The giant mitochondria of ctenophore comb-plates

By G. A. Horridge

(From the Gatty Marine Laboratory and Department of Natural History, the University, St. Andrews, Fife)

With 8 plates (figs. 1 to 8)

Summary
The elongated cells which bear the continually active giant cilia of the combs contain numerous large mitochondria, up to 8 μ by 6 μ in size, which are filled with irregular tubular cristae. The ciliated cells are up to 100 μ long, but only 10 μ wide, and from their centrally situated nucleus can be traced a succession of stages, tentatively interpreted as the formation, growth, erosion, and final dissolution of mitochondria. Small ones occur near the nucleus in the region of the nuclear membrane, which may there be irregular, puffy, and electron dense. Some small mitochondria are surrounded by amorphous material which stains heavily with lead; others lie against the nuclear membrane, as if in intimate relation with it. Cristae of mitochondria which are interpreted as juvenile are filled with an amorphous material; some of the cristae open to the outside of the mitochondrion. Towards the ciliated end of the cells the appearance of the mitochondria suggests that they are breaking down; this is the region where food particles are eroded and where the cilia consume energy. Here the mitochondria are shrunken and around them are numerous vesicles; their cristae are fewer and they open into the cytoplasm. Similar vesicles, which are apparently of mitochondrial origin, are extruded between the cilia from the cells. The proposed cycle of generation and disintegration of mitochondria, based upon morphology, is so far an unproved hypothesis.

Introduction
There has recently been a great deal of speculation, backed by observation on diverse material, as to the origin of mitochondria. They have been investigated in cells which have developed them anew, such as maize meristematic cells (Lund and others, 1958) and mammalian spermatocytes (André, 1962), and also in liver cells, where a rapid turn-over of mitochondria is suggested from biochemical evidence of turn-over rates (Fletcher and Sanadi, 1961). Less has been heard of the possible fate of senescent mitochondria. In nerve-cells, if there is universally an axoplasmic flow towards the terminations as Weiss (1962) has found in vertebrates, it is probable that, unless they are attached to the axolemmal membrane, as they are in some crustacean axons, mitochondria must be irreversibly swept along. Weiss finds that the axoplasm moves at 1 mm/day. Therefore, it is not surprising that accumulations of mitochondria are consistently found at nerve-terminals in a wide range of animals from mammals (Boycott and others, 1961) to ctenophores (Horridge and Mackay, 1964). However, I have found only one instance in the literature where a cycle of appearance and subsequent growth of mitochondria has been set up as an hypothesis based upon observations. This
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is in the cortex of the 1 to 3 mm oocyte of the chicken (Schjeide and McCandless, 1962) where mitochondria are supposed to be formed from the highly convoluted oocyte cell membrane, although the authors state in a footnote that a large proportion of the rapidly increasing numbers of mitochondria migrate outwards from the centre of the oocyte. The oocyte material is so peculiar, and the conclusions so much at variance with those of others, that the hen oocyte mitochondria will not be further considered.

The comb-plates of ctenophores have long been known for their large cilia, which grow up to 2 mm long in common planktonic species such as Pleurobrachia and Beroë (Chun, 1880). The cilia are carried on the peripheral ends of cushions of elongated specialized epithelial cells known as polster cells, which are arranged as a series of groups, each group bearing a fused mass of cilia in the shape of a flat plate. The plates are arranged in 8 rows along the sides of the animal, which is radially symmetrical, with the long axes of the plates at right angles to the line of the combs, as the rows are called. Continual waves of ciliary beats pass along the comb-rows, usually towards the aboral end of the animal. The mechanism of transmission of the wave of activity through the tissue is not understood: this is the example to which the term 'neuroid transmission' was first applied by Parker (1905). The ciliary wave, and the spontaneous activity of isolated pieces of comb-rows, can be stopped by excitation of a nerve-net which spreads ubiquitously, and fairly homogeneously, over the ectodermal surface of the whole animal. During a search for the terminals of the nerves which inhibit and co-ordinate the ciliated cells (Horridge and Mackay, 1964) the great size of the mitochondria of the comb-plate cells attracted attention. The numerous mitochondria presumably contain the mechanism which produces available energy from food supplies at a considerable rate, although I have not been able to find any quantitative figures on their performance. However, giving a vivid introduction to the topic, one can suppose that the leg muscles of a horse contain about one pound (dry weight) of mitochondria which are supplying the available energy for muscular work when the horse is performing at one horse power. This is the same order of energy production per pound weight as is supplied by the engines of a jet plane in a vertical climb.

Intense activity, on any theory of ageing, is bound to wear out the mechanism. The membranes of mammalian mitochondria have been shown to be the sites of the electron transport system involved primarily in the oxidation of food, namely DPNH and TPNH, choline and succinate cytochrome c reductase, cytochrome c oxidase and glucose-6-phosphatase (Chance, 1963). A high density of mitochondria is characteristic of tissues which are capable of a high energy output. From the continual activity of their enormous cilia it is clear that the ctenophore ciliated cells have a high energy production. Considering that the animals are relatively large and depend entirely on cilia for locomotion, it is understandable that the ciliated cushions are almost entirely filled with large mitochondria. This paper presents the salient features which make ctenophore comb-plates attractive material for a study of mitochondria.
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Methods

The material was the same as that used for the earlier work on nerve endings. Pieces of comb-plate of Pleurobrachia pileus were fixed in a mixture of 2% osmic acid with an equal volume of sea-water. Blocks were embedded in araldite and the sections were stained with lead acetate by the alcohol-ether method already described (Horridge and Mackay, 1964). On account of the watery nature of the material, consistently good fixation is difficult to achieve, and different cytoplasmic constituents cannot be fixed simultaneously, no matter what combinations of added sucrose and dilution with water are tried.

Observations

Normal mitochondria

The mitochondria have a consistent appearance in section (fig. 1). The size varies rather systematically as one goes along the elongated cells from the nucleus to the ciliated edge of the comb-plate. Many details of the long polster cells, which are up to 100 \( \mu \) long and approximately 10 \( \mu \) wide, are illustrated in fig. 2 and in a previous paper (Horridge and Mackay, 1964). The polster cells are closely packed with mitochondria in the region around the nucleus, but are relatively free from them at the two ends (a) in the region near the cell bases, which stand upon the mesogloea below, and (b) in the distal region up to 10 to 20 \( \mu \) from the cilia. Mitochondria never lie in close association with the bases of the cilia. The mitochondria are not of equal size, and while there is a considerable variation a general trend can be discerned, with small ones near the nucleus, then larger ones and finally small ones again where they are most distant from the nucleus. The cytoplasmic contents are very consistent from cell to cell and analysis is simplified because the only components are those illustrated in this paper.

The pattern of the contents of single mitochondria in section is quite different from that of any other mitochondria of which I have seen figures although, especially in the related ctenophore Eucharis multicorns, there is a resemblance to some protozoan mitochondria. The central region is filled with a mass of round and wormlike shapes in sections, as shown in fig. 1 and other figures. This pattern is interpreted as arising from a mass of irregularly arranged tubes within the mitochondrion. Occasionally examples can be found with a spiral or more obvious lamellar structure as in figs. 6, B and 7, B, C. The cristae are rather uniformly spaced, suggesting a stiff arrangement of tubes rather than a bag of vesicles, and there is never close packing in one region or shaking down as might be expected with vesicles. It has been shown that a section through a random system of straight cylinders contains a high proportion, in fact 86%, of elliptic shapes with ratio of axes less than 2 to 1 (Elias, Sokol, and Lazarowitz, 1954). The tubes of the cristae are thick as compared with the section thickness, and where they are caught in side view they are clearly contorted and of irregular thickness, so that
statistical methods based on uniform shapes are not applicable. In a lobate ctenophore, *Eucharis multicornis*, parallel membranes are often found, showing that many of the tubes are flattened in cross-section. Most sections show a few lengths of tube (t in fig. 1) 0.1 to 0.3 μ in diameter, enough to suggest that most of the central mass consists of irregularly coiled tubes, though there may be vesicles as well. The internal membranes appear as single when fixed in osmic acid and stained with lead acetate; here and there the tube walls are continuous with the inner of the two enveloping membranes, as in mitochondria elsewhere. The tubes are, therefore, topologically similar to cristae of normal mitochondria. This arrangement of their membranes is the principal criterion for calling these structures mitochondria in the first place. The spaces in the background between the cristae are hardly more electron-dense than the cytoplasm outside. Here and there, scattered on the membranes of the cristae, are electron-dense particles. The distance between the mitochondrial outer membranes is not uniform, showing that they do not adhere in the regular fashion that membranes in contact sometimes show.

Tubular cristae of about 50 μ diameter are characteristic of the amoeba *Pelomyxa* (Pappas and Brandt, 1959), but the tubes are arranged in a regular zigzag array, not haphazardly as in ctenophore polster cells. Tubular cristae, less well developed than those of ctenophores, occur in *Sagitta* and in coelenterates.

Tissues other than the comb-plates have mitochondria of the same form but of normal size, and they make up a much smaller fraction of the contents of the cell.

**Extrusion of vesicles between the cilia**

Most vertical sections through the comb-cells show vesicles of about 100 μ diameter near the bases of the cilia and sometimes within them, as seen by Afzelius (1960). But there are vesicles of a similar size outside the cells too, in sea-water. These are most clearly distinguished as being outside the cilia where there is a small gap between the membranes of adjoining polster cells at their ciliated surface, as in figs. 3, B and 6, A. The vesicles are hollow;
Fig. 3

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those that lie outside in the sea-water commonly have a double membrane. Comparison between the sizes and appearance of the vesicles inside and outside suggests that the vesicles sometimes acquire an extra membrane as they pass out through the cell membrane. Groups of polster cells which have many vesicles outside have correspondingly more inside than do other cells without vesicles outside. These points suggest that vesicles are extruded from the inside, not formed in both directions at the membrane.

The erosion of mitochondria

Apart from the distal region which extends for about 10 to 15 μ beneath the cilia, the polster cells are filled with mitochondria. The peripheral limit of mitochondria is rather sharp and runs at about the same level across all the cells, as in figs. 2 and 3. This is the region of disintegration of lipoidal droplets, which are very electron-dense round bodies when fixed with osmium and stained with lead (fig. 4). They are scattered throughout the tissues of ctenophores and are presumably the food bodies which are passed between cells. The rapid expenditure of energy in the ciliated cells has to come from some large and obvious internal source since there are no blood-vessels, interstitial cells or transport cells. The present conclusion is that the dense droplets are the food reserves and that these progressively disintegrate. They shrink, lose their bounding membrane, and finally break up into small droplets as in fig. 4. In the region where this is happening the most peripheral mitochondria are small and also appear to be breaking down, as can be seen in fig. 4 and, more particularly, in fig. 5.

Signs of disintegration can also be seen at the peripheral ends of the most distal mitochondria, i.e. those nearest to the cilia. The outer membrane is puffy or sometimes missing in parts, and tubules or cristae can be found opening through the outside membrane of the mitochondrion, not merely through the inner one. This could be significant if we imagine their contents to be reacting with cytoplasmic constituents. In the surrounding cytoplasm there are numerous single-walled vesicles of 50 to 250 μ. The appearance of the membrane and contents of these vesicles is exactly like that of the

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**Fig. 3 (plate).** The peripheral ends of the ciliated cells.

A, a region where typical large mitochondria, almost filling the width of the cell, are more abundant than in fig. 2. Some of them (tc) show more obvious signs than others of a tubular form of the cristae. Lipoidal droplets of various sizes occupy their characteristic position.

B, the region of the roots of the cilia, showing vesicles. In some instances it is obvious that the vesicles are outside the membranes of the cilia.

cil, cilia; cm, cell membrane; d, vesicle with double membrane; l, lipoidal drops; r, root of cilium; sw, outside sea-water between cells; tc, tubular cristae.

**Fig. 4 (plate).** A region across 4 cells where the furthest peripheral mitochondria lie near 4 disintegrating lipoidal droplets. The latter, interpreted as food reserves, are in a different state of disintegration in each cell.

cm, cell membrane; iv, invagination, which is interpreted as the opening of the internal cavity of the cristae to the outside of the mitochondrion; l, lipoidal droplets in various degrees of disintegration; m, mitochondria, also showing signs of disintegration to various degrees; v, vesicles.
mitochondrial cristae. In this region there are in some cells groups of vesicles and tubules which appear to be the final remains of a recently disintegrated mitochondrion. Mitochondria in this region are different from those in the other region of small mitochondria, near the nucleus 50 μ away. They appear shrunken and many examples have fewer cristae than normal, as in fig. 2 (em). Moreover they are deficient in mitochondrial dots, which are the small black spots occurring in photographs on the tubules of mitochondria nearer the nucleus.

In ctenophores the mitochondria and cilia, between which there must be a rapid transfer of energy, have no close morphological relationship, such as Olsson (1962) found in Amphioxus, where the cristae of mitochondria are arranged in line with the transverse bars of the cilia rootlets against which they rest. In ctenophores the roots of the cilia are bundles of long fibres which extend down the polster cells as far as the nucleus.

Mitochondria in proximity to the nucleus

The nuclei lie about midway along the elongated polster cells. In this region the cytoplasm is crowded with mitochondria, which, judging from their shape, are tightly squeezed together. A very much elongated mitochondrion may lie alongside the nucleus, pressed between it and the cell membrane. The resting nucleus has a continuous sharply defined double membrane, with nuclear pores 80 mμ in diameter and from 100 to 500 mμ apart. The nucleus is frequently elongated along the length of the cells and lobed at its ends. Structures which suggest activity of the nuclear membrane are of several types, some of which may be related to possible origins and growth of mitochondria. Frequently lobes of the nuclei project for distances of 1 to 2 μ in the direction of one or other of the ends of the cell. There is sometimes a heterogeneous group of vesicles of different sizes in this area, as in figs. 6, C and 7, A. These vesicles do not contain the granular substance typical of the contents of the nucleus but it is quite possible that they are formed by a bubbling of the outer nuclear membrane. The nuclear membrane appears puffy, with a relatively indistinct separation between the two membranes. Sometimes the cytoplasm in this area contains irregular masses of electron-dense material, as in figs. 7, B and 8, C, D. In contrast to the nerve-cells, and cells thought to be luminescent cells, there are no cytoplasm structures which, in comb-plate cells, can be equated with Golgi membranes or endoplasmic reticulum.

Small mitochondria, which are sometimes encountered pressed closely against the nuclear membrane, differ in several ways from ordinary mitochondria which also lie in chance contact. These mitochondria are small, with few cristae, which are crowded unusually close together. The mitochondrial wall is thick and dense, the cristae appear solid, or at least partially filled with an amorphous substance. These features serve to characterize mitochondria which are interpreted as being immature. The same features have been considered as characteristic of immature mitochondria in
meristematic cells of *Zea* (Lund and others, 1958). Some of these small mitochondria are in contact with the nuclear membrane in a way suggestive of a special relationship which can be interpreted as transfer of material. The membranes of the cristae are frequently fused with the outer membrane in such a way that the cristae appear to open to the outside of the mitochondrion. This can be seen at the invaginations marked (iv) in figs. 7 and 8. It is a feature of mitochondria interpreted as juvenile in a variety of vertebrate embryonic cells (Dempsey, 1956).

In tracing mitochondria back to smaller and smaller examples it is quite clear that ones such as x, y, and z in figs. 7, B, C, and 8, A, C, D, are mitochondria, although they may be juvenile. There are even smaller examples, with a few tiny cristae, like the one illustrated by s in fig. 8, A. Smaller than this, one finds, as possible precursors, only peculiar granules such as q in fig. 8, A. If their growth is rapid, few examples of the smallest ones can be expected. Growth through the early stages must involve the accumulation of a great deal of material, judging from the densely filled cristae of those interpreted as immature. Based upon these findings it is possible to propose that small mitochondrial seeds come in contact with the nuclear membrane and are pumped up with products secreted by the nucleus. This in turn suggests that some of the cristae originate at the points of contact of the growing mitochondrion with the nuclear pores, and that the invaginations of the inner mitochondrial membrane stem from this process.

**Discussion**

The one piece of evidence which indicates a dynamic situation in what is otherwise a series of static pictures is that vesicles extruded between the cilia are being continually lost from the animal. The animal lacks an excretory system and it is most probable that the vesicles are a waste product. The mutual breaking down of the food and mitochondria appears to be the process which forms these vesicles. The mitochondrial material must be replaced, and the size distribution of the mitochondria, with small and apparently developing ones in the neighbourhood of the nucleus, suggested that they are continually formed. In fact, as soon as attention was directed at the elongated polster cells as a system of energy production behind the giant cilia, the suggestion of a mitochondrial assembly and breakdown line presented itself as a likely hypothesis. To be fair it is not proved in any way; vesicles could be formed in both directions from the external membrane, and some could pass into the cell; mitochondria could approach the nucleus and be disappearing into the nuclear membrane. The morphological background merely sets the stage; a test of the hypothesis must be based upon further work of an experimental nature. An attempt to break into the cycle by starving the animals led to nothing, because the animals die after 1 to 2 weeks of starvation, without a noticed change in the cytology of the ciliated cells.

As known up to that time, observations on the origin of mitochondria have been summarized by Dempsey (1956) who gives reasons for questioning
division of mitochondria as the general mechanism of increase of numbers. Dempsey mentions work on mitochondria of developing kidney, heart, and nervous tissue and suggests that mitochondria may develop anew from other unknown structures. However, the opposite view, that mitochondria multiply by fission, still finds experimental support. Following radiocholine labelling of *Neurospora* mitochondria, later extracted, Luck (1962) found that the total labelled isotope initially present is preserved through 3 successive generations of doubling the numbers of mitochondria, and all mitochondria of the increased number share the labelling, though this might possibly be explained in a variety of ways.

The development of the numerous mitochondria in rat spermatocytes has been traced from precursor mitochondria which are few (André, 1962). The cristae become more numerous as the mitochondria grow in small groups, which are surrounded by a diffuse dark-staining structureless substance and accompanied by various sizes of small endoplasmic vesicles. Because the groups form round already existing mitochondria, André concludes unnecessarily that pre-existing mitochondria must precede development of new ones, but says no more. Kilarski (1962) finds that small round or oval dense bodies are probably mitochondrial precursors in rat liver sarcoma and in embryos.

In the amoeba *Pelomyxa*, the limiting membranes of mitochondria and post-division nuclei are often apparently continuous, and the appearance strongly suggests an exchange of material (Brandt and Pappas, 1959). After 3 days enucleate amoebae lost their ability to utilize carbohydrate and fat (Brachet, 1955) perhaps because their mitochondria wear out. Further evidence comes from other diverse material demonstrating that the mitochondrial co-enzyme DPN is synthesized in the nucleus (Hogeboom and Schneider, 1952), that the relevant synthetic enzyme for this is localized in the nucleolus (Baltus, 1954), and that the nucleolus moves to a point on the

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**Fig. 5** (plate). A to D. Small mitochondria which are the ones nearest to the ciliated end of their cells. All are interpreted as being in an advanced state of erosion. Some of the cristae appear to open to the outside of the outer membrane, and there are many vesicles in the cytoplasm.

*cm*, cell membrane; *cr*, crista of mitochondrion; *in*, invagination of mitochondrion surface as if the crista here opens to the outside; *mv*, empty vesicle; *t*, tubules in cytoplasm, resembling neurotubules.

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**Fig. 6** (plate). A, a length of the ciliated edge showing the large numbers of vesicles ejected between the cilia. The boundaries between the cells are indicated by marks down the right-hand edge.

B, an abnormal mitochondrion with cristae in the form of concentric lamellae. Examples of this type are common in the ctenophore *Eucharis multicornis*.

C, enlargement of the area outlined in black in fig. 2, to show groups of vesicles around the peripheral extensions of the nuclei of 3 adjacent cells. These vesicles seem to be unrelated to mitochondria. The small black specks are lead contamination due to faulty technique, and the section has been figured only because it was suitable for the low power view in fig. 2.

*cm*, cell membrane; *d*, vesicles with double membranes ejected in the sea-water; *m*, mitochondrion; *n*, nucleus; *nv*, group of vesicles associated with the lobes of the nucleus; *p*, pores of the nuclear membrane in face view and in section; *r*, root of cilium; *sw*, sea-water; *t*, tubules which resemble neurotubules; *z*, concentric lamellae, see also fig. 7, C.
Fig. 5

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nuclear membrane adjacent to an external mitochondrion (Chéremont and Frederic, 1951). Many of my pictures show such a situation in *Pleurobrachia*, for example in fig. 7, E. There is evidence that certain mitochondria contain RNA (Simpson and others, 1957) but perhaps in the long polster cells of ctenophores and certainly in long neurones they get no opportunity to renew it by actual contact with the nucleus.

The general conclusion from these varied studies is that mitochondria grow from small bodies and that the nucleus is essential in some way. Biochemical evidence shows a relatively short life for mitochondria of rat liver cells. Here the separately measured turn-over rates of the protein, the lipid and the cytochrome C, labelled with both $^{35}$S methionine and $^{14}$C acetate, give a half-life of 10-3 days for each of the 3 components, suggesting that mitochondria are single units with this half-life (Fletcher and Sanadi, 1961). Apart from the fact that ctenophores grow rapidly to maturity in a few weeks, nothing is known of the origin or life expectancy of the comb-cells or of their cytoplasmic inclusions. The numbers of growing and disintegrating mitochondria, and especially the numbers of vesicles which are ejected, suggest that the turn-over of mitochondria must be rapid.

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**Fig. 7 (plate).** Possible origins of mitochondria.

A, longitudinal section through an elongated nucleus showing what appears to be production of vesicles by the breaking up of lobes of the outer nuclear membrane at its two ends.

B, small mitochondria closely associated with the nuclear membrane in two adjacent cells. Above the nucleus of the cell on the left is an indefinite area containing pieces of nuclear membrane and small irregular pieces of mitochondria with densely filled cristae and indistinct membranes. These are interpreted as embryonic mitochondria. In the cell on the right the mitochondria are more distinct but still different from mature ones. Opposite to the smallest of them the nucleolus is against the nuclear membrane.

C, small mitochondria, which differ from mature ones, pressed closely together in the vicinity of the nucleus.

D, enlargement of the small mitochondrion against the nuclear membrane, from fig. 8, D.

E, enlargement of the small mitochondrion against the nuclear membrane, marked x in fig. 7, B.

cm, cell membrane; g, apparent gap in mitochondrial membrane; iv, invagination which is interpreted as a crista opening to the outside of the mitochondrion; l, lipoidal droplet; m, mitochondrion; n, nucleus; nl, nucleolus; mv, vesicles bunched near the ends of the nucleus; p, pore in the nuclear membrane; w, x, y, z, various forms of small mitochondria, interpreted as immature.

**Fig. 8 (plate).** Small mitochondria in apposition to the nuclear membrane.

A, a very small mitochondrion (s) and two peculiar vesicles (q). The mitochondrion (x) has denser than normal cristae.

B, this mitochondrion is perhaps only incidentally in contact with the nucleus, because the membranes look oblique in the area of contact and the mitochondrion has cristae which appear mature.

C, 3 mitochondria, the smallest of which (x) looks embryonic.

D, 3 small mitochondria which are embedded between 2 terminal lobes of the nucleus. The nuclear pores (p) show regions where the nuclear membrane is seen flat and not in section.

cm, cell membrane; iv, invagination interpreted as a crista opening through the wall of the mitochondrion; m, mitochondrion; n, nucleus; nl, nucleolus; p, pores in the nuclear membrane; q, peculiar vesicles, to which the search for smaller and smaller mitochondria leads; s, small mitochondrion; x, small mitochondrion with indistinct membrane and dense cristae in contact with the nucleus.
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It cannot be too strongly emphasized that the interpretation of mitochondrial formation and dissolution given here is highly tentative. All that has been presented is an anatomical background which throws the problem into focus in all its complexity, and I am well aware of possible criticisms of 'artistic speculation' (Sjöstrand, 1962). However, the giant mitochondria of ctenophores may prove to be of interest to others who have critical or experimental procedures to apply. Occasionally a primitive invertebrate can be found with a structure particularly suited to the solution of a general physiological problem.

These mitochondria were discovered during a search for quite a different object, the synapses upon the ciliated cells. I am grateful to my former associate B. Mackay for the part he played in these two studies, and to Professor H. G. Callan and Dr. H. C. Macgregor for electron microscope facilities in St. Andrews.

References