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AN ULTRASTRUCTURE AND IMMUNOHISTOCHEMICAL STUDY ON THE CHICKEN'S ENDOCRINE HEART

(With 7 Figures)

By

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دراسة التركيبات الدقيقة ومناعة كيمياء الانسجة للخلايا العضلية الهرمونية فى قلب الدجاج

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تمت هذه الدراسة على قلوب خمس دجاجات لجهورن حيث تم أخذ العينات من الاجزاء المختلفة للقلب مثل الأذينين والبطينين والحاجز الفاصل بين الاذينين والبطينين. تم تجهيز هذه العينات للدراسة بواسطة الميكرسكوب الضوئى الالكترونى كما تم دراسة مناعة كيمياء الأنسجة وذلك باستخدام الأجسام المضادة لهرمون قلب الانسان أوضحت هذه الدراسة وجود خلايا هرمونية فى قلب الدجاج حيث توزعت هذه الخلايا توزيع عشوائى خلال مناطق القلب المختلفة بدون اى تركيز فى جزء معين من اجزاء القلب المختلفة. كذلك كان الجهاز الأفرازى لهذه الخلايا غير متطور ومضمحل وقد تم الأستدلال على ذلك من خلال الاعداد القليلة للحبيبات الأفرازية فى سيتوبلازم هذه الخلايا. عند معاملة هذه الخلايا بالأجسام المضادة لهرمون قلب الانسان أعطت الحبيبات الأفرازية تفاعلا سلبيا مما قد يدل على اختلاف طبيعة هرمون قلب الدجاج عن هرمون قلب الأنسان.

SUMMARY

The hearts of different mammalian and submammalian species that have so far been examined all appear to be the source of a novel peptide hormone, variously termed atrial natriuretic peptide (ANP), cardiodilatin (CDD) and/or cardionatrin. Despite the well-established role of ANP as an important regulator of body fluid and electrolyte homeostasis in mammals, the endocrine nature of the avian heart has not yet been fully elucidated. In this

study, five leghorn chicken hearts were examined. The intensity, distribution and ANP immunoreactivity of the myoendocrine cells in the different heart regions of the heart were investigated with light and electron microscopy, and through immunogold labeling of ANP with goat anti-human ∝-ANP antiserum. We have confirmed the existence of myoendocrine cells in the different regions of the avian heart. Myoendocrine cells were randomly scattered within the different heart regions without any predictable localization pattern. They had very small numbers of mature secretory granules and an atrophied secretory apparatus. In comparison to controls weak to nrgative ANP immunoreactivity was seen. These results might indicate a difference in amino acid sequence between avian and human ANP, or diminished role of ANP as a regulator of body and electrolyte homeostasis in birds.

Key words: Chicken heart-ultastructure-Immunohistochemistry.

INTRODUCTION

Recent studies have proved that the heart of the different mammalian and many submammalian species is an important source of new class of hormone (Jamieson and Palade (1964); Schnermann, (1979); Reinecke et al. (1985) and Forsmann (1986).

Reflecting their remarkable diuretic and natriuretic effects, different names have been suggested for this hormone. Atrial Natriuretic Peptide (ANP); Atrial Natriuretic Factor (ANF) (Kangawa and Matsuo, 1984); Cardiodilatin (CDD), Forssmann et al (1983);Cardionatrin, Forssmann et al (1984) and Aureculin are among the most common names.

Despite of the well-established endocrine nature of mammalian heart, the secretory nature of the avian heart is not precisely yet determined. Some studies had completely denied the existence of any secretory activity within the cardiocytes forming the different regions of the avian heart (De Bold and Salerno, 1983). Other studies reported some sort of secretory activity within the avian myocardiocytes (Reinecke et al., 1988).

Therefore, the objective of this study was to provide a clear morphological evidence about the existence or absence of secretory activity within the avian heart. Also, to determine whether the avian cardiac hormone is related in its nature and amino acid sequences to human cardiac hormone. Confirming homology to human cardiac hormone is a prerequisite for the use

of avian form as a promising substitute to the human one in the treatment of many disease condition i.e. hypertension, congestive heart failure.

MATERIAL and METHODS

Five hearts from apparently normal Leghorn chicken were used.Small pieces from the different heart regions (right and left atria; right and left ventricles; interatrial and interventricular septum) were fixed in 2.5% glutaraldehyde in cacodylate buffer at PH 7.4 for 12 hours. The samples were washed in 0.1 M cacodylate buffer, post fixed in 1.0% osmium tetroxide in 0.1 M cacodylate buffer for 1 hours, washed in buffer again, dehydrated in ascending ethanolic series. cleared in propylene oxide and embedded in Erlandson's maraglas, D.E.R. 732 embedding medium (Caceci, 1984).Semithin sections were cut using glass knives and stained with 1% toluidine blue in 1% sodium borate for 30 seconds followed by 0.5% safranin in 0.5% sodium borate for 10 seconds. Thin sections were double stained with lead citrate and uranyle acetate and examined with transmission electron microscope at 80 KV. For immunohistochemistry, we had used the gold labelling as specific immunomarker. The fixed tissues embedded in L.R. white resin,and the goat anti-alpha human ANP were used as a specific primary antibody. For controls, the primary antibody was replaced by the buffer.

RESULTS

At the light microscopic level, the secretory nature of the atrial and ventricular myocardiocytes was not evident. Electron microscopic examination had disclosed a clear similarity in the structure of the atrial and ventricular myocardiocytes.

The endocrine secretory activity of the avian heart was evident mainly within the myocardial layer.

Ultrastructurally, three cell types were recongnized among the atrial and ventricular myocardial layer: 1) working cardiocytes, 2)conduction cells, 3) granules-containing contractile cells.

The working cells constituted much of the atrial and ventricular myocardium. They were characterized mainly by the entire absence of any secretory activity in their cytoplasm. They were elongated with an oval, centrally located nuclei. The nuclear chromatin was dispersed throughout the nuclear matrix with some aggregations along the inner side of the nuclear

envelope (Fig. 1). The major part of their cytoplasm was filled with myofibrill with the usual sarcomere components (Fig 1). The contiguous cell ends were connected to each other through a well-developed intercalated discs showing the usual structure of fascia adherents, maculae adherence (Fig. 2) and gap Junctions. A dense mitochondrial population was evident within the different sarcoplasmic regions especially the interfibrillar (Fig 3) and perinuclear spaces. The smooth sarcoplasmic reticulum was evident in most cases as vesicular structures mostly seen in close apposition to the T-tubules at the z-band levels. A dense glycogen aggregations, lysosomes and multivesicular bodies were also recognized especially within the perinuclear cytoplasm.

The second cell type was the avian conduction cell which were identified mainly among the ventricular myocardiocytes. They were similar to their counterparts in other mammalian species. In addition, a comparatively well defined secretory apparatus was evident in some cells. It was composed of a scarce rER tubules, and prominent Golgi complex associated with considerable number of progranules and mature secretory granules (Fig. 4). In comparison to working cells, the mitochondrial population was characterized by less densly packed cristae (Fig. 4)

The third cell type was the granules-containing contractile cells. The ultrastructural features of these cells were to large extent similar to those previously described for the working cells. The secretory apparatus composed of scarce rER. A limited number of mature secretory granules was usually recognized, in most cases only one or two granule were seen mainly within the perinuclear regions (Fig. 5). However, migration of these granules to the interfibrillar (Fig. 6) was sometimes observed. Other organelles included a prominent Golgi complex and dense mitochondrial population with densly packed cristae (Fig. 6). The mature secretory granules were spherical or ovoid membrane-bound with a diameter of 100-250 nm, and contained homogenous electron dense matrix which were separated from the surrounding membrane by an electron-lucent hallo (Fig. 6). No exocytotic figures or any evidence indicate the way by which the mature secretory granule is released from the cell were identified.

Treating the sections with anti-alpha hyman ANP has disclosed the absence of any reactivity within the granules matrices of all cell types(Fig. 7).

DISCUSSION

The present study aimed primarily to confirm the secretory nature of the avian heart. We have tried to examine the different regions of the avian heart and to determine which area have the highest population of the myoendocrine cells. The distribution of the avian myoendocrine cells appear to be different from those previously described for the other mammalian species (Bompani et al. (1959); Bloch, et al. (1985); Forssmann (1988) and Marei (1994). Moreover, their secretory activity was not pronounced. The avian myoendocrine cells were recognized as single endocrine units interposed among the atrial and ventricular myocardiocytes. They were randomly scattered without any predictable localization. Concerning the distribution of myoendocrine cells in other mamalian species investigated so far, the distribution of the avian myoendocrine cells was clearly different. A comparatively large body of evidence has asserted that the mammalian myoendocrine cells were localized mainly among the cells forming the atrial wall, especially within the right atrium (Herbest, et al ., 1988 and Reinecke et al., 1988). However, the presence of ventricular myoendocrine cells were also reported (Bloch et al., 1985; Back et al., 1986 and Nemer et al., 1986).

In our previous studies we have studied the distribution of myoendocrine cells within the different regions of the camel heart (Marei, 1994). We have disclosed the existence of myoendocrine cells within the atrial wall, right ventricular wall and interventricular septum. No myoendocrine cells were identified among the cells forming the left ventricular wall or the papillary muscle. These results were completely different from those reported for the avian heart. On the other hand, our result were similar to those of Reinecke et al. (1988) who reported that in contrast to mammals, myoendocrine cells occur only rarely in the avian heart. Chapeau et al. (1985) had the opinion that myoendocrine cells distribution is a phylogenetic matter. In mammals, birds and reptiles, ANP-producing cells are mainly restricted to the atria, while in the lower vertebrate classes such cells occurred in considerable number in the ventricles as well.

The secretory activity of the avian heart was also extended to include some conduction cells. A. prominent secretory apparatus was evident with a considerable number of mature secretory granules. similar findings were reported for other mammalian species Forssmann (1986) in human and Marei (1994) in camel. However, the exact role of conduction cell secretory nature was not precisely determined yet.

Treating the sections with the monoclonal antibody directed against the human cardiac hormone has revealed weak to completely negative reactivity within the granule matrix of the myoendocrine cells, conduction cells and non-contractile granule containing cells. The exact role of the avian cardiac hormone is still a subject of controversy. In this study the rudimentary secretory activity as evident by few mature secretory granules, and the negative ANP-immunoreactivity might suggest a less important role of cardiac hormone to the avian species. Moreover, the negative ANP-reactivity might also indicate that the chicken heart substance may be different from mammalian ANP. This result agrees with the statement given by De Bold and Salerno (1983) that in avian species the cardiac hormones may have lost their physiological importance, since in the avian hearts studied ANP-immunoreactive myoendocrine cells were found only infrequently compared to other vertebrate species.

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LEGENDS

Fig. (1): Woking mycardiocytes. Note nucleus (N), mycofibrils (Y), mitochondria (I). No secretory granules X 10 000.

Fig. (2): Working mycardiocytes. Note myofibrils (O), and macula adherens (arrow) X 19 000.

Fig. (3): Working mycardiocytes showing interfibriller mitochondria (arrow) with densly packed cristae X 10 000.

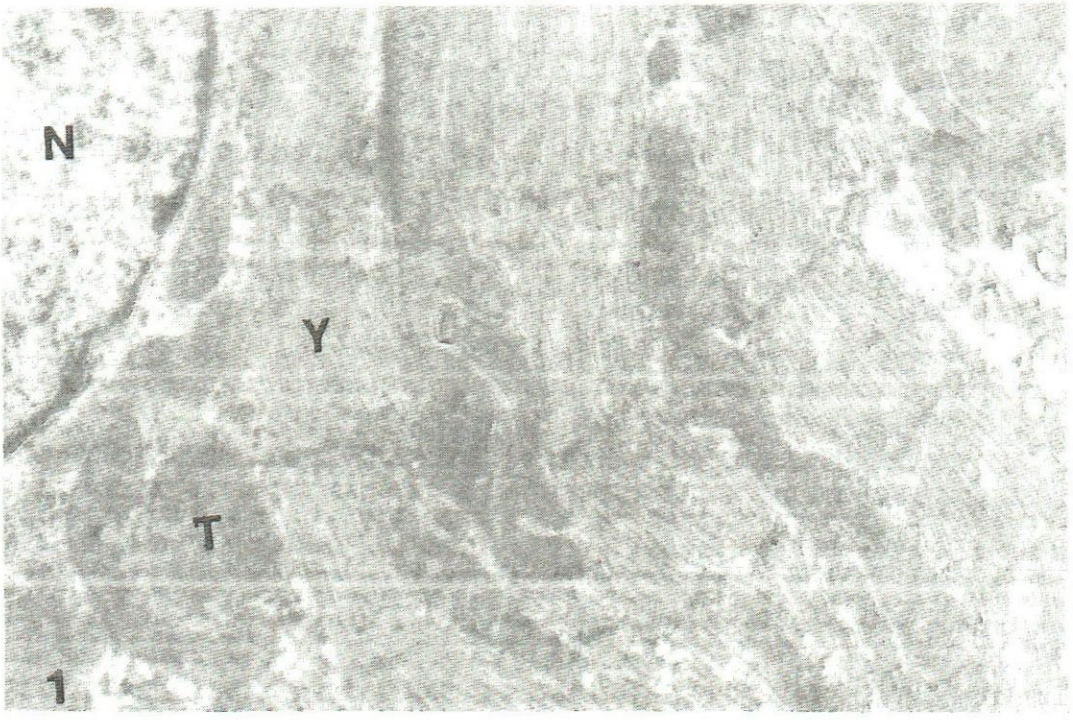
Fig. (3): C.S. in Purkinje-conduction cell. Note nucleus (N), mycofibrills (O), mitochondria with few cristae (T) and mature secretory granules (arrow) X 14 000.

Fig. (4): C.S. in Purkinje-conduction cell. Note nucleus (N), mycofibrills (O), mitochondria with few cristae (T) and mature secretory granules (arrow) X 14 000.

Fig. (5): Granules-containing contractile cell. Note nucleus (N), giant mitochondria (T), myofibrils (O) and only one secretory granule (arrow) X 29 000.

Fig. (6): C.S. in granules containing contractile cells showing myofibrils (O), mitochondria. (T), Golgi (C) and mature secretory granules (arrows) X 19 000.

Fig. (7): Ultrastructural immunohistochemical labelling of the storage of human ANP in chickenn's myoendocrine cells. Note negative reaction (arrow). X 36 000.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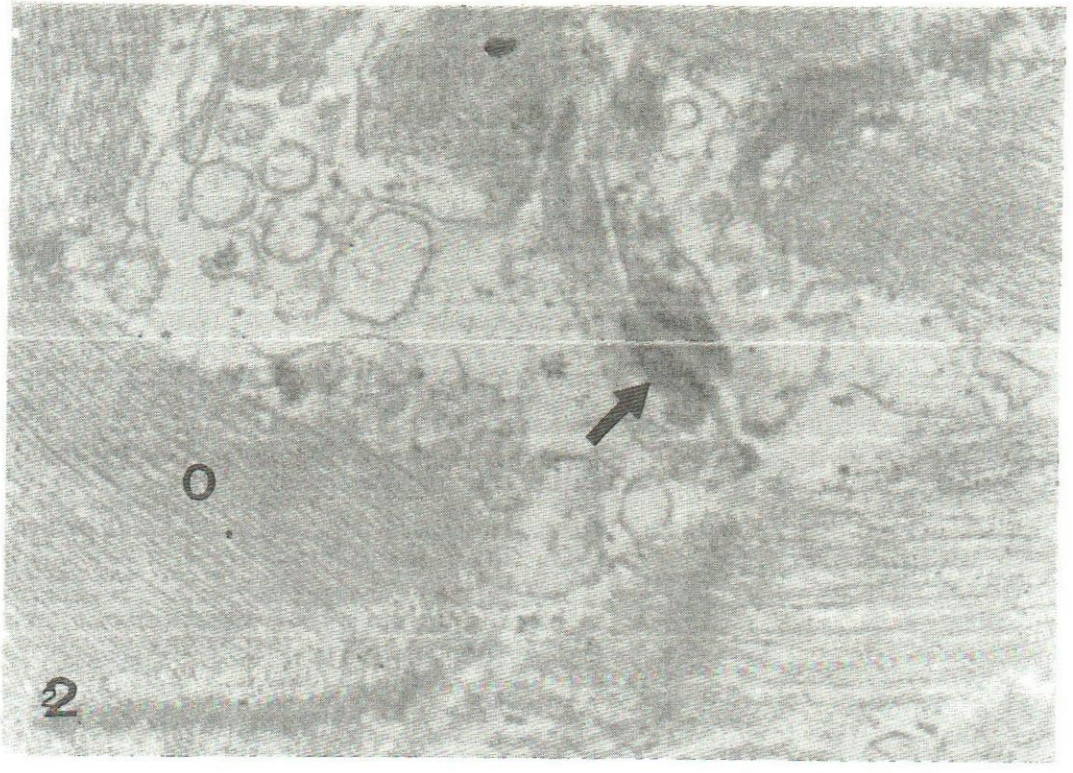
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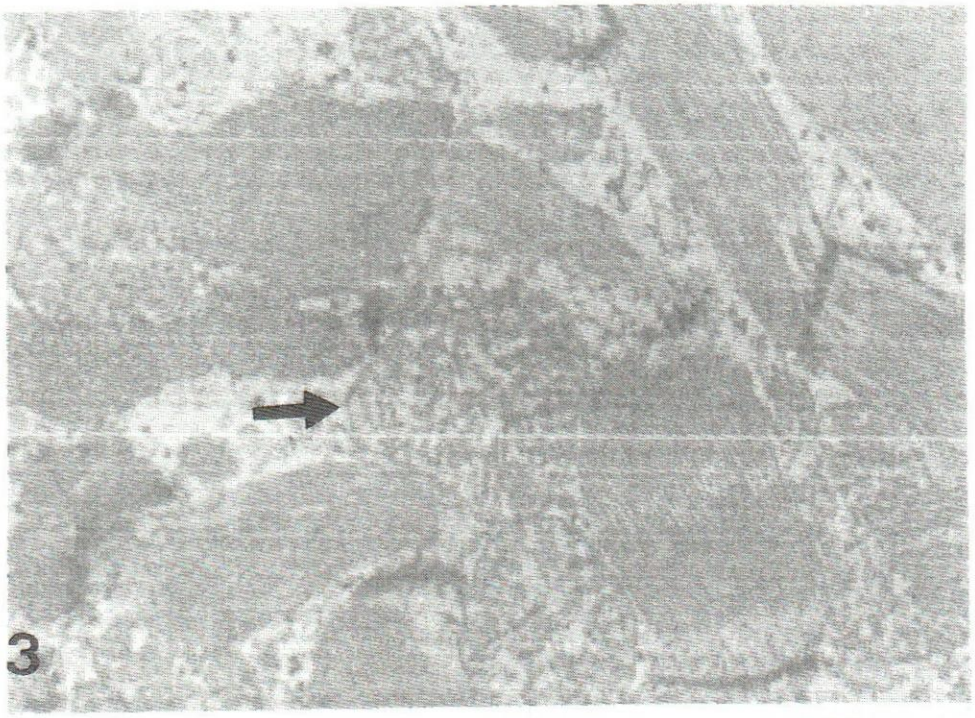
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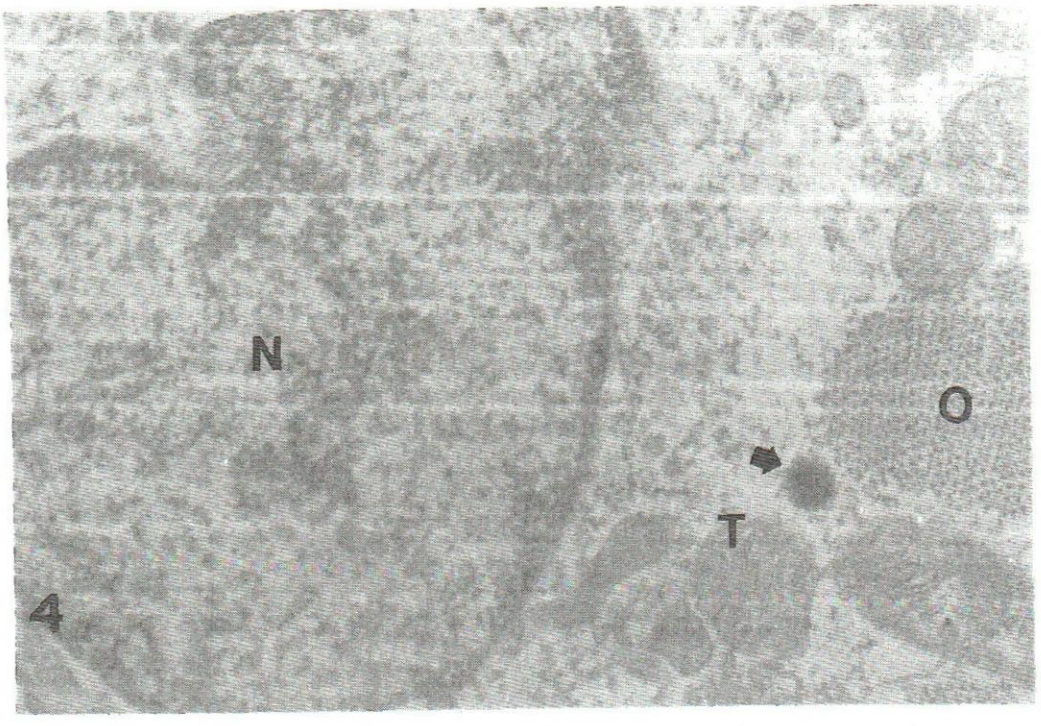
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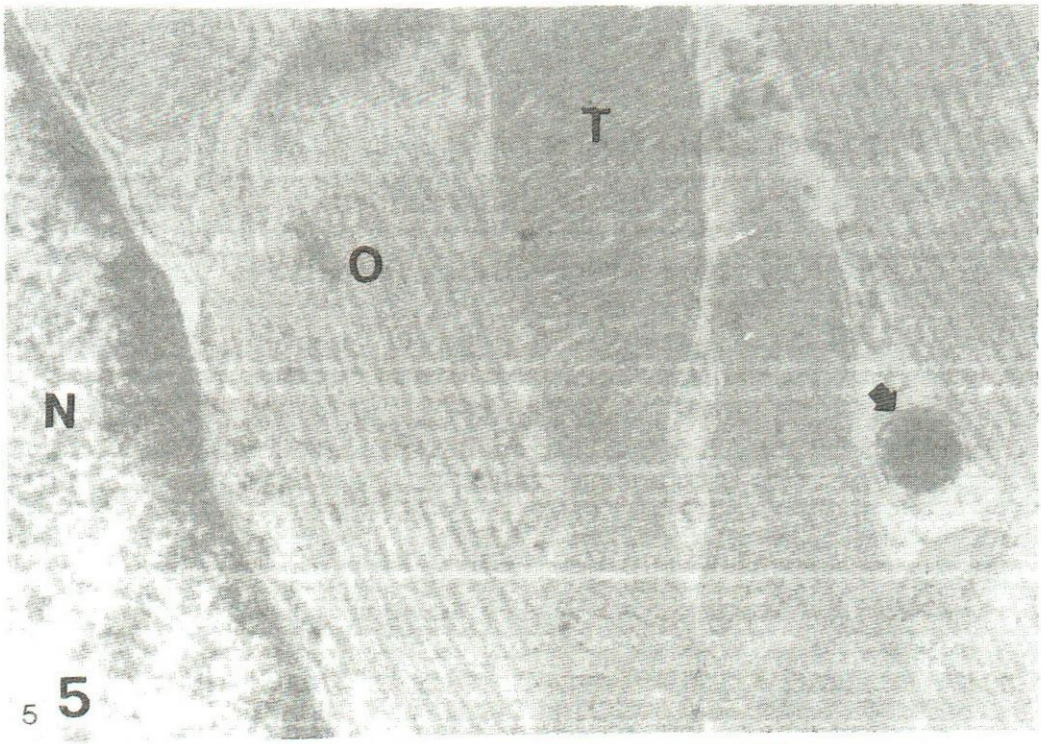

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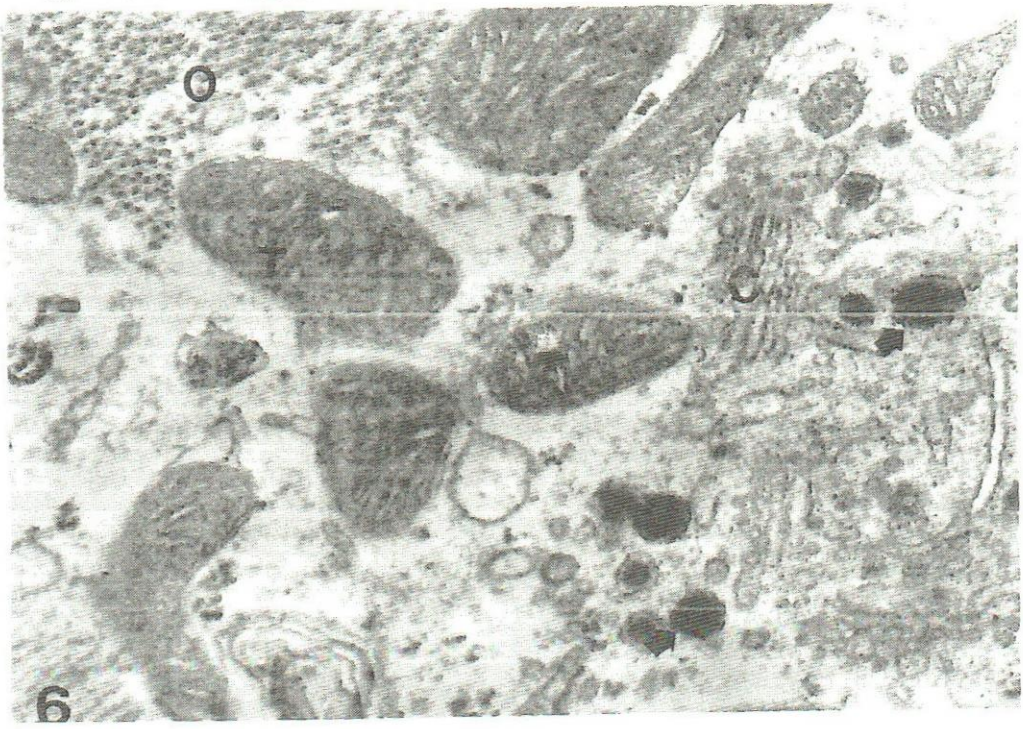

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