, at various stages of the life-cycle, these bacteria-like bodies stained a bluish green when treated *intra vitam* with a Janus green B solution of about 1:10,000. It has been frequently shown by various authors that this *intra vitam* method does not appear to have any corresponding selective action upon bacteria, while Cowdry (1) and many other investigators have found that mitochondria in living cells stain specifically with Janus green B. Various other tests for mitochondria were also carried out, similar to those I have already described in some of my previous
papers (4, 5, 6, 7, 8, 9, 10), and the results show quite clearly that these protoplasmic bodies are true mitochondria reacting in the same manner as mitochondria present in other animal and plant cells.

**Mitochondrial Behaviour during the Life-cycle.**

When the adult organism or trophozoite, cut in longitudinal section and stained with the Heidenhain process, is examined under high magnifications, the cytoplasm is found to contain a large number of bent rod-shaped and spherical mitochondria, scattered irregularly throughout the protoplasm of the medullary region of the parasite (see fig. 1, Pl. 9). A closer study of these mitochondria shows that they increase in number by means of transverse binary fission. A more detailed observation of the general arrangement of these bodies will reveal a dense aggregation of mitochondria in the vicinity of the nucleus (see fig. 1, Pl. 9) lying in close contact with the outer surface of the nuclear membrane. When dealing with this phenomenon in previous papers (9, 10), I have pointed out that it may possibly be a surface-tension effect which is apparently dependent upon the phosphatidal nature of the mitochondria. When stained sections of the next stage of the life-cycle—the early sexual phase—are examined, the cytoplasm of the encysted gametocytes is observed to contain numerous rounded mitochondria, often varying in size; while on the other hand the number of rod-shaped bodies, such as were seen in the previous stage, have undergone a considerable decrease in number (see fig. 2, Pl. 9). These larger rounded bodies probably arise through a fusion of several mitochondria. This apparent fusion of mitochondria has been observed by several authors, among whom is W. J. M. Scott (13), who describes a similar phenomenon occurring in the pancreas; and Cowdry believes that the larger spherical bodies are the result of a coalescence of several or more mitochondria. Later, I was able to demonstrate the actual fusion of mitochondria in living amoebae (7). This constant fusion of mitochondria during certain phases in many cells may possibly be explained in terms of surface-tension, and may be similar to the
fusion of oil-drops in suspensions of oil in water, and it does not seem likely that any physiological function can be attached to it. If sections showing the small gametes within the cyst are now examined, it will be noticed that the protoplasm of these minute cells contains small spherical mitochondrial bodies varying in size (see fig. 8).

During the true sexual phase of the life-cycle of the organism, when union of the gametes occurs, a noticeable fusion of mitochondria takes place (see fig. 3, Pl. 9), since the rounded mitochondria during this process tend to lose their spherical form and become irregular clumps. I have observed this effect on several occasions, and it is of interest to note that the same phenomenon occurs during the sexual phase in the binucleate Opalina (5) when conjugation of the gametes occurs.

The protoplasm of the two encysted gametocytes is not entirely used up to form the young gametes, and a surplus of residual cytoplasm is always left over and is termed by many authors the 'cystal residuum'.

An examination of this surplus cytoplasm within the cyst reveals the presence of several types of granules within it (see fig. 3, Pl. 9), mitochondria, lipoidal droplets, together with a third type of granule, which appears light blue after staining with Heidenhain's iron haematoxylin. These latter types of grains have the appearance of vegetative bodies. As the cyst becomes more mature these granules disappear as the residual cytoplasm becomes absorbed (see fig. 4, Pl. 9).

In order to ascertain the nature of these granules within the 'cystal residuum' the sperm-sacs of many earthworms infected with Monocystis were placed in a saline solution, teased, and examined for cysts. A portion of the sperm-sacs, containing several fairly large cysts, was removed on to a clean slide, and with the aid of needles the cyst walls were then ruptured. In order to test for mitochondria a Janus green solution of about 1:10,000 was introduced under the cover-slip containing the cyst. Several granules (the mitochondria) stained a bluish green, while other granules within the residual protoplasm, presumably the lipoidal and vegetative grains, did not show
a selective action to this stain. In order to ascertain the nature of the former type of granule a solution of Soudan III was run under the cover-slip containing another such ruptured cyst, and in this case the fatty droplets were observed to stain a light yellowish red, which demonstrated their lipoidal nature.

If the young zygote is examined in a fairly mature cyst the cytoplasm is seen to contain a variable number of rounded mitochondria. But later examination of the young spores, which are derived from the zygotes before the chitinous case is fully secreted around them, reveals that the mitochondria during this stage have undergone a notable decrease in number, and when finally the spore has reached maturity it is found to be entirely devoid of mitochondria. Sections cut through many such mature cysts show the complete disappearance of mitochondria both within the spores themselves and also within the cyst, and the 'cystal residuum' at this stage of the life-history of the parasite has become completely absorbed.

It cannot be suggested that this effect is due to a faulty fixation or technique, nor that the fixing fluids were unable to penetrate the chitinous case of the mature spores; because in every case the individual nuclei in each spore stained sharply and clearly, while the protoplasm of the sporozoite stained a palish blue when treated with the Heidenhain process.

In several cases material was fixed in a solution containing 4 per cent. osmic instead of the usual 2 per cent., and each time the same results were obtained. Material was also left in both Champy and osmo-chromic fixatives for a period of forty-eight hours, and even in over-stained cells no traces of mitochondria could be seen.

The next step in the life-history occurs when in another earthworm the mature spore-coat finally ruptures and the young sporozoites within are liberated. They immediately become active and make their way to the developing sperm, where they gradually increase in size, and develop into the adult individual or trophozoite.

Stained sections of these young organisms on examination once more reveal the presence of small rounded and rod-shaped
mitochondria scattered irregularly throughout the medullary region of the cell. As the sporozoite gradually develops into the trophozoite the mitochondria increase in number. Continued observation of progressive stages of development has shown that the awakening of the cell is accompanied by a reformation and rapid reproduction of the mitochondria.

The behaviour of the mitochondria during the life-cycle of this organism, and especially their apparent origin de novo during chemical resynthesis, do not lend any support to Wallin's (14) contention that mitochondria are symbiotic organisms.

**ON THE ROLE PLAYED BY MITOCHONDRIA DURING CELLULAR METABOLISM.**

That mitochondria play an important part in the metabolism of the cell was first put forward by Guillermond (3), who suggested that starch and other plastids produced in plant cells owe their origin indirectly to mitochondria. Cowdry (2) also formulated the theory that these substances are originally formed within the mitochondria which later enlarge to form the body of the plastid, while Regaud (12) suggested that mitochondria are able to select certain materials from the surrounding protoplasm of the cell and build them up into various products. Later, while studying the behaviour of mitochondria within a common endoparasite protozoon—Opalina (5)—I was able to detect vegetative granules arising in close connexion with the surface of each single mitochondrium. These observations tend to support the speculation that mitochondria are the loci of protein and of general protoplasmic synthesis within the living cell. More recently the work of Marston (11) has suggested that the mitochondria contain concentrated within them the enzymes which bring about cell synthesis, their action being to build up protein at their surface. Moreover, the behaviour of mitochondria appears to illustrate the capacity of enzymes to reverse their activity in accordance with conditions, i.e. synthesis or hydrolysis, according to the concentration of the substrate. For example, in Opalina we see that mitochondria
synthesize vegetative products at their surface; while in *Paramoecium* (6), *Nyctotherus* (8), and *Amoeba* (7) the function of mitochondria is also one of hydrolysis, where it is believed that the mitochondria which come to be included within the food vacuoles bring about the digestion of the food.

We see that *Opalina* and *Monocystis* feed in the same way by means of endosmosis through the cuticle of the protoplasm. One would therefore expect to observe a similar phenomenon in *Monocystis*. But observations on this organism show that the actual origin of these food-storage products from the surface of the mitochondria cannot be detected. The mitochondrial grains present in the protoplasm of *Monocystis* are very much smaller than those of *Opalina*, and it is quite probable that the latter organism, owing to its large size, is the more favourable object for studying this phenomenon. But it is highly suggestive that the vegetative granules, which we previously observed in the residual protoplasm of the early cyst, arise in the same manner as similar plastid and vegetative products in other animal and plant cells.

Bearing in mind the evidence that mitochondria appear to be the centres of general protoplasmic synthesis within the living cell, it is of interest to observe that in *Monocystis* there occurs a stage in the life-cycle of this organism—namely, the spore phase—wherein synthetic activity and digestive activity do not occur, and moreover, at this period previous observations have shown that mitochondria can no longer be detected within the protoplasm of the cell. The study of the behaviour of mitochondria throughout the life-history has shown that these bodies make their reappearance in the free active sporozoite at a time when chemical synthesis takes place. Later, the growth of the sporozoite into the adult individual is accompanied by an increase in the number of cell mitochondria. This evidence seems to suggest that the reawakening of cellular activity within the spore is dependent upon the reformation of mitochondria.
**Summary.**

1. Observations show that mitochondria are capable of arising de novo in the freshly liberated sporozoite stage of the life-cycle of *Monocystis*.

2. Mitochondria are present in large numbers throughout the course of the asexual phase of the life-cycle. During the conjugation of the gametocytes the rod-like mitochondria give rise to numerous spherical bodies. At fertilization the mitochondria within the gametes appear to fuse, resulting in the formation of larger clumps.

At the commencement of the spore phase the mitochondria gradually decrease in numbers, and are totally absent within the mature spore. Later, the growth of the liberated sporozoite or young trophozoite is accompanied by a reformation and rapid reproduction of mitochondria.

3. The disappearance and reformation of mitochondria during certain stages of the life-cycle may be correlated with their apparent synthetic activity.

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**References.**

2. —— 'General Cytology.' Chicago, 1924.
5. —— Ibid., p. 167.
6. —— Ibid., vol. iii (1926), p. 89.
7. —— Ibid., pp. 89, 149.
9. —— Ibid., p. 75.
10. —— Ibid., p. 187.

DESCRIPTION OF PLATE 9.

Sections of Monocystis at various stages of the life-cycle. All figures are drawn from material fixed in either Flemming's solution without acetic or else in a Champy fixative and stained with Heidenhain's iron haematoxylin, and occasionally counterstained in eosin.

Fig. 1.—Longitudinal section of adult individual or Trophozoite, showing mitochondria scattered throughout the medullary region of the organism. Note mitochondria lying close to and in intimate contact with outer surface of nucleus.

Fig. 2.—Section showing association of two adult organisms within the cyst. Note the increase in the number of spherical mitochondria, which have arisen by fusion of the rod-shaped mitochondria of the previous stage.

Fig. 3.—Section through cyst showing mitochondria within the protoplasm of the gametes, showing conjugating gametes and larger granular clumps (x and z) formed by fusion of the mitochondria. Within the residual protoplasm are seen the three types of granules. M, mitochondria; L, lipoidal droplets; v, vegetative grains.

Fig. 4.—Section showing mature cyst. The residual protoplasm has been completely absorbed. Note the absence of mitochondria within the spores. The nuclei of spores are stained clearly.

Fig. 5.—Longitudinal section through two spores as seen under higher magnification. Note how the nuclei of each spore stains clearly, and also the total absence of mitochondria.

Fig. 6.—Section cut through a sporozoite showing the reappearance of mitochondria.

Fig. 6 A.—Section of same as seen with higher magnification. Note the rod- and spherical-shaped mitochondria in cytoplasm, together with sharply stained nucleus.