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MITOCHONDRIAL FORMS IN THE SECRETORY ACINAR CELLS OF THE PAROTID GLAND OF THE ONE-HUMPED CAMEL (CAMELUS DROMEDARIUS)

(With 5 Plates)

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أشكال المتقدرات في الخلايا العنيبية الإفرازية للغدة النكفية في الجمل وحيد السنام

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أجرى هذا البحث على الغدة النكفية لثمانية جمال بالغة وسليمة ظاهرياً (خمس ذكور وثلاثة إناث) باستخدام المجهر الإلكتروني النافذ. أظهرت الدراسة تميز الخلايا العنيبية للغدة النكفية بإحتوائها على عدد ضخم من المتقدرات التي شغلت نسبة كبيرة من السيتوبلازم. وجد بالإضافة إلى المتقدرات المألوفة ، متقدرات أخرى كبيرة ومختلفة الأشكال يصل طول بعضها إلى ١٣,٥٨ ميكرون وعرضها إلى ٥,٤ ميكرون . أوحظ أن توزيع هذه المتقدرات يختلف تبعاً لدورة الإفراز ، حيث كانت المتقدرات ذو الأشكال المختلفة أكثر شيوعاً خيال مرحلة تكوين الحبيبات الإفرازية. ومن أشكال المتقدرات الأكثر شيوعاً مايشبه تقريباً العصاه الطويلة، والثعبان، والأرقام العربية مثل 2 ,3 والحروف مثل Y, U, T, S, P, O, L, e وكذلك علامة الإستفهام والفصلة. وقد لوحظت أشكال أخرى لهذه المتقدرات مثل البطة ، السمكة ، حصان البحر ، القلب ، البلطة ، البوق ، مفتاح صمولة ، الغليون ، السندان ، رباعي الأضلاع ، القطر والعدسة المقعرة. هذا وقد شوهدت متقدرات مجنحة وأخرى ملفوفة أو مفصصة. وكان وجود هذه المتقدرات بأشكالها المختلفة وحجمها الكبير وأعرافها الكثيرة دليل على احتياج الخلية لكميات كبيرة من الطاقة ، وهذا يعكس بدوره كفاءة الخلايا العنيبية في الغدة النكفية على إنتاج كمية هائلة من اللعاب اللازم لتندية المأكولات النباتية بالمناطق القاحلة.

SUMMARY

The present study was carried out on the parotid gland of eight adult apparently healthy camels (5 males and 3 females) by using the transmission electron microscope. The most obvious cytological feature of the parotid acinar cells was the vast number of mitochondria, which occupied a large proportion of the cytoplasm. Besides the ordinary

mitochondrial-shapes, another large mitochondria of different forms could be also demonstrated. The latter varied in distribution in relation to the secretory cycle, and were particularly common at the onset of the stage of intensive secretory vesicle formation. The most commonly observed mitochondrial shapes resembled more or less elongated sticks, snaks, Arabic numerals as 2 or 3 or letters as e, L, O, P, S, T, U and Y as well as question marks or commas. In addition, another mitochondrial shapes like a duck, fish, sea horse, heart, ax, trumpet, spanner, tobacco-pipe, anvil, quadrilateral, penicillate and biconcave lens were observed. Winged, coiled and segmented mitochondria were also demonstrated. Those mitochondria with their numerous cristae and large size (reaching 13.58 µm in length and 5.4 µm in width at their maxium dimensions) were an evidence of high-energy amounts required. This may reflect the efficiency of the parotid gland acinar cells in producing enormous amounts of watery saliva required for moistening the dietary plants of arid districts.

Key words: *Mitochondrial shapes, secretory acinar cells, parotid gland, camel.*

INTRODUCTION

Most eukaryotic cells contain many mitochondria, which volumetrically occupy up to 25 percent of the cytoplasm (Lodish, Berk, Zipursky, Matsudaira, Baltimore and Darnell, 2000). Mitochondria are the major site of cellular adenosine triphosphate (ATP), since it supplies the cell with most of its usable energy. Mitochondria display a variety of shapes and sizes but they are usually ciruclar, rod or crescentic in shape with a width of about 0.5 µm – 1 µm (Kerr, 2000). However, with regard to the general cells activity (secretion production), not only the number but also the size and shape of mitochondria may vary considerably. Within the same cell and even during hours, these semi-autonomous cell organelles move, change their shape (e.g. by elongation, shortening, branching, buckling, swelling) divide and or fuse (Johnson, 1992; Cross and Mercer, 1993; Bereiter-Hahn and Voth, 1994; Yaffe 1999b; Young and Heath, 2000). Variation in mitochondrial shapes and sizes was observed in various mammalian tissues (Sohal, McCarthy and Allison, 1972; Bereiter-Hahn and Voth, 1994; Salem, 1996). In this connection, the present investigation is concerned with an electron microscopic study of the various mitochondrial forms in correlation to the secretory state of the parotid acinar cells.

MATERIAL and METHODS

Specimens of the parotid gland of eight apparently healthy adult camels (5 males and 3 females) were obtained from Assiut Slaughterhouse. Small pieces of the gland were fixed by immersion in a mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1M Na-cacodylate buffer (pH 7.2) for 4 hours at 4°C (Karnovsky, 1965). Then they were washed in 0.1M Na-cacodylate buffer and post-fixed in 1% Osmium tetroxide in 0.1M Na-cacodylate for further 2 hours at 4°C. The samples were then dehydrated in ethanol and embedded in Epon-Araldite mixture (Anderson and Andre, 1968). Semithin sections were cut on LKB ultratome and stained with toluidine blue for orientation for ultrathin sections. The latter were stained with uranyl acetate and lead nitrate and examined under JEOL 100 CX II transmission electron microscope.

RESULTS

The parotid acinar cells of the camel were more or less pyramidal-shaped joined together apically with zonulae occludentes, zonulae adherents and desmosomes. Along the lateral plasma membranes, intercellular canaliculi provided with numerous microvilli and sometimes contained secretory materials, could be observed. The basal plasma membrane of the secretory cells showed large basal infoldings. Well-developed myoepithelial cells were demonstrated between the basal border of the acinar cells and basal lamina (Figs. 1a & b). At the onset of the formation of secretory vesicles, the acinar cells were characterized by the presence of well-developed Golgi- apparatus (2-3 complexes and associated vesicles), abundant rough endoplasmic reticulum, in addition to the vast number of mitochondria, which occupied a large proportion of the cell cytoplasm (Figs. 1a & b). Beside the ordinary mitochondria that appeared round, oval or rod-shaped, these secretory aciner cells contained also many large mitochondrial forms. Their maximum dimensions reached about 13.58 µm in length and 5.4 µm in width. These mitochondria exhibited numerous cristae, densely-stacked, occasionally penetrated far deeper and often extending across almost the entire width of the organelle. The most obviously observed mitochondrial forms resembled more or less elongated stick (Fig. 2a) and snake (Fig. 2b), Arabic numerals as 2 or 3 (Figs 3 & 4) and letters as e, L, O, P, S, T, U and Y (Figs. 5a-h) as well as question marks or commas (Figs. 6a &b). Additional mitochondrial shapes looking as a

duck, fish, sea horse, heart, ax, trumpet, spanner, tobacco-pipe, anvil, quadrilateral, penicillate and a biconcave lens were demonstrated (Figs. 7a-l). Winged, coiled and segmented mitochondria were also observed (Figs. 8a-c). The secretory acinar cells contained, in addition, numerous ribosomes, few lipid droplets, lysosomes as well as cytoplasmic filaments.

At the end of the secretory stage, the acinar cells presented numerous homogenous electron-lucent mature secretory granules and more or less ordinary shaped mitochondria (Fig. 9a). After the release of the secretory material into the acinar lumen (Fig. 9b), the acinar cells showed again numerous mitochondria, filling most of the cytoplasm, assuming the same patterns as those at the onset of secretory granule formation (Fig. 10).

DISCUSSION

The present study revealed various mitochondrial shapes within the secretory acinar cells of the parotid gland of the camel. These mitochondria attained larger-size (reaching 13.58 µm in length and 5.4 µm in width) and were characterized by their numerous cristae. The most commonly observed mitochondrial patterns, especially at the onset of the stage of intensive secretory vesicle formation, resembled nearly elongated sticks, snakes, Arabic numerals (2 or 3), letters (e, L, O, S, P, S, T, U and Y) as well as question marks and commas. In addition, another mitochondrial shapes like a duck, fish, sea horse, heart, ax, trumpet, spanner, tobacco-pipe, anvil, quadrilateral, penicillate and biconcave lens were demonstrated. Winged, coiled and segmented mitochondria were also demonstrated. Unique mitochondrial forms were also observed resembling H-, L-, O-, S-, T- and Y- shape in the von Ebner's glands of the camel (Salem, 1996), C-, U- and O- shape in the adrenal cortex of hamsters and normal hepatocytes of rat and dog (Ghadially, 1997), and snake-like tubular as well as branched tubular – shape (Yaffe, 1999b).

The C-, U- and O- shaped mitochondria may represent sections of various planes through the cup-shaped organelle (Ghadially, 1997). However, the H- shaped mitochondria may result from side to side fusion of parallel organelles, while the ring-shape variety may be formed as a result of fusion of the smaller mitochondria (Sohal *et al.*, 1972).

On the other hand, large-sized (giant) mitochondria of variable shapes were recorded in human myocardium (Kraus and Cain, 1980) and in the secretory and ciliated cells of the gerbil trachea and bronchioles

(Spicer, Parmley, Boyd and Schulte, 1990). These giant mitochondria with numerous cristae appeared at the beginning of the secretory phase, indicating the increased intensity of oxidative phosphorlation as a functional adaptation of increased energy, needed for the cells (Sengel and Stöbner, 1970).

Alterations of mitochondrial morphology are also found in certain pathological conditions as tumors. These alterations include pyknotic mitochondria, mitochondria with longitudinal instead of the usual transverse cristae as well as mitochondria with fewer or swollen cristae (Ghadially, 1997).

Alterations in mitochondrial shape occur during normal cell growth, cellular differentiation and development (Scheffler, 1999, Yaffe, 1999a), where a Dnml protein acts as key molecular mediator of changes in mitochondria morphology (Bleazard, McCaffery, King, Bale, Mozdy, Tieu, Nunnari and Shav, 1999).

In the present study, the increased number of mitochondria, especially all these remarkable different shapes (with numerous cristae) that found at the onset of the stage of secretion production in the parotid acinar cells of the camel are evidence of high-energy amounts required. Slautterback (1965); Schwarzmann, Hoppler, Kayar and Weibel (1989) and Heppelmann, Meblinger, Neiss and Schmidt (1994), supported this suggestion, where they found a linear relationship between the oxidative capacity of the tissue and the mitochondrial mass and the complexity of the cristae.

In the present study, such demands are met by mitochondrial multiplication, and growth, as specially connected here with elongation, sprouting and fusion. In view of the fact that the distribution and from development of mitochondria is determined by interaction with cytoskeleton, and microtubules in particular, the broad spectrum of unique mitochondrial shapes can be explained. In the parotid secretory cells, subnuclear microtubule orientation and/or arrangement is rather net-like, so that the mitochondria may generate different more or less twisted filamentous profiles as well as shorter and variable smaller or larger fusing structures within narrow space (Ghadially, 1988; Alberts, Bray, Lewis, Raff, Roberts and Watson, 1994; Bereiter-Hahn and Voth, 1994). Nevertheless, whatever the reasons of varying shapes of the mitochondria may be, an effective adaptation of energy production to an enormous intracellular metabolism (production of large amounts of watery, electrolyte-rich secretion for this animal to adapt the adversed climatic conditions) is reflected anyway.

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LEGENDS

Plate I:

Figs 1a & b: Electron micrographs of the secretory acinar cells of the parotid gland of the camel at the onset of the secretory stage, showing various mitochondrial forms in addition to the ordinary variety, well developed Golgi-apparatus (arrow), immature secretory granules (asterisk) and rough endoplasmic reticulum cisternae (arrowhead). Nucleus (N), lumen (L) with long microvilli, myoepithelial cell processes (My), intercellular canaliculi (Ic), basal plasma membrane with basal infoldings (double arrowheads). X a: 6720; X b: 8333.

Plate II-V:

Figs 2-8: Electron micrographs of the most common mitochondrial patterns found within the secretory acinar cells of the parotid gland of the camel at the onset of the secretory stage.

Plate II

Fig. 2a: Elongated stick-like. X 23692.

Fig. 2b: Snake-like. X 23333.

Fig. 3: 2-like. X 40588.

Fig. 4: 3-like. X 28000.

Plate III

Figs. 5a-h:

(a) e- like. X 35787. (b) L- like. X 29556.
(c) O- like. X 35467. (d) P- like. X 21636.
(e) S- like. X 23333. (f) T- like. X 32667.
(g) U- like. X 40526. (h) Y- like. X 28000.

Fig. 6a: Question mark-like. X 42000.

Fig. 6b:Comma-like. X 39000.

Plate IV:

Figs. 7a-h:

(a) Duck-like. X 23756. (b) Fish-like. X 19833.
(c) Sea horse-like. X 23333. (d) Heart-like. X 28000.
(e) Ax-like. X 28000. (f) Trumpet-like. X 16667.
(g) Spanner–like. X 22000. (h) Tobacco pipe-like. X 37894.
(i) Anvil-like. X 36923. (j) Quadrilateral-like. X 37778.
(k) penicillium-like. X 11429. (l) Biconcave lens. X 35714.

Plate V:

Figs. 8a-c:

(a) Winged-mitochondria. X 42000.
(b) Coiled-mitochondria. X 22400.
(c) Segmented mitochondria. X 24500.

Fig. 9a: Electron micrograph of a parotid gland acinar secretory cell, with mature secretory granules in the supranuclear region (asterisk), containing mitochondria mostly of ordinary shape (M), ill-developed Golgi-complex (arrow) and few, short rough-endoplasmic reticulum cisternae (arrowhead). Lumen (L), intercellular canaliculi (Ic), nucleus (N), basal infoldings (double arrows). X 5263.

Fig. 9b: Electron micrograph showing the release of the secretory materials (arrow) into the acinar lumen (L) by exocytosis. X 16000.

Fig. 10: Electron micrograph of a secretory cell of the parotid gland of the camel after the release of their secretory materials. Notice that the abundunt mitochondria resembling those at the onset of the secretory stage. Golgi-complex (arrow), rough endoplasmic reticulum cisternae (arrowhead), nucleus (N), lumen (L), myoepithelial cell process (My). X 7000.

plate I

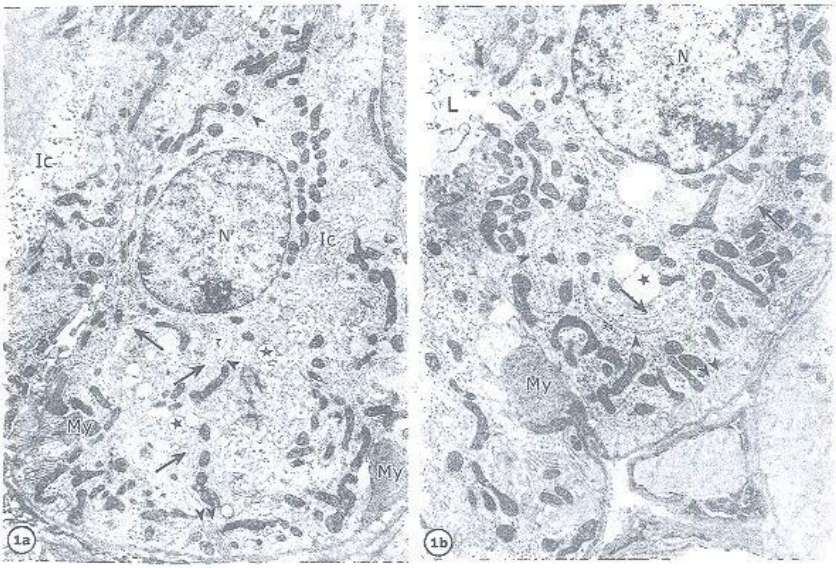
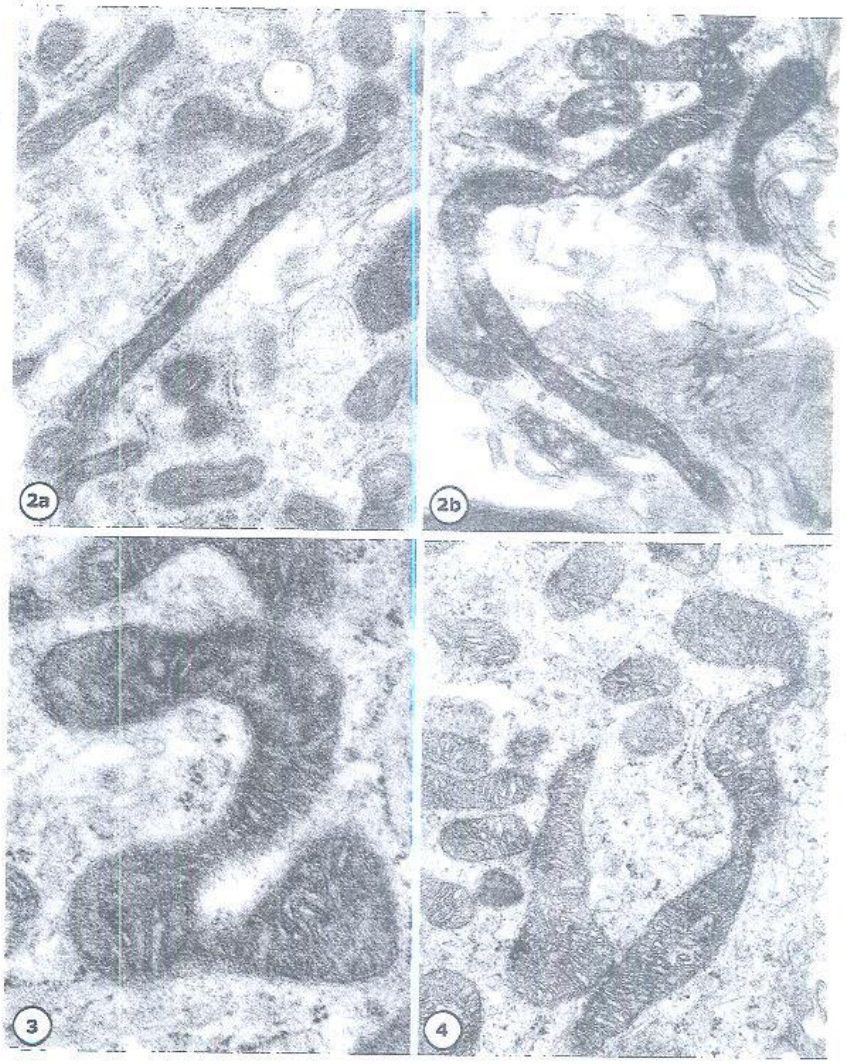


plate II



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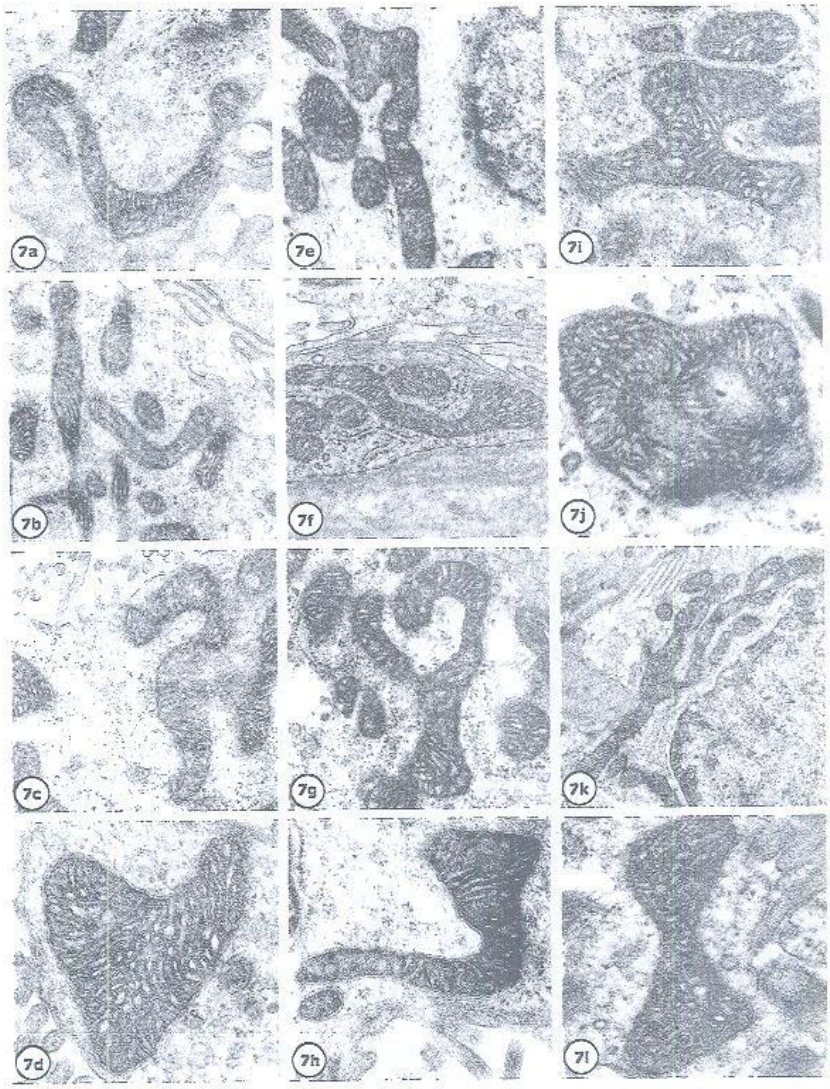

plate III

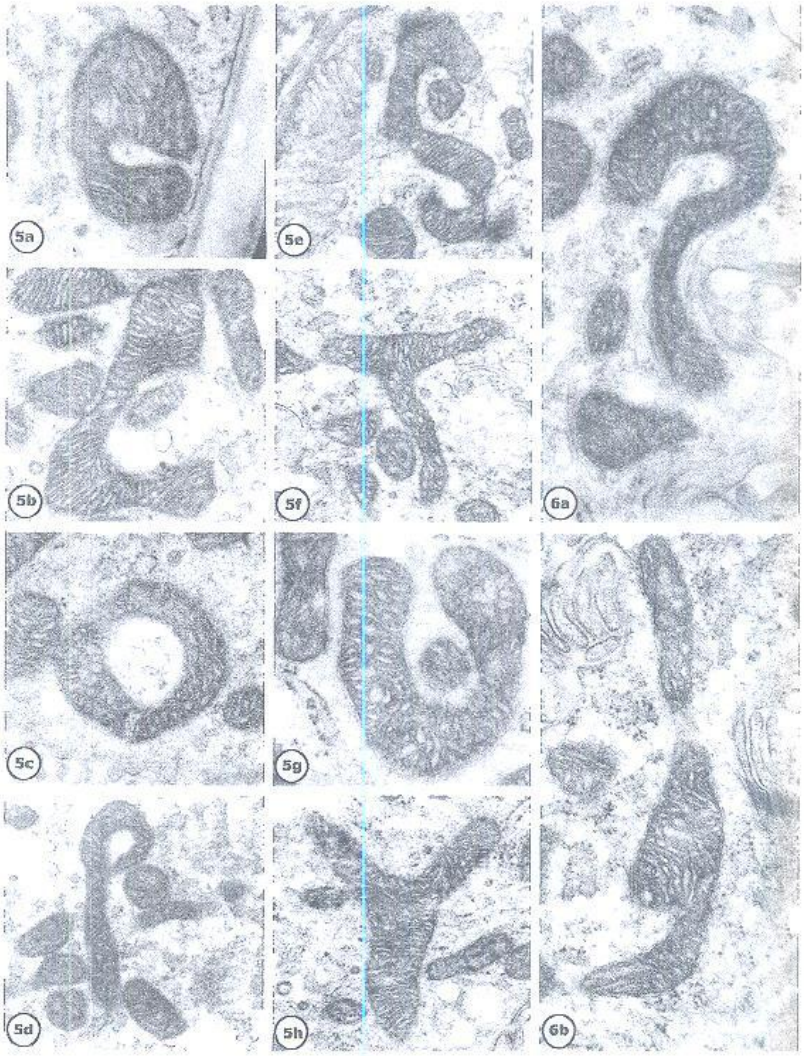

plate V