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CONTRIBUTION TO THE PROBLEM OF THE SPECIFICITY OF THE ZIO IMPREGNATION*

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Introduction

As the results of the ultrastructural investigations accepted almost universally, in the later years evermore perfect varieties of the electron microscopic methods of fixation, embedding and impregnation have been elaborated. Nevertheless, as compared with the number of visualizing procedures tried and found suitable for light microscopes, there are comparably few methods adapted to electron microscopic examinations. Some fundamental methods (OsO_4, $KMnO_4$, aldehydes, negative ways of staining, etc.) are suitable for fixing all the components of the animal and plant tissues and cells recognized so far with a by and large unequivocal result. In the later decades electron histochemistry became a wide-spread way of investigation in order to reveal the chemical structure of the components of cells. Having recognized many components of tissues and cells fundamentally, the importance of histochemistry is recently growing for the methods often enable us to study the structures biochemically, enzymatically, pharmacologically, etc.

The procedure of zinc iodide — osmium tetroxide impregnation (ZIO) investigated by us is similarly of light microscopic origin (MAILLET, 1959; 1962; JABONERO, FABRA, MOYA and JABONERO, 1961; JABONERO, 1964; THIES, 1964; RODRIGUEZ-PÉREZ, 1964), applied lately also on electron microscopic level (AKERT and SANDRI, 1968; PELLEGRINO DE IRALDI and GUEUDET, 1968; 1969; MARTIN, BARLOW and MIRALTO, 1969; NIEBAUER, KRAWCZYK, KIDD and WILGRAM, 1969; LAMPARTER, STEIGER, SANDRI and AKERT, 1969; KAWANA, AKERT and SANDRI, 1969).

Since the beginning of the application of ZIO impregnation, the researches have been interested in the problem, what is in fact, impregnated by ZIO, resp. in wich sense this method may be considered to be specific. The most literary data report only on ZIO impregnated nerve components (nerve fibers, synaptic vesicles), but there are also results that have not supported this kind of specificity of the reaction. In our present work we want to make known our results obtained in various nerve structures concerning the specificity of ZIO impregnation.

* This investigation was performed while the Electron Microscope Laboratory was in the framework of the Faculty of Sciences, Attila József University, Szeged.

Plate I

Light microscopic picture of a rat iris after ZIO impregnation for five hours. The arrows point to the varicosities of the nerve fibers. (x 520)

Materials and Methods

For determining the specificity of ZIO reaction we have performed structural investigations on the corpus pineale and iris of full-grown rats, as well as on the superior cervical ganglion (SCG) of the cat. The investigations were carried out by impregnating with ZIO solution, prepared according to AKERT and SANDRI's (1968) method at a temperature of 0—4°C for different lengths of time (1, 2, 5 and 7 hours). The way of preparing the ZIO solution is as follows: the reaction of 7,5 p.c. Zn + 2,5 p.c. J_2 being realized in a wet medium, the solution obtained is mixed with the 2 p.c. diluted solution of OsO_4 in a ratio of 4 to 1, immediately before application. Here we note that in earlier investigations there were generally longer impregnations (16—20 hrs) applied and this method is followed by MADARÁSZ and co-workers, too, in the

Anatomical Institute of the University Medical School of Budapest during their similar investigations. The matter prepared for the electron microscopic study after the usual alcoholic dehydration was embedded in Araldit (PEASE, 1964). It was contrasted in block (during dehydration) with 3—4 p.c. uranyl acetate dissolved in 70 p.c. ethanol, then on the section according to REYNOLDS's (1963) method and our pictures were taken by electron microscope Tesla BS 242 D, as well as by Tesla BS 413. We have carried out light microscopic examinations on iris removed from rat in physiological NaCl solution and impregnated in ZIO for five hours.

Results

After impregnating the iris of the rat with ZIO — separating sharply from the adjacent elements of the connective tissue — we could reproduce light microscopically the staining of nerve fibers (Fig. 1). The nerve fibers run in bundles, in some places we could well observe the ZIO positivity of the so-called varixes in the nerve fibers, as in the case of silver impregnation. After a ZIO impregnation for 2 hours in the electron microscopic pictures of the corpus pineale of the rat, the electron-dense granules that demonstrated the site of ZIO positivity were found mainly in the vesicular regions of the nerves (Fig. 2). The average size of the ZIO-positive granules is 300—500 Å, often — but not always — localized in the wall or interior of the synaptic vesicles. Also in case of the two-hours impregnation we have observed the mitochondria being moderately swollen, supposedly owing to the low pH of the impregnating medium.

An impregnation lasting for a longer period of time considerably decreases the specificity of ZIO reaction and its elective axonal localization, changing even its granularity of usual size. In this case we often get reaction product of finer distribution of appearing in larged spots. After an impregnation of three hours, in the superior cervical ganglion of the cat the ZIO positivity has appeared, apart from the nerve fibers, on the nuclear membrane of Schwann cell, too (Fig. 3). Besides the granules, however, positivity also appears in spots, mainly cytoplasmatically. The size of ZIO-granules that can sometimes be observed in the perinuclear sites, as well, approaches that of the granules which are connected with the clear synaptic vesicles. At the same time, activity may be observed in the axolemma and in the myelin sheath, as well, appearing in the form of minor clods and being expressed particularly on the internal lamellae (Fig. 4).

After being impregnated for seven hours, the reaction of the endoplasmatic reticulum in the ganglion is remarkable (Fig. 5). It is well discernible in photographs of higher magnification (Fig. 6) that the ZIO-granules that are situated in the cysternae of the endoplasmatic reticulum do not fill compactly the tubules but there are ZIO-reactive elementary units of about 80 Å in diameter surrounding non-reacting units that may sometimes have 300—400 Å diameters. We want to notice that earlier Csillik and KNYIHÁR (1968), studying the electron microscopic localization of AChE similarly in the ganglion of the cat, described inactive endoplasmatic units. On the basis of the similar morphological pictures, it can be imagined that the same endoplasmatic units were visualized with the ZIO impregation, as well. In these preparations of ours also the perinuclear ZIO positivity is strong, appearing on the membrane forming both walls of the cysterna and in the gap itself, too. Also some mitochondria got stained and there may be observed in the cytoplasm also a large number of ZIO-positive granules that cannot be localized in a structural element.

Discussion

Several literary data are available to evaluate the specificity of ZIO reaction. The method of impregnation used, after the original KJ—OsO_4 staining (CHAMPY, 1913) was modified, in the form of ZnJ_2—OsO_4 first on light microscopic level (MAILLET, 1959), proved to be suitable mainly for visualizing the nerve fibers and nerve terminals (MAILLET, 1959, 1962; JABONERO, FABRA, MOYA and JABONERO, 1961; JABONERO, 1964). JABONERO (1964) emphasises that he has not succeeded in staining the neurons of the superior cervical ganglion. RODRIGUEZ—PÉREZ (1964), however, seems to have already stained in his adrenal gland preparation not only pericellular plexuses but cytoplasm, as well. THIES (1964) showed the so-called dendrite cells and the melanocytes, too, with ZIO in the human skin.

After applying the ZIO impregnation electron microscopically, AKERT and SANDRI (1968) reveal nerve impregnations. Later PELLEGRINO DE IRALDI and GUEUDET (1968, 1969) discuss the ZIO positivity observed also in the layer of rods and cones in the photoreceptor cells in the retina of the rat. NIEBAUER, KRAWCZYK, KIDD and WILGRAM (1969) stained the granules of the epidermal LANGERHANS cells, GOLGI zone and the nuclear membrane at patiens suffering from the LETTERER-SIWE disease. Simultaneously with the above investigations, the question of the specificity of ZIO was also raised. MAILLET (1959, 1962) and, following him, NIEBAUER, KRAWCZYK, KIDD and WILGRAM consider to be probable that the lipid-like matters are stained. According to AKERT and SANDRI (1968) the cholinergic mechanism is supposedly indicated by ZIO positivity and, essentially, this supposition is supported also by the work of MARTIN, BARLOW and MIRALTO (1969), opposite to the more recent investigation of KAWANA, AKERT and SANDRI (1969). It is worth noticing that the dense-core vesicles do not take up the stain while reserpin inhibits the ZIO positivity of the nerve fibers (PELLEGRINO DE IRALDI and GUEUDET, 1968).

In our present investigations, with an impregnation lasting only for a short time, we have succeeded in showing electively ZIO positivity localized in the nerve fibers and mainly in the clear synaptic vesicles of the corpus pineale of the rat. With the help of that, apart from the impregnation of nerve fibers by a long-lasting staining, we have demonstrated the ZIO activity appearing in the ganglion cells of the superior cervical ganglion of the cat that presented

Abbreviations:

Ax	axon	N	nucleus
Coll	collagen	M	mitochondrium
Cy	cytoplasm	My	myelin
Er	endoplasmatic reticulum	Sch	Schwann cell

Plate II

Corpus pineale of the rat. In the dilated gap of the connective tissue that separates the pinealocytes from each other several nerve fibers can be observed. ZIO positivity can be found exclusively in the nerve fibers. (x 32 000)

Plate III

Superior cervical ganglion of the cat after ZIO impregnation for three hours. The ZIO positivity can be found in „aspecific" sites, too: in the perinuclear cysterna of Schwann cell, on the axolemma and, non-bound to structure elements in the cytoplasm (arrows). (x 40 000)

Plate II

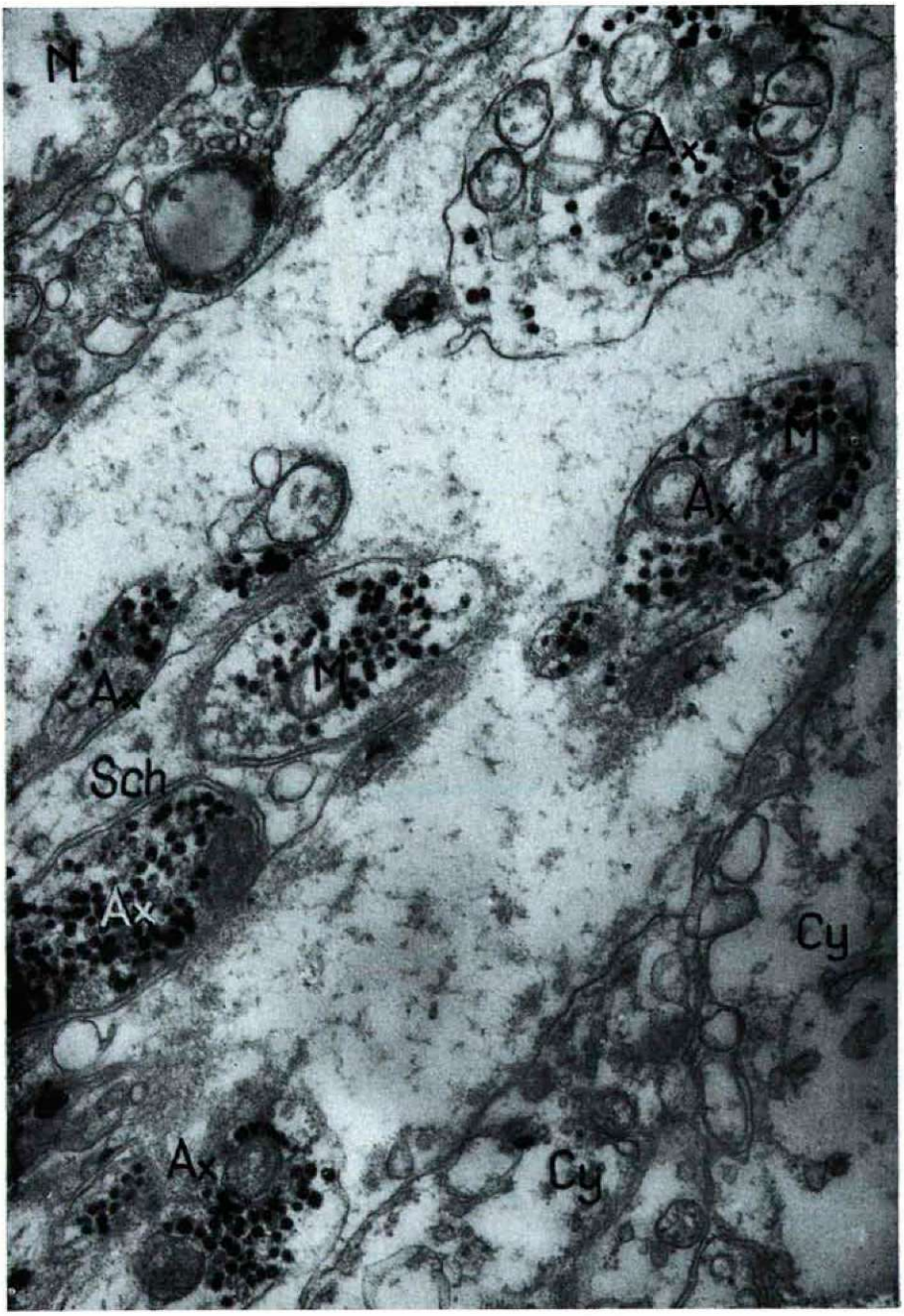
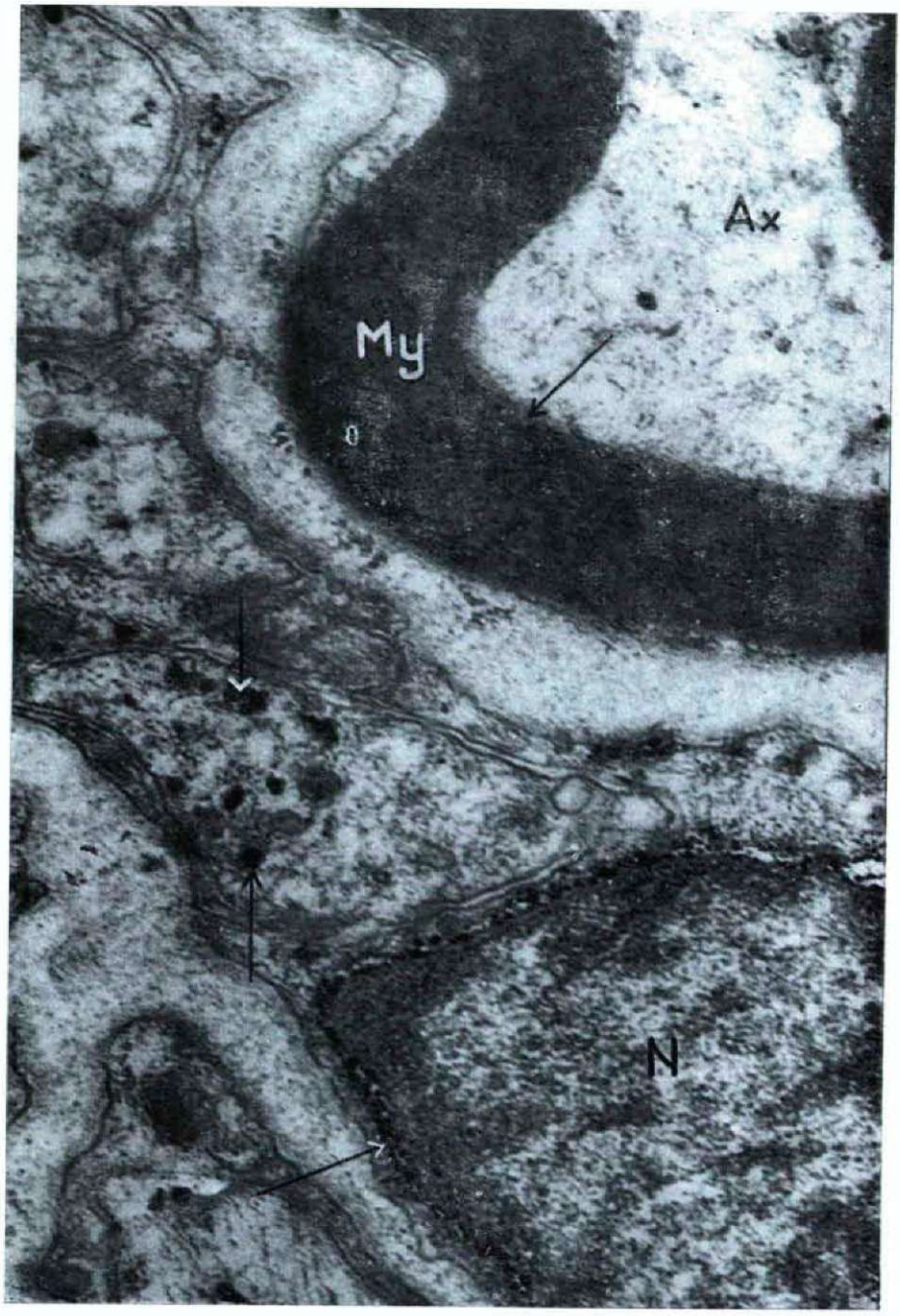


Plate III



itself strongly in the perinuclear cysterna and in the endoplasmatic reticulum, and it could be observed also mitochondrially, as well as in the cytoplasm, too, sporadically. In the some place, also nuclear membrane and processes of Schwann cells gave a strong reaction, and also the lamellae of the myelinated nerve fibers showed themselves active.

As compared with the literary data we may summarize the results of our investigations so that the ZIO method stains not only the nerve fibers and synaptic vesicles specifically, but after an impregnation for a longer period of time, it stains, in addition to the above mentioned ones, also several other elements of cells and nerve fibers. At any rate, further investigations are needed for recognizing the similarity of the chemical structure wich equally results in the ZIO positivity of the structures of different functions.

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Plate IV

SCG of the cat, after ZIO impregnation for three hours. The ZIO-positive electron-dense precipitate is bound, besides the synaptic vesicles of the axons, to several structure elements (arrows). (x 40 000)

Plate V

A: Ganglion cell from a cat's SCG, after ZIO impregnation for seven hours. ZIO positivity can be observed freely in the perinuclear cysterna (arrows), the endoplasmatic reticulum of the ganglion cell, as well as in the cytoplasm (arrow marked with an asterisk). (x 21 000)
B: Picture of a SCG ganglion cell of the cat after ZIO impregnation for seven hours. The granules of ZIO positivity having, on the average, 80 Å diameter surround in some places non-reacting units of a size 300—350 Å (arrows). (x 130 000)

Plate IV

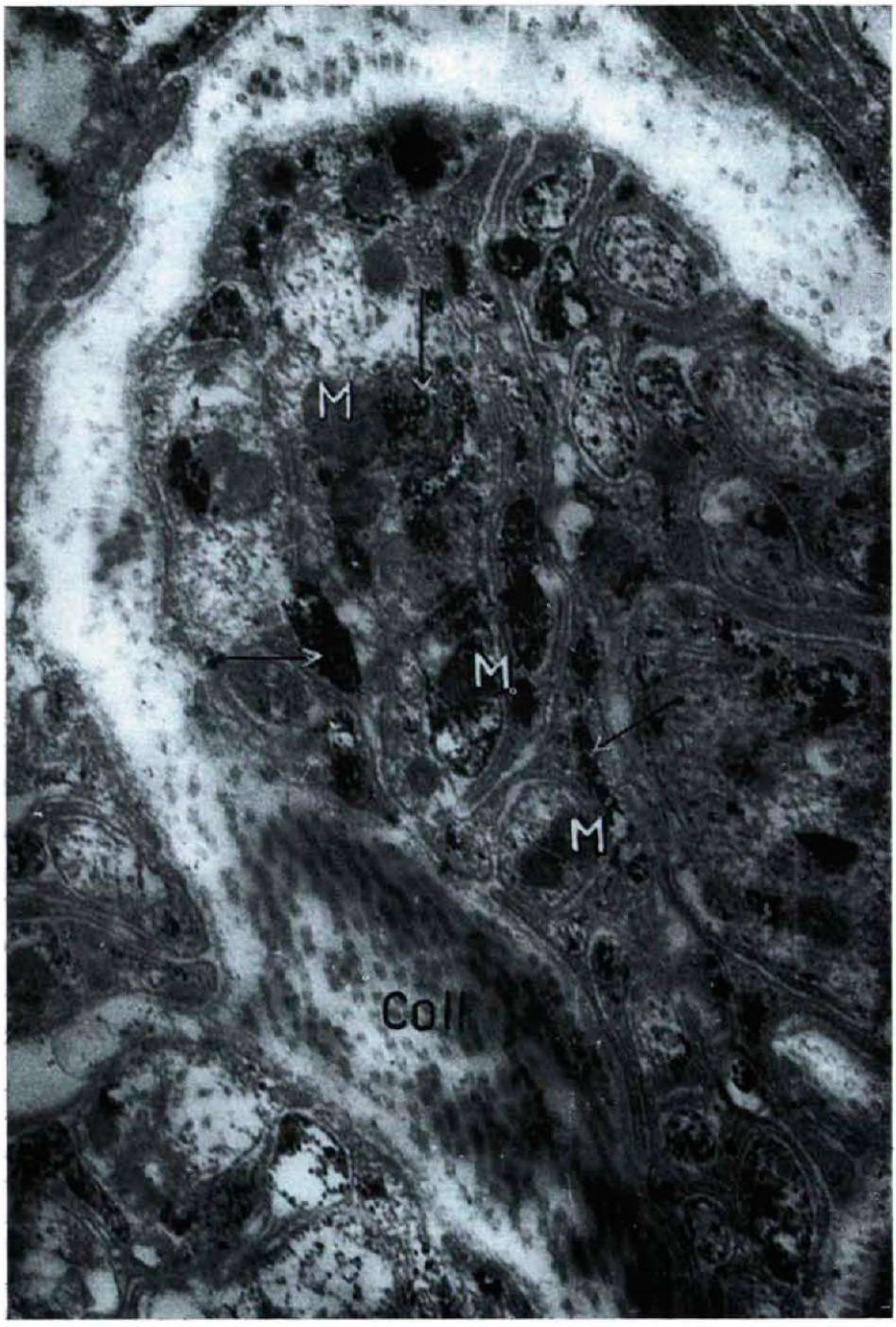
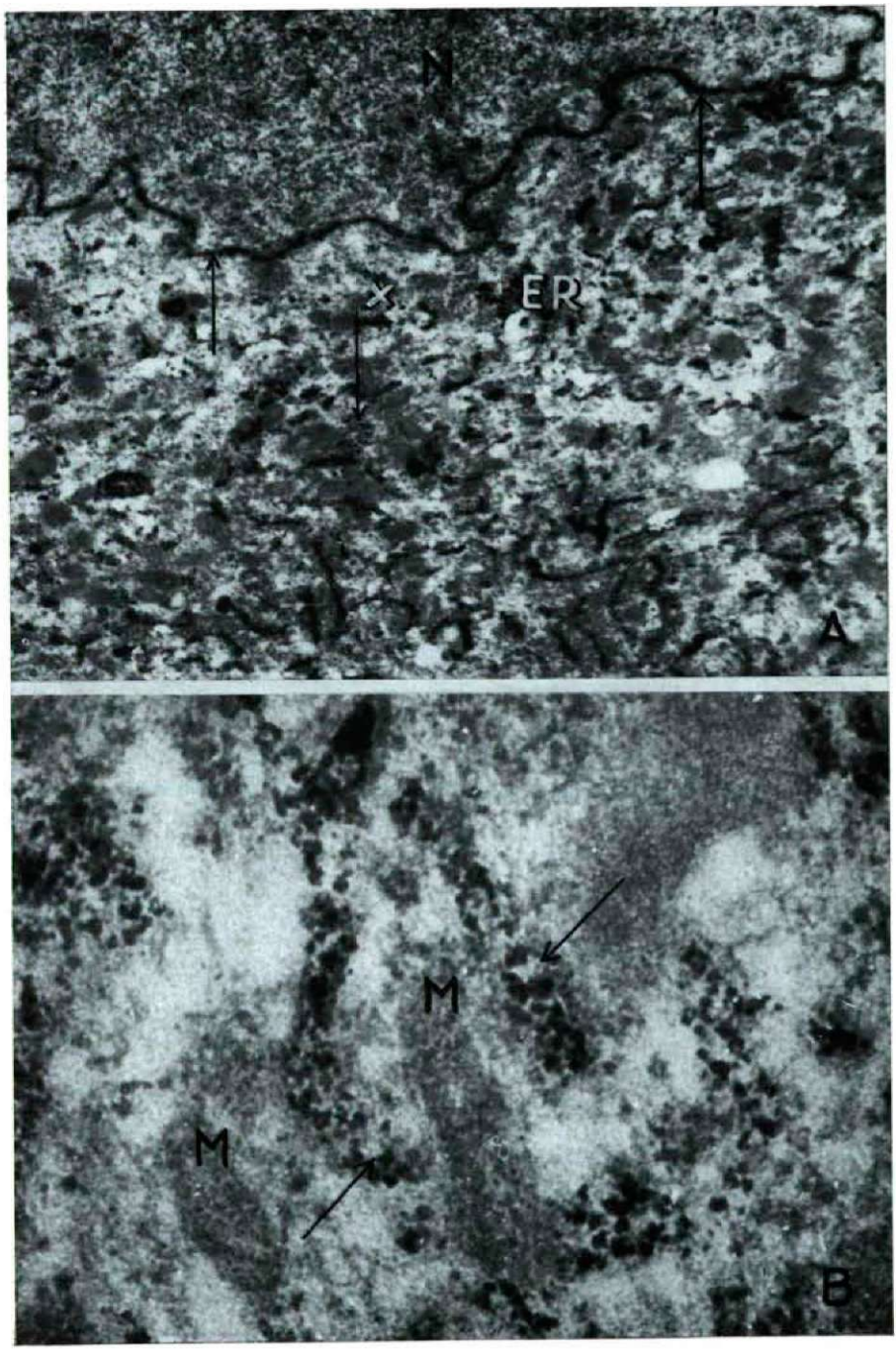


Plate V



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