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Electron microscopic observations on the structure of the rat adrenal medulla

I. The ultrastructure and organization of chromaffin cells in the normal adrenal medulla

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INTRODUCTION

Reports on the ultrastructure of chromaffin cells in adrenal glands include those of Lever (1955) and Eränkö & Hänninen (1960) on the rat, Sjöstrand & Wetzstein (1956) and Wetzstein (1957) on the mouse, cat and guinea-pig, De Robertis & Vaz Ferreira (1957*a*, *b*) on the rabbit, De Robertis & Sabatini (1960), Yates, Wood & Duncan (1962) and Michel-Béchet, Cotte & Haon (1963) on the hamster, Kano (1959) and Fujita, Kano, Kunishima & Kido (1959) on the chick, while Burgos (1959) described the normal structure of the frog adrenal. In these works chromaffin cells were recognized in all specimens examined by virtue of their content of osmophilic secretion granules; they also contained the usual cytoplasmic organelles including mitochondria, Golgi apparatus and endoplasmic reticulum. Lever (1955) described light and dark cells which he concluded represented different secretory states of a chromaffin cell. Three forms of chromaffin cells were identified by Eränkö & Hänninen (1960); these were said to differ in their relative contents of chromaffin granules, mitochondria and 'vesicle-containing bodies'. These three forms were thought to represent different secretory stages of chromaffin cells. Lewis & Lever (1958) reported a variation in amount of endoplasmic reticulum as well as in electron-dense contents of chromaffin granules during the chromaffin cell secretory cycle.

Wetzstein (1957) claimed to have identified two types of chromaffin cells in the mouse, guinea-pig and cat adrenal medulla. The most numerous form was referred to as the chief cell (Hauptzellen) while the other was named the accessory cell (Nebenzellen). Accessory cells were said to have fewer, but larger chromaffin granules, a more osmophilic nucleus and more closely packed endoplasmic reticulum than the chief cells. Wetzstein suggested that the chief cells were adrenaline-storing elements and that the accessory cells stored noradrenaline. Yates, Wood & Duncan (1962) have also claimed to have distinguished two types of cells in the hamster adrenal medulla, one being adrenaline-storing and the other noradrenaline-storing. The adrenaline-storing (A-cells) were said to have slightly electron dense cytoplasm, osmophilic secretion granules of 700–1000 Å diameter and to be mainly peripheral in distribution. The cytoplasm of the noradrenaline-storing cells (N-cells) was said to show greater electron density and to contain larger chromaffin granules (1000–3500 Å).

Michel-Béchet *et al.* (1963) described four types of cells in the hamster adrenal.

Three of these were thought to represent different functional states of chromaffin elements while the fourth type was an adreno-cortical cell. These latter workers were unable to distinguish between A- and N-cells and suggested that a gradual transition from one cell type to the other occurs.

Cilia were observed arising from chromaffin cells in the hamster adrenal by De Robertis & Sabatini (1960), but few structural details were given.

In the earlier works on the electron microscopy of the mammalian adrenal medulla little or no attention was paid to the adrenocortical cells which form a constant feature of the medulla in the various species examined. These cortical elements were, however, recognized by Michel-Béchet *et al.* (1963) in the hamster adrenal and described in some detail.

The present work is concerned primarily with the ultrastructure and general arrangement of the parenchymal elements of the normal rat adrenal medulla. Though some reference to nervous structures must inevitably be made, these elements will be considered in detail in a companion publication (Coupland, 1964).

MATERIALS AND METHODS

In this work adult male Wistar strain rats (200–250 g. weight) were used. Adrenal glands were removed from animals immediately after sacrifice by a blow on the head or from living animals anaesthetized by an intraperitoneal injection of Veterinary Nembutal. Immediately after removal from the animal the adrenal gland was sectioned with a thin stainless steel razor blade and slices of *c.* 1 mm. in thickness of the whole gland prepared with minimal trauma and immediately immersed in ice-cold fixative.

A variety of fixatives was employed during the course of this work. The most satisfactory were a sucrose-buffered osmium tetroxide mixture (Caulfield, 1957) or 5% buffered glutaraldehyde. The glutaraldehyde was buffered with either 0·1 M cacodylate or phosphate; this fixative has some advantages over osmium tetroxide since it will penetrate slices of 2–3 mm. in thickness, has less tendency to overfix the periphery of the tissues and, when followed by immersion in osmium tetroxide, allows one to differentiate between the adrenaline- and noradrenaline-storing elements (Coupland, Pyper & Hopwood 1964). In order to carry out this histochemical reaction for noradrenaline the tissues should be fixed for a minimum of 4 hr. in buffered glutaraldehyde before being transferred to cold buffered 1% osmium tetroxide (pH 7·3); the optimum time for immersion in osmium tetroxide is 1 hr. Following fixation by either of the above techniques, tissues were dehydrated and embedded in araldite and sectioned on the Cambridge–Huxley ultramicrotome. Sections were stained on the grid with 1% aqueous potassium permanganate and differentiated with 2·5% aqueous citric acid. Some tissues were fixed only in buffered glutaraldehyde.

RESULTS

The rat adrenal medulla is composed of two major types of parenchymal elements, viz. chromaffin cells and cortical cells which are associated with vascular, nervous and connective tissue elements.

General topography

Chromaffin cells are polyhedral (Pl. 1, fig. 1) and arranged in a compact epithelial fashion so that where these cells abut, they are usually separated from each other by a gap of only 150–200 Å in width. In regions where three or more chromaffin cells approach each other the 200 Å gap usually widens to form a junctional intercellular space of variable width (Pl. 1, figs. 1–3). This junctional space may contain granular material, microvilli, cilia and nerve fibres (Pl. 2, fig. 5), but does not contain connective tissue elements. Smaller intercellular spaces which may contain granules and microvilli are occasionally observed between two cells only and again represent an increased separation of plasma membranes of adjacent chromaffin cells which are usually only *c.* 200 Å apart. These smaller spaces may join the junctional spaces in adjacent areas. Junctional intercellular spaces may be traced towards the connective tissue spaces which lie adjacent to blood vessels, nerve fibres and cortical islands in the medulla, but the junctional space is always separated from the milieu of the connective tissue elements by a distinct basement membrane, which bridges the end of the space (Pl. 2, fig. 7).

Where chromaffin cells are in close apposition, desmosomes are frequently encountered (Pl. 1, fig. 1) and interlocking zones occasionally occur (Pl. 3, fig. 12). Desmosomes are often observed at the margin of junctional spaces (Pl. 1, figs. 2, 3) though this arrangement is not sufficiently constant to suggest that in this situation they form terminal bar-like structures.

Other aspects of chromaffin cells are related to blood vessels and connective tissue elements, nerve fibres, nerve cell bodies and groups of cortical cells (Pl. 2, figs. 4, 6, 7). The faces of chromaffin cells bordering connective tissue spaces are always covered by a distinct basement membrane which separates the two elements. In contrast cortical cells are not covered by a basement membrane and hence come into direct contact with the contents of connective tissue spaces (Pl. 2, fig. 6).

Bundles of nerves composed of both myelinated and unmyelinated elements (Pl. 2, fig. 6; Pl. 8, fig. 41) are surrounded by a variable amount of connective tissue stroma including fibroblasts, collagen fibres and an occasional macrophage. Nerve fibres are surrounded by Schwann cell cytoplasm throughout the majority of their course and are only bare at zones of contiguity (synapses) or for short distances within junctional intercellular spaces. Some Schwann cells (Pl. 3, fig. 13) send attenuated tongue-like processes between neighbouring chromaffin cells to reach and envelop distant nerve fibres. These Schwann cell processes are covered externally by a basement membrane where they are in contact with connective tissue elements. The basement membrane is absent where Schwann cytoplasm lies adjacent to nerve fibres or chromaffin cells. The constant association of the basement membrane with any aspect of a Schwann cell process which is in contact with a connective tissue space is of value in distinguishing between these structures and attenuated portions of fibroblasts (Pl. 2, figs. 6, 7; Pl. 8, fig. 41). Fibroblasts possess a better developed endoplasmic reticulum with associated ribosomes than do Schwann cells and do not envelop nerve fibres; they are not clothed by a basement membrane, but are commonly seen lying adjacent to large numbers of collagen fibres.

Collagen fibres are frequently encountered between the various parenchymal elements and show the typical structure. A few finer fibres which exhibit periodicity are also observed in some regions in glutaraldehyde-osmium-fixed material and may represent micro-collagen fibres (Pl. 6, fig. 27). The connective tissue stroma alongside blood vessels and nerve bundles is composed of collagen fibres and fibroblasts; these elements are also observed separating cortical and chromaffin cells (Pl. 2, figs. 4, 6; Pl. 6, fig. 29; Pl. 8, fig. 41). Macrophages are occasionally observed in the connective tissue stroma.

Blood vessels

The adrenal medulla is a highly vascular structure and blood vessels are a frequent occurrence in most sections. Three varieties of vessels may be recognized. First, channels lined with endothelium and separated from chromaffin cells only by a subendothelial space of 350–500 Å, an external basement membrane, connective tissue space free from collagen fibres, the basement membrane of the chromaffin cell and the submembranous space of 350–400 Å. In some regions the two basement membranes appear to fuse (Pl. 6, fig. 28). These vessels are continuous with the second type in which the endothelial lining is separated from chromaffin cells by the same number of layers, but in this form the connective tissue space is wider and contains collagen fibres and processes of fibroblasts and occasionally macrophages, which lie between two basement membranes. The first type of vessel corresponds to capillaries, the second to venous sinuses which are continued as veins. The transition from capillary to sinus to vein is insensible and associated with a gradual increase in supporting connective tissue. In some regions one side of the vessel has the characteristics of a type of one vessel (capillary) while the other side has the structure of a venous sinus or vein (Pl. 3, figs. 8, 9).

The third type of channel is readily distinguished from the others. It consists of an endothelial layer with associated basement membrane and is surrounded by a distinct layer of smooth muscle cells and with a variable amount of external connective tissue (Pl. 3, fig. 10). These vessels are the arteriae medullae. In these vessels each smooth muscle cell is surrounded by distinct basement membrane except in occasional areas where adjacent smooth muscle cells approach to within 200 Å of each other (Pl. 3, fig. 11).

Chromaffin cells

The plasma membranes show specializations in the form of microvilli (Pl. 2, fig. 5) localized invaginations and evaginations (Pl. 3, figs. 12, 13; Pl. 4, fig. 15) cilia (Pl. 7, figs. 30–34) and desmosomes (Pl. 1, figs. 1–3). The structure of the cilia will be considered in detail below. Microvilli are relatively few in number, sessile, usually on surfaces closely related to blood sinuses or junctional spaces and most numerous in the latter regions. Small localized invaginations of the cell surface may represent a point of previous discharge of a chromaffin granule, or pinocytosis. The larger invaginations may be produced by pre-terminal nerve fibres or nerve endings or together with corresponding evaginations of adjacent chromaffin cells represent a zone of cellular interlocking. Desmosomes usually take the form of simple thickenings of opposed surfaces, and less commonly exhibit a quintuple-layered form. They are frequently encountered.

In tissues fixed in buffered 1% osmium tetroxide, only one type of chromaffin cell may be identified with certainty and slight variations in the form of chromaffin granules or the appearance of nuclei and other organelles may usually be correlated with distance from the surface of the block and hence with degree of fixation. The majority of artefacts due to under- or overfixation with osmium tetroxide may be overcome by the use of buffered glutaraldehyde as this has better powers of penetration and less tendency to overfix. Furthermore, if fixation is allowed to proceed for 2–4 hr. subsequent transference to osmium tetroxide not only reveals details of cell membranes, but also acts as a histochemical method for the identification of noradrenaline-storing granules, the contents of which become homogeneous and intensely electron dense, while the adrenaline-storing elements show internal granularity and moderate electron density. Caulfield's buffered osmium tetroxide has a tendency to cause mitochondrial shrinkage, while glutaraldehyde may cause slight swelling and occasionally vesication of these elements.

In glutaraldehyde-osmium tetroxide fixed material the majority of cells contain granules which show only slight to moderate electron density; these are the adrenaline-storing cells (A-cells) (Pl. 2, figs. 4, 6; Pl. 3, figs. 8, 9; Pl. 5, figs. 20, 21).

The noradrenaline-storing cells (N-cells) are less numerous, occur singly or in small groups and contain typical intensely electron dense granules (Pl. 3, fig. 9; Pl. 4, figs, 17, 18). Both types of granules are membrane-bound and the A-cell granules are symmetrical with usually only a narrow peripheral halo separating the granular contents from the external membrane. The inclusion granules of the N-cells not uncommonly show asymmetry of contents which are homogeneously stained and non-granular (Pl. 6, fig. 23; Pl. 8, fig. 41), and which may adhere to the inside of the external membrane; the latter remains intact. In tissues fixed in buffered osmium tetroxide all chromaffin granules are rounded or ovoid and their granular electron dense constituents are usually symmetrically arranged. In tissues fixed in glutaraldehyde, with or without subsequent osmication, the granules of N-cells are asymmetrical and their electron-dense constituents are often displaced towards the periphery and adhere to the limiting membrane. The asymmetry of N-cell granules in glutaraldehyde-osmium tetroxide treated material must, therefore, result from the action of glutaraldehyde on the noradrenaline-storing granules and probably results from the chemical reaction which takes place between the aldehyde and the primary amine. The development of a noradrenaline-glutaraldehyde complex has recently been confirmed chemically (Coupland & Hopwood, unpublished observations) and details will be presented elsewhere. In glutaraldehyde-osmium tetroxide treated sections the entire contents of some A- and N-cells may be more electron dense than that of others (Pl. 2, fig. 4). The significance of this finding is unknown and it may reflect degree of fixation or osmication and hence be artefactual, or may reflect cell function.

Each A- and N-cell contains a single nucleus, which is usually rounded after fixation in buffered osmium tetroxide, and which may be rounded or show slight indentations in tissues fixed initially in glutaraldehyde (Pl. 1, fig. 1; Pl. 2, fig. 4; Pl. 4, figs. 16–18; Pl. 5, fig. 20). The nucleoplasm shows moderate diffuse granularity and there is often a narrow zone of greater electron density immediately within the inner nuclear membrane; this is most evident in glutaraldehyde-osmium

tetroxide-fixed material. Nucleoli are well defined, have the typical structure and up to three have been observed in a single cell (Pl. 4, fig. 16). The nuclear membrane has the typical three-layered structure and nuclear pores are commonly observed (Pl. 4, fig. 16; Pl. 5, figs. 20, 22). There is no difference in the staining properties of A- and N-cell nuclei (Pl. 4, figs. 17, 18). In some sections continuity between the outer layer of the nuclear membrane and endoplasmic reticulum has been noted.

The chromaffin cells contain a full complement of cytoplasmic organelles. These include mitochondria, multi-vesicular bodies, centrioles, Golgi membranes, endoplasmic reticulum and ribosomes, as well as dark bodies.

Mitochondria

Mitochondria are elongated bodies of 375–500 μ in width and up to 1 μ in length. The membranes show the typical arrangment and cristae are commonly observed and may often be shown to be continuous with the internal membrane (Pl. 5, fig. 19). The mitochondrial matrix is moderately electron dense (Pl. 5, figs. 19–21).

Endoplasmic reticulum

Endoplasmic reticulum occurs in the form of cisterns and tubules, the former being aggregated in some parts of the cell (Pl. 4, fig. 16; Pl. 5, fig. 20). The external aspect of these membranes is studded with ribosome granules of *c.* 150 Å diameter, Granules of a similar appearance may also be observed scattered irregularly in the cytoplasmic sap; these may also represent ribosomes.

Golgi membranes

The Golgi complex consists of a collection of smooth-surfaced membranes of tubular or vesicular profile which may be loosely arranged or aggregated. It lies in a para-nuclear position (Pl. 5, figs. 21, 22; Pl. 6, fig. 23). While many of the Golgi membranes enclose no electron-dense material, electron-dense particles may be observed in some small tubules or vesicles where they appear to accumulate initially in a peripheral position adjacent to the Golgi membrane (Pl. 5, fig. 22). Some tubular Golgi membranes show a terminal dilatation and here the membranes may be slightly thickened and show increased electron density (Pl. 5, figs. 21, 22; Pl. 6, fig. 23); some of these dilatations are surrounded by a narrow granular zone of increased electron density. Some of the membrane-bound collections of electron dense material associated with the Golgi complex have an appearance similar to small chromaffin granules. In tissues fixed in glutaraldehyde-osmium tetroxide, the membrane-bound collections of electron-dense substance in the Golgi zone of A-cells resemble chromaffin granules (Pl. 5, fig. 21). In the N-cells, however, some of this electron-dense material fails to show the homogeneity and intense electron density of the N-cell granules (Pl. 6, fig. 23).

Multi-vesicular bodies

One or two multi-vesicular bodies are commonly observed in a chromaffin cell irrespective of its functional type. These bodies show a single peripheral membrane and contain a variable number of small vesicles and not uncommonly a patch of

moderately electron-dense granular material (Pl. 5, fig. 21; Pl. 6, fig. 24; Pl. 7, fig. 32); the amount of granular material appears to be inversely proportional to the number of small vesicles.

Dark bodies

Dark bodies are also bounded by a single peripheral membrane, contain granular material of varying degrees of electron density and organization (Pl. 1, fig. 1; Pl. 3, fig. 13; Pl. 6, fig. 25) and in some cases small vacuoles. Vacuolation of these bodies is more marked in stimulated cells (Coupland, unpublished observations) and the general form and appearance of these structures would suggest that they correspond to lysosomes. An electron-histochemical study of these elements is currently being pursued.

Chromaffin granules

The most typical feature of the normal chromaffin cell is the presence of numerous membrane-bound cytoplasmic granules which contain a variable quantity of electron-dense material. As indicated above the appearance of the granules depends to some extent on the method of fixation. In tissues fixed in buffered osmium tetroxide, the granular material is moderately electron dense and usually arranged in symmetrical fashion within a limiting membrane. In the glutaraldehyde-osmium fixed tissues the contents of the N-cell granules are intensely electron dense and usually homogeneous and often eccentrically situated within the membrane (Pl. 6, fig. 22; Pl. 8, fig. 40). A-cell granules resemble those seen in osmium-fixed material. In ultrathin sections of tissues fixed in buffered osmium tetroxide, the electron-dense constituent of many chromaffin granules is distinctly granular and has a particle size of *c.* 100 Å diameter. The limiting membrane has a typical three-layered (unit membrane) structure; this may be in direct contact with the contents or may be separated from the electron-dense internal substance by a peripheral halo of variable width.

The chromaffin granules are scattered throughout the cytoplasm, except in regions occupied by specific organelles, and they do not have a specific association with endoplasmic reticulum. The external membranes of some granules, which lie in peripheral parts of cells, show varying degrees of contact and fusion with the plasma membrane of chromaffin cells (Pl. 6, figs. 26, 27). In other regions true fusion is evident and the granular contents can be observed being discharged in the immediate vicinity of the cell (Pl. 6, figs. 28, 29). Opposite the point of discharge, the adjacent basement membrane not uncommonly shows a slight invagination or thinning. In consequence of this method of discharge only the granular contents of the individual chromaffin granules are released. Since this discharged material is never observed lying free in the perivascular connective tissue spaces or within the endothelium of the vessel walls it would appear that once released it is rapidly broken up. This method of discharge has been observed on the free surface of chromaffin cells adjacent to blood vessels and connective tissue spaces as well as on those surfaces which border junctional intercellular spaces; hence granule contents may enter the latter spaces. Small caveolae (Pl. 4, fig. 15) without evidence of recent discharge of granular material, may be observed on all aspects of chromaffin cells,

including those which border a 200 Å intercellular space; these may represent pinocytosis or a late stage in granule extrusion. In the rat there is no marked difference between the size of granules in A- and N-cells and both show a range of 50–350 μ. The apparent size of granules as estimated from electron micrographs depends upon the plane of section as well as absolute size of individual granules. Some granules have an oval rather than a rounded profile (Pl. 5, figs. 20, 21). Some of the smallest granules are found in the vicinity of the Golgi complex.

Cilia

Single cilia have been observed in association with both the A- and N-cells (Pl. 7, figs. 35, 36). They are of frequent occurrence in any section through the adrenal medulla and, allowing for the geometric factors involved in sectioning, their frequency suggests that the majority if not all chromaffin cells possess a single cilium.

Some cilia have been observed arising deep within chromaffin cells (Pl. 7, fig. 33), the more distal centriole usually being modified to form a basal body (Pl. 7, fig. 34). Others arise from a more peripheral site where the cilium is attached to a distinct basal body which may or may not be associated with a definite centriole. Cilia which have a deep origin reach the surface of the chromaffin cell by passing through an intracellular tunnel (Pl. 7, figs. 32–36), the outer wall of which is formed by the plasma membrane of the chromaffin cell; they eventually protrude into a junctional intercellular space. The space which surrounds the shaft of a cilium as it passes through the tunnel is directly continuous with that of the junctional space. Cilia which arise more peripherally run almost immediately into a junctional intercellular space (Pl. 7, figs. 30, 31). All cilia observed in association with chromaffin cells have possessed a central pair of fibrils and six to eight pairs of peripheral fibrils, the 8 + 2 configuration being the more common (Pl. 7, figs. 35–37). Since the plane of section is commonly oblique it is sometimes difficult to make an exact count of peripheral fibrils. The peripheral fibrils may be followed to a basal body and are continuous with the peripheral fibrils of this structure (Pl. 7, figs. 31, 33, 34) which is often a modified distal centriole. Granular electron-dense material surrounds the fibrils of the basal body, though no base plate exists. Lateral extensions of fibrillar or granular nature may be traced from the basal body into the surrounding cytoplasm (Pl. 7, figs. 31, 33).

Centrioles

Centrioles have been observed in chromaffin cells, but where two have been identified in the one cell, one of these has always acted as basal body for a cilium. Proximal centrioles are not modified to form basal bodies and possess the usual nine groups of three peripherally situated fibrils (Pl. 8, fig. 38).

Intracellular fibrils or tubules

Small numbers of randomly orientated elongated profiles which may be either fibrils or tubules have been observed in the cytoplasm of both A- and N-cells (Pl. 6, fig. 23; Pl. 8, figs. 38, 41). These do not show any definite periodicity and have a diameter of 160–180 Å. They may occur in any part of the ergastoplasm.

Cortical cells

Cortical cells may be readily identified since their cytoplasm is mainly occupied by large rounded or ovoid mitochondria (Pl. 2, figs. 4, 6; Pl. 8, figs. 39, 41) while inclusions are fewer and larger than chromaffin granules and usually contain moderately electron-dense material of a lipid nature. They occur in small groups and some exhibit uniform diffuse moderate electron density while others are less dense; the terms 'dark' and 'light' cells may, therefore, be applied to these elements (Pl. 8, fig. 39). The mitochondria of all cortical cells show well-defined double peripheral membranes and numerous internal tubules or vesicles rather than cristae. The Golgi zone is well defined. Membrane-bound dark bodies are numerous and of similar structure to those present in the non-stimulated chromaffin cell; they must be distinguished from chromaffin granules. The lipid inclusions are membrane-bound and the majority of the contents are only slightly electron dense and are homogeneous or finely granular; occasionally small, irregular, highly electron-dense masses are present in these inclusions along with the other material (Pl. 8, fig. 39). Endoplasmic reticulum is scanty and ribosomes few in number.

The free surfaces of these adrenocortical cells are often studded with microvilli (Pl. 8, fig. 41). Since there is no covering basement membrane cortical cells are in direct contact with adjacent connective tissue spaces.

Each cortical cell possesses a single round or ovoid nucleus which is bounded by a distinct double nuclear membrane and usually contains a single nucleolus. The nucleoplasm is diffusely granular and in some areas, in particular around the periphery, zones of increased electron density may occur and often lie adjacent to the nuclear membrane (Pl. 2, fig. 4; Pl. 8, fig. 39). A patchy density of this degree is not usually observed in chromaffin cells and the nuclei of cortical elements are, therefore, often more electron dense than those of chromaffin elements. The perinuclear space of cortical cells is wider and more electron translucent than that of the chromaffin cells; this difference is best seen in tissues fixed by the glutaraldehyde-osmium tetroxide technique.

DISCUSSION

The appearance of electron-dense granular material within the Golgi membranes, its apparent transformation into chromaffin granules, and the discharge of the electron-dense contents of chromaffin granules at the cell surface with fusion of the granule membrane with that of the plasma membrane was noted by De Robertis & Vaz Ferreira (1957*a*) and De Robertis & Sabatini (1960). Present results are in accord with this hypothesis on the method of formation and discharge of chromaffin granules.

In material fixed in glutaraldehyde and subsequently stained with osmium tetroxide, two types of membrane-bound chromaffin granules may be distinguished in the normal rat adrenal medulla (Coupland *et al.* 1964). One is concerned with the storage of noradrenaline, the other with storage of adrenaline; as indicated above, these granules may differ in both staining properties and appearance. The noradrenaline-storing granules contain a highly electron-dense and usually homogeneous substance which is often arranged eccentrically within the limiting mem-

brane. Only the high electron density is a constant feature since some granules do not show eccentricity. The apparent concentration of electron-dense material in one part of the granule may be due to shrinkage of the contents away from the limiting membrane or to distension and distortion of the membrane without overall change in diameter of the electron-dense contents, or to a combination of both factors. It is, however, an artefact due to the reaction between glutaraldehyde and noradrenaline since it is observed in granules fixed only in glutaraldehyde or by the glutaraldehyde-osmium tetroxide sequence, but not in those fixed in buffered osmium tetroxide.

The granules of adrenaline-storing cells show a fine internal granularity, are less electron dense and the contents more symmetrically arranged; the concentration of these fine internal granules is often greater near the centre than at the periphery and a clear halo may separate them from the limiting membrane. It is of interest to note that in the A-cells, no granules are observed which react for noradrenaline and hence it would appear that, in these cells, the granule-bound catecholamine is stored largely, if not exclusively, as adrenaline. These findings suggest that during catecholamine synthesis noradrenaline either has an extremely short half-life, or exists in a relatively unbound form in the cell sap.

The occurrence of membrane-bound granular material of similar electron density to that of the A-cell granules in the Golgi region of the N-cells is also of interest. In order to explain this occurrence or, indeed, to consider the various possibilities it is necessary to consider the chemical composition of chromaffin granules. Hillarp (1959) has shown that the non-aqueous part of bovine chromaffin granules is composed of 20% catecholamine, 35% protein, 15% ATP and 22% lipids and it would seem reasonable to assume that rat chromaffin granules have a similar chemical composition, even though the proportions may vary. Physicochemical considerations suggest that the protein and ATP of the granules may function as binding substances for the catecholamines.

Comparison of material fixed in glutaraldehyde alone with that fixed in glutaraldehyde-osmium tetroxide indicates that A-cell granules are no more electron dense after the combined treatment. The electron density of A-cell granules in these preparations does not, therefore, depend on the reaction between catecholamine and osmium tetroxide and furthermore it is no greater than that of many cell membranes or other cell contents. The moderate density of the A-cell granules in glutaraldehyde-osmium tetroxide fixed material may, therefore, be due to binding substances.

If the granular material of moderate electron density present in the membranes and vesicles of the Golgi zone of N-cells represents an early stage in chromaffin granule formation the substance present may be binding material less catecholamine or binding material associated with adrenaline. From the work of Goodall & Kirschner (1957) on the sequential conversion of dopa→dopamine→noradrenaline→adrenaline it would appear that the former possibility is the more likely. Hence it is possible that the binding substance may begin to accumulate before noradrenaline is added though this in no way excludes the possibility that the two elements are added simultaneously to larger granules. Some membrane-bound collections of granular substance found in the Golgi zone may represent an early stage in the formation of either dark bodies or multivesicular bodies.

Apart from the difference in staining properties of chromaffin granules in the A- and N-cells, no specific difference has been observed between the size range of chromaffin granules or staining properties of cell organelles associated with the two cell types. Thus both possess mitochondria, endoplasmic reticulum, ribosomes, a well-defined Golgi zone, multivesicular bodies and dense bodies. The multivesicular bodies are small, bounded by a single unit membrane, and in addition to small vesicles many contain a variable quantity of moderately electron-dense granular material; vesicles and granular material show an inverse relationship to each other. They do not resemble the structures in the rabbit adrenal referred to by De Robertis & Vaz Ferreira (1957*b*) as 'multivesicular catechol-containing bodies'; since these had a double external limiting membrane they may well have been nerve endings or pre-terminal portions of nerve fibres containing the two types of synaptic vesicles (Coupland 1964). The multivesicular bodies described and illustrated by Eränkö & Hänninen (1960) closely resemble cortical mitochondria, and in this respect it should be noted that mixing of the contents of chromaffin and cortical cells may readily occur as a result of mechanical trauma during slicing and fixation of tissues. In damaged preparations of this type plasma membranes may not always appear to be disrupted in a single thin section, though serial sections reveal discontinuity. Hence cortical mitochondria may occasionally be seen within what otherwise appears to be an intact chromaffin cell or chromaffin granules may be observed within an apparently intact cell which contains many cortical organelles. These artefacts may be largely prevented by allowing the adrenal slices to fix for 5–15 min. at 0–4° C., before cutting the tissues into smaller pieces.

Dense bodies are present in all chromaffin and cortical cells and are bounded by single external membrane. Morphologically they resemble the elements now commonly referred to as lysosomes (for review see De Duve, 1959; Novikoff 1961). The association of lysosomes with hydrolytic enzymes has been clearly demonstrated by the above workers and it is possible that the dark bodies of chromaffin cells serve as the site of intracellular storage of acid phosphatase, an enzyme known to be present in large quantities in the adrenaline-secreting cells of the rat adrenal (Eränkö, 1955). Having regard to the marked difference in the intensity of the acid phosphatase reaction in A- and N-cells, one might expect that, if the dark bodies do store acid phosphatase, the A-cells would contain a greater number of these bodies than the N-cells. No evidence in favour of such a variation has been obtained and further work on the problem is currently in progress.

Endoplasmic reticulum is often inconspicuous in sections through adrenal chromaffin cells. This is not surprising since the cells are not concerned with the synthesis of large amounts of protein. In some regions, however, tubular profiles or cisterns of endoplasmic reticulum, studded with ribosomes, are aggregated more or less in parallel with each other. These aggregates are often para-nuclear and since their overall dimensions are not great, may be missed in any particular plane of section; they may, however, be a constant feature of normal rat chromaffin cells. Though aggregations of endoplasmic reticulum in chromaffin cells are less numerous than those in neurones which are responsible for the Nissl staining properties, the form and appearance of the material in the two cell types is not dissimilar.

The occurrence of linear profiles in the cytoplasm of chromaffin cells is of interest. They are often more clearly seen in glutaraldehyde-osmium-fixed material though they do occur in tissues fixed in buffered osmium tetroxide. The diameter of the elements is similar to that of neurotubules. Since they are usually sparsely distributed it is difficult to be sure that any particular rounded profile of the correct transverse diameter represents a transverse section of one of these elements and hence a decision as to their tubular or fibrillar nature has as yet not been possible.

Cilia may be identified in virtually all sections of the rat adrenal medulla. They arise from distinct basal bodies which may be situated deep within the cell substance or more superficially. Deep basal bodies usually lie adjacent to a distinct centriole and themselves represent a modification of the distal centriole. Only one out of six superficial basal bodies has been seen lying adjacent to a proximal centriole. This finding may reflect the plane of section, distance of the proximal centriole from a modified distal one, or the fact that some superficial basal bodies have no association with centrioles. Although cilia on chromaffin cells were recognized by De Robertis & Sabatini (1960), these authors gave no details of their internal structure. In the present work all the cilia have possessed a central pair of filaments together with six to eight peripheral pairs; eight pairs being usual. They differ, therefore, from those described by Barnes (1961) in association with the parenchymal cells of the pars distalis of mouse pituitary and those observed by Sorokin (1962) arising from fibroblasts in tissue culture. Cilia illustrated by both these authors were devoid of an axial pair of filaments. They also differ from those observed by Grillo & Palay (1963) arising from Schwann cells in the adult rat which possessed nine pairs of peripheral filaments and a central tube. The cilia observed in the present work resemble those described by Grillo & Palay (1963) in so far as they do not reach the milieu of true connective tissue spaces, but are always separated from these by a distinct basement membrane. The functional significance of the cilia associated with chromaffin cells is difficult to assess. Having regard for their size, relative to that of chromaffin cells and the random nature of the plane of section employed in the preparation of the material, it seems possible that all rat adrenal chromaffin cells may possess a cilium. The cilia of chromaffin cells differ from those normally considered to be sensory in so far as they possess a pair of axial fibrils, while they differ from the typical mammalian motile cilium in having only six to eight peripheral pairs of fibrils. All cilia appear to protrude into a junctional intercellular space and in this region, which presumably contains some fluid, the shaft of the cilium may be intimately associated with bare nerve fibres which are approaching the target chromaffin cells, microvilli, and granular material. The presence of a well-defined axial pair of fibrils together with the well-developed basal body, from which some fibrils and electron-dense aggregates extend laterally into the surrounding cytoplasm, is in keeping with a motile function of these projections and it is tempting to suggest that they may be involved in the movement of fluid in what would otherwise be a relatively stagnant backwater, viz. the junctional intercellular space.

Cortical elements form a constant feature of the adrenal medulla of the rat and may represent cord-like projections from the inner aspect of the reticular zone or discrete islands. They constitute part of the juxta-medullary zone of the adrenal

cortex (Celestino da Costa, 1951) or interlocking zone (Roaf, 1935). Though the cells vary in overall electron density and may be designated light or dark, they contain what appear to be normal healthy cytoplasmic organelles and inclusions. Microvilli are numerous and have been described previously in relation to inner zone cortical cells by Carr (1961). In marked contrast to chromaffin and Schwann cell elements, they are not separated from connective tissue spaces by a distinct basement membrane. They are, however, separated from adjacent chromaffin cells or Schwann cells by a layer of connective tissue of variable thickness and by the basement membrane associated with the latter structures.

SUMMARY

Chromaffin cells of the rat adrenal medulla are organized in a compact epithelial fashion with a gap of *c.* 200 Å between adjacent elements. In some regions this gap widens to form a junctional intercellular space.

The granular substance of chromaffin granules appears to accumulate initially in the membranes of the Golgi zone and is finally extruded from the cell surface. Cilia are often observed arising from chromaffin cells and may form a constant feature of all chromaffin elements in the rat adrenal medulla.

Adrenaline- and noradrenaline-storing cells may be differentiated by fixing sections initially in glutaraldehyde and then transferring to osmium tetroxide.

Apart from chromaffin cells, myelinated and unmyelinated nerve fibres with associated Schwann cells, adrenocortical cells, blood vessels and connective tissue elements form constant features of sections through the adrenal medulla.

I wish to thank the Wellcome Trust and the Scottish Hospital Endowments Trust for their generous financial support which made this work possible.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Group of chromaffin cells in rat adrenal medulla. Note 200 Å gap between cells with occasional desmosomes, junctional spaces (*J*) and nerve ending (*N*). Fixed in buffered osmium tetroxide (B.O.T.). ×6750.

Fig. 2. Junctional intercellular gap containing nerve ending and Schwann cell process. Note desmosome. B.O.T. ×25,600.

Fig. 3. Junctional intercellular space with desmosomes at two of the three extremities. B.O.T. ×25,600.

PLATE 2

Fig. 4. Cortical cells lying adjacent to blood sinus to left and adrenaline-storing chromaffin cells (centre and right). Fixed by glutaraldehyde-osmium tetroxide sequence (G.O.T.). ×1690.

Fig. 5. Junctional intercellular space associated with four chromaffin cells, containing cilium (*C*), nerve ending (*N*) and microvilli. Note also cilium in right lower chromaffin cell. B.O.T. ×13,650.

Fig. 6. Cortical cell (bottom left), chromaffin cell (top right) separated by myelinated and unmyelinated nerve fibres and fine connective tissue. Note basement membrane covering surfaces of Schwann cells and chromaffin cells exposed to connective tissues. No membrane covers the cortical cell. G.O.T. ×9000.

Fig. 7. Fibroblast lying between chromaffin cells. Note basement membrane is associated with the free surface of chromaffin cells but not that of the fibroblast. Junctional intercellular space (*J*) approaches connective tissue space but end is bridged by basement membrane of adjacent chromaffin cells. B.O.T. ×6700.

PLATE 3

Fig. 8. Small blood vessel in rat adrenal medulla, right side having characteristics of a capillary, left side that of a venous sinus. Note basement membranes and proximity of adrenaline-storing chromaffin cells. G.O.T. × 9000.

Fig. 9. Blood vessel with mixed (capillary/venous sinus) characteristics in rat adrenal medulla. N-cell above, A-cells below (right and left). Note prominent perinuclear space in endothelial cell after G.O.T. fixation. × 5400.

Fig. 10. Transverse section through arterial medulla of rat adrenal. Note inner layer of endothelium is surrounded by smooth muscle cells. B.O.T. × 2630.

Fig. 11. Section through wall of medullary artery of rat adrenal. Basement membranes surround smooth muscle cells except for limited areas where adjacent smooth muscle cell plasma membranes are approximated and separated only by a gap of 200 Å. The basement associated with endothelial cells is separate from others. B.O.T. × 6300.

Fig. 12. Interlocking region of adjacent chromaffin cells, associated with desmosomes and near to a junctional intercellular space which contains granular material. B.O.T. × 19,200.

Fig. 13. Attenuated Schwann cell process lying between three chromaffin cells. Note absence of basement membranes, 200 Å gap between cells and nerve ending to left. Schwann cell cytoplasm covers non-synaptic face of nerve ending. Dark body showing variable electron density and tendency to vacuolation in upper chromaffin cell. B.O.T. × 9600.

PLATE 4

Fig. 14. Chromaffin cell (below) lying adjacent to endothelial cell (above). Note microvilli and basement membranes. B.O.T. × 22,500.

Fig. 15. Chromaffin cell (below) adjacent to blood capillary (above). Note attenuated cytoplasm (diaphragms) of endothelial cells, basement membranes of endothelial and chromaffin cells which almost fuse and narrow zones between the two basement membranes and between these and the adjacent cells. Caveolus on free surface of chromaffin cell. B.O.T. × 33,700.

Fig. 16. Chromaffin cells showing one nucleus with three nucleoli. Also notice aggregations of endoplasmic reticulum, mitochondria, nuclear pores (arrows), cell membranes with desmosomes and junctional intercellular spaces. B.O.T. × 7500.

Fig. 17. Noradrenaline-storing cells. G.O.T. × 4500.

Fig. 18. Adrenaline-storing (below) and noradrenaline-storing cells (above) G.O.T. × 2630.

PLATE 5

Fig. 19. Chromaffin cell mitochondria. B.O.T. × 52,500.

Fig. 20. Adrenaline-storing chromaffin cells. Note plasma membranes and intercellular spaces; nerve ending (*N*) lies in lower space. Aggregation of endoplasmic reticulum and ribosomes below nucleus. Note well defined mitochondria. G.O.T. × 12,000.

Fig. 21. Section through Golgi zone of A-cell. Note tubules, some with electron dense terminal dilatations (arrow). Occasional tubules of endoplasmic reticulum with associated ribosomes. Multivesicular body (*M*) contains vesicles and granular material. G.O.T. × 25,200.

Fig. 22. Golgi zone of chromaffin cell. Note accumulation of electron dense material in some Golgi tubules or vesicles. Nuclear membrane, sectioned obliquely, shows pores. Note granular nature of contents of some chromaffin granules. B.O.T. × 27,000.

PLATE 6

Fig. 23. Golgi zone of N-cell. Note some membranes enclose homogeneous material which has high electron density while others contain moderately electron dense granular material (*G*). G.O.T. × 36,000.

Fig. 24. Multivesicular body in chromaffin cell. Note vesicles, granular material and single limiting (unit) membrane. B.O.T. × 45,000.

Fig. 25. Two dark bodies of different electron densities lying adjacent to chromaffin granules (smaller). Single limiting (unit) membrane. B.O.T. × 60,000.

Fig. 26. Chromaffin cell lying adjacent to blood vessel. Note diaphragms in endothelial cell but no discontinuity. Two chromaffin granules are fusing with the plasma membrane (arrows). Note collagen fibres in space between the two basement membranes, also spaces between membranes and adjacent cells. B.O.T. $\times$ 21,000.

Fig. 27. A-cell showing continuity between external limiting membrane of chromaffin granule and plasma membrane. Granular material is passing through region of basement membrane which is less electron dense at this point and may be discontinuous. G.O.T. $\times$ 42,000.

Fig. 28. Chromaffin granule discharging. Endothelial cell cytoplasm above. Note fused basement membranes of chromaffin and endothelial cells. B.O.T. $\times$ 42,000.

Fig. 29. Chromaffin granule discharging towards connective tissue space. Note attenuation of basement membrane over point of discharge. Some collagen fibres stain heavily. Cortical cell on left. B.O.T. $\times$ 24,000.

PLATE 7

Fig. 30. Cilium arising from peripheral part of chromaffin cell. Shaft passes directly into junctional intercellular space. Golgi complex (*G*) is adjacent to basal body. B.O.T. $\times$ 9000.

Fig. 31. Longitudinal section through cilium and basal body. Note fibrils in shaft and basal body also electron-dense substance associated with latter. Fibrils pass laterally from basal body. B.O.T. $\times$ 30,000.

Fig. 32. Cilium entering tunnel which contains granular material and a microvillus. Note also multivesicular bodies and fibrils (or tubules) in cytoplasm. B.O.T. $\times$ 16,800.

Fig. 33. Cilium entering intracellular tunnel. Golgi zone of chromaffin cell *G*. Note electron dense fibrils extending from the basal body into adjacent cytoplasm. B.O.T. $\times$ 21,000.

Fig. 34. Proximal and distal centrioles of a chromaffin cell. The distal centriole is modified to form a basal body and the cilium enters the intracellular tunnel. G.O.T. $\times$ 36,000.

Fig. 35. Transverse section through cilium lying in tunnel within an N-cell. Note extreme electron density of contents of chromaffin granule and $8+2$ fibril pattern in cilium. G.O.T. $\times$ 52,500.

Fig. 36. Transverse section through cilium lying in tunnel within an A-cell. Note contents of chromaffin granule are only moderately electron dense and granular. Cilium has 8×2 fibril pattern. G.O.T. $\times$ 75,000.

Fig. 37. Oblique section through cilium lying within junctional intercellular space. B.O.T. $\times$ 52,500.

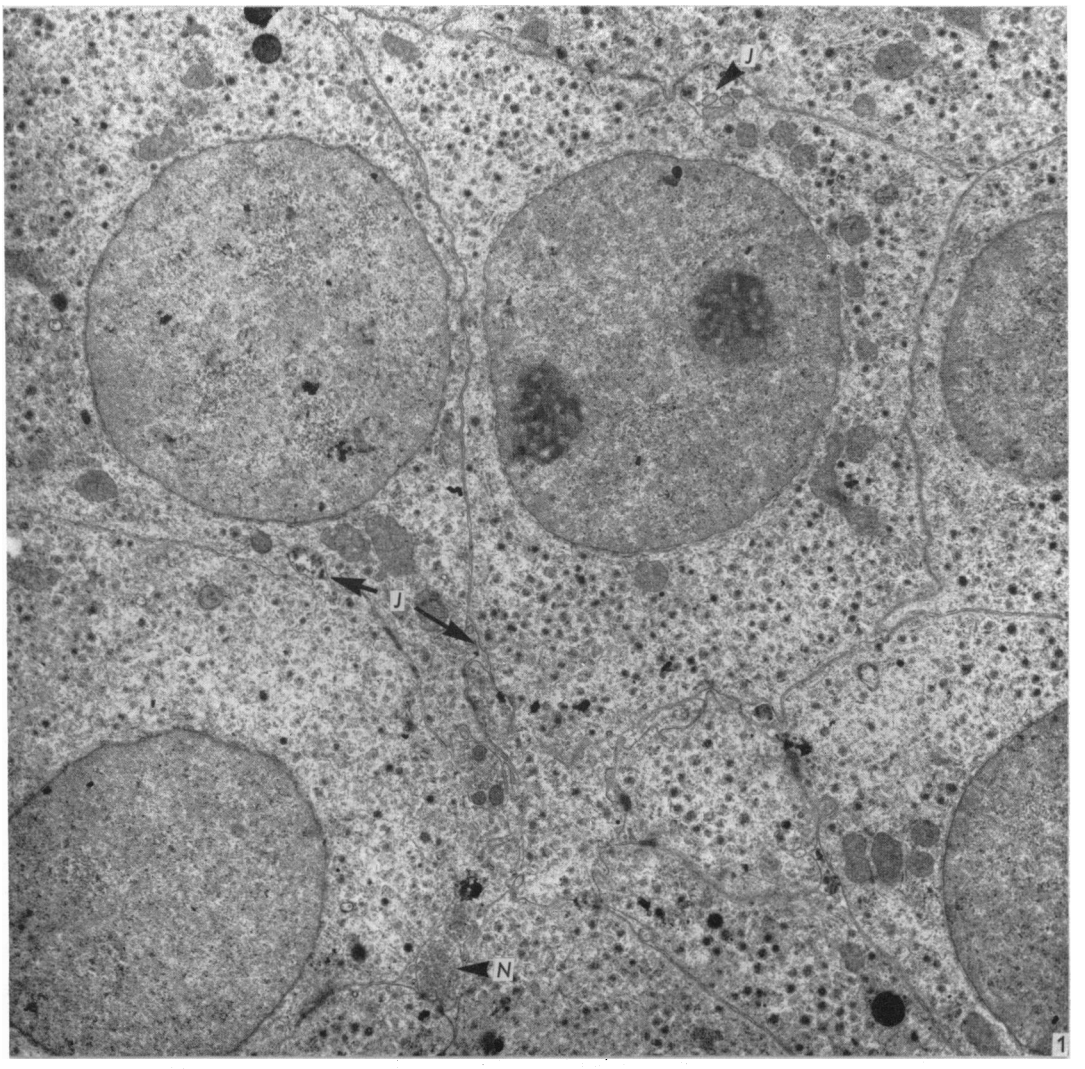
PLATE 8

Fig. 38. Transverse section through proximal centriole of chromaffin cell showing nine groups of triple fibrils. Golgi membranes lie to right. Scattered groups of ribosomes also visible. G.O.T. $\times$ 56,200.

Fig. 39. Light and dark cortical cells containing typical mitochondria and inclusions. Note electron translucent perinuclear space. G.O.T. $\times$ 2800.

Fig. 40. Elongated profile of fibril or tubule in cytoplasm of a noradrenaline-storing cell. G.O.T. $\times$ 90,000.

Fig. 41. Section through myelinated and unmyelinated nerve fibres and associated Schwann cells, dark cortical cell with microvilli, part of N-cell (bottom left) and A-cell (top right) and a fibroblast (bottom right). Note collagen fibres in connective tissue spaces and basement membranes which lie adjacent to chromaffin and Schwann cells but not to cortical cell and fibroblast. G.O.T. $\times$ 9000.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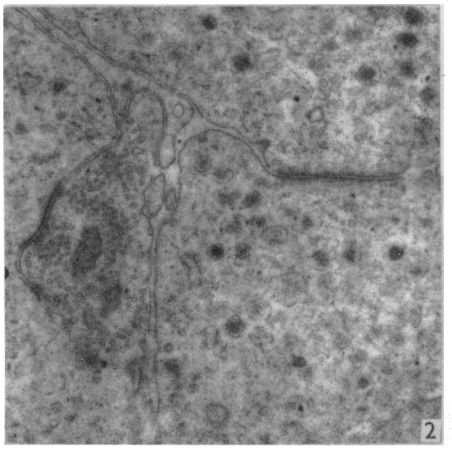
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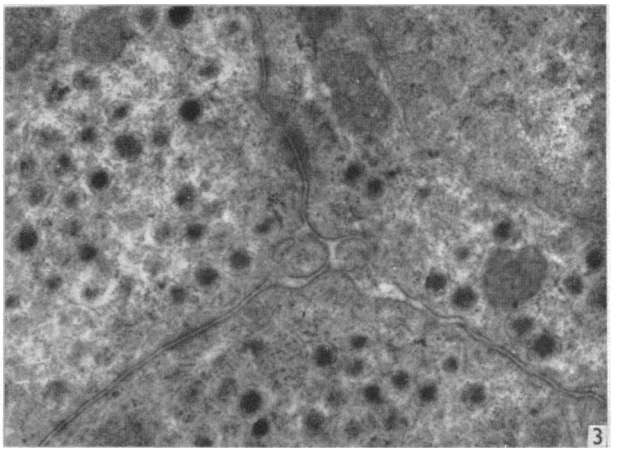
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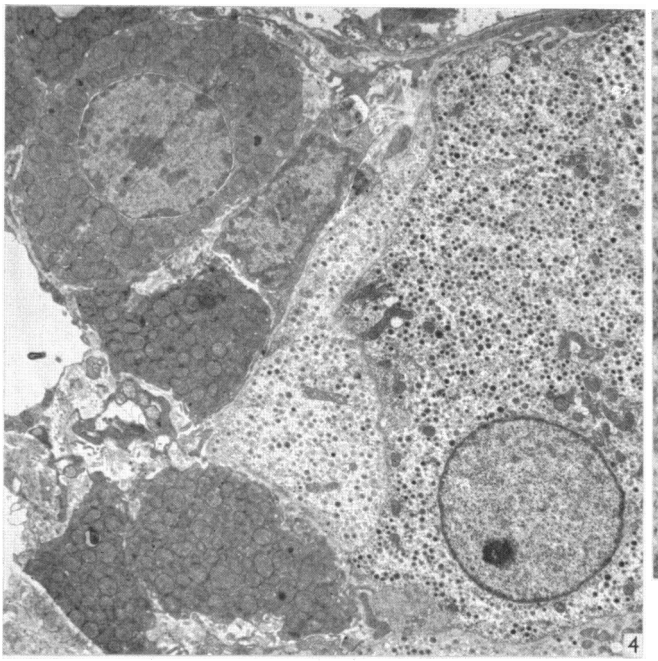

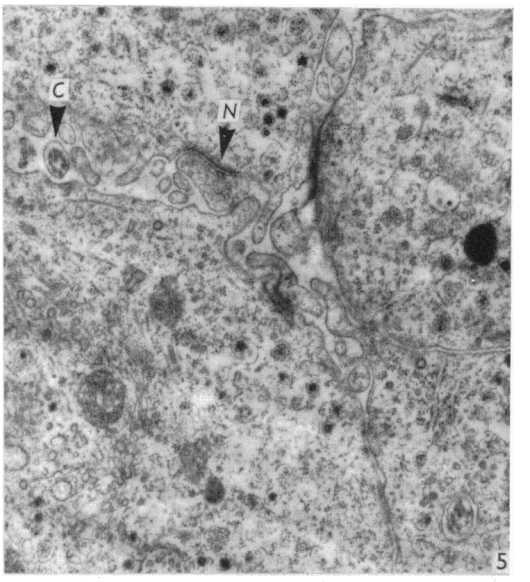
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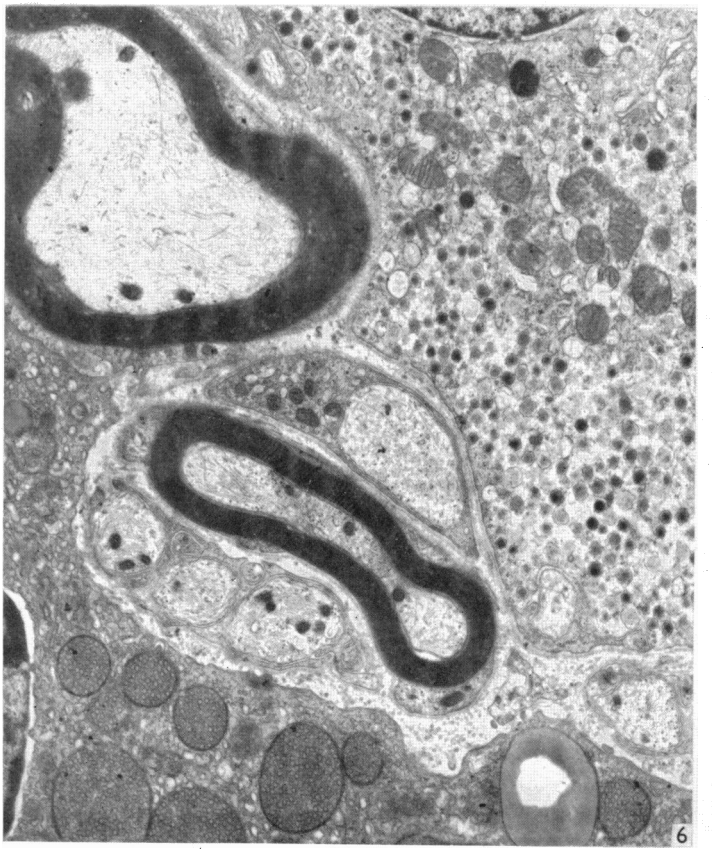

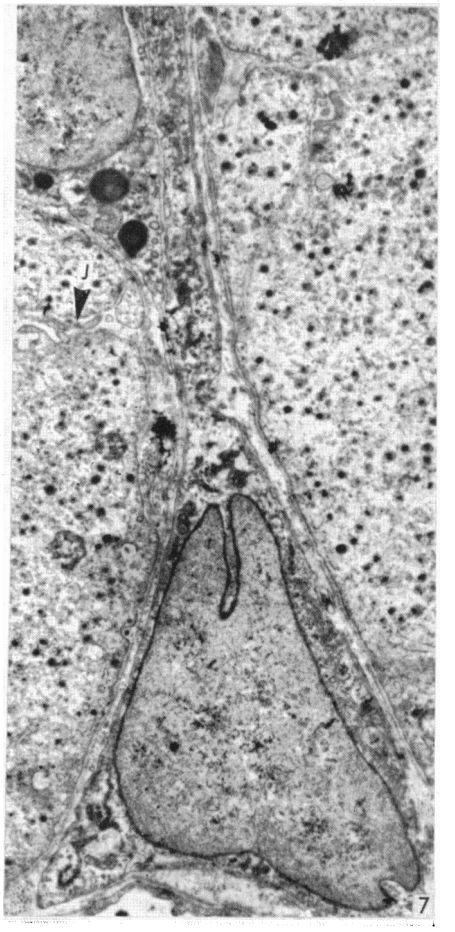
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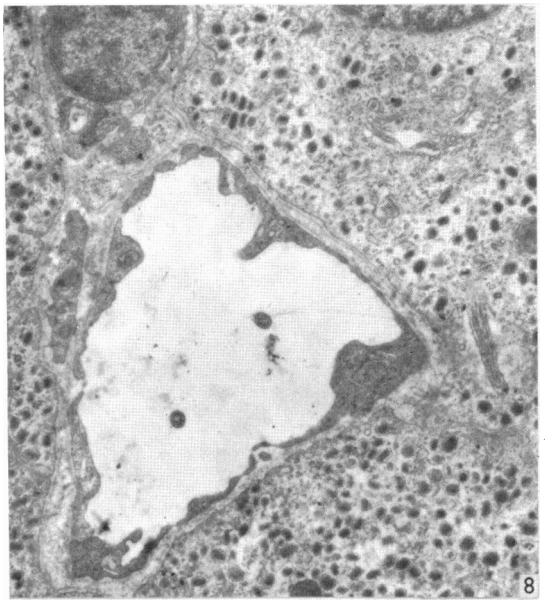
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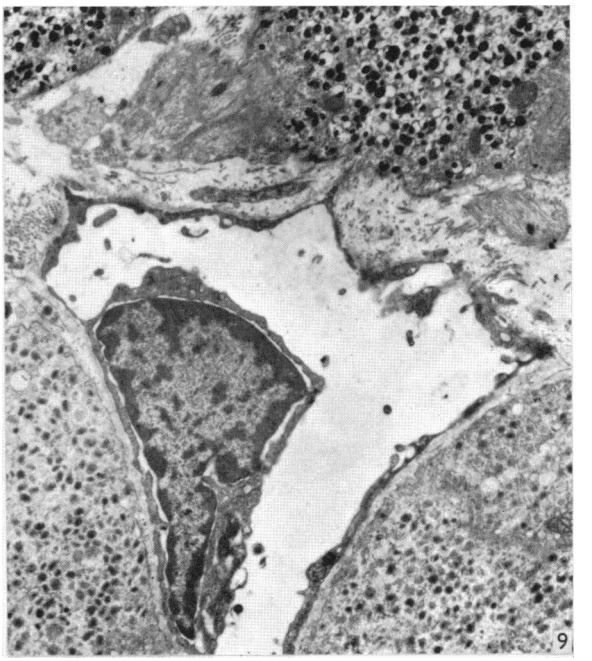
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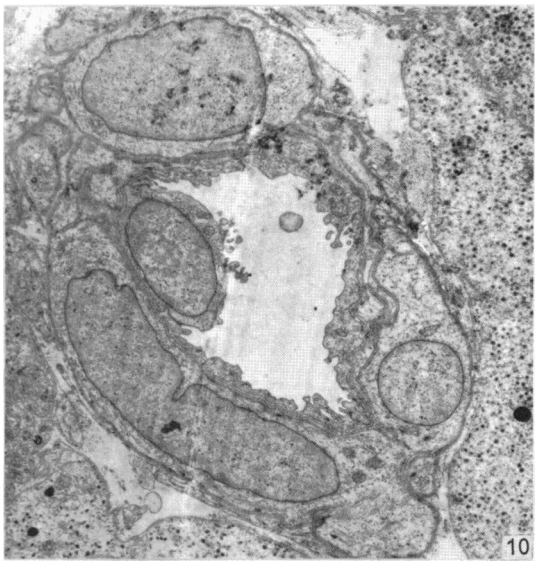
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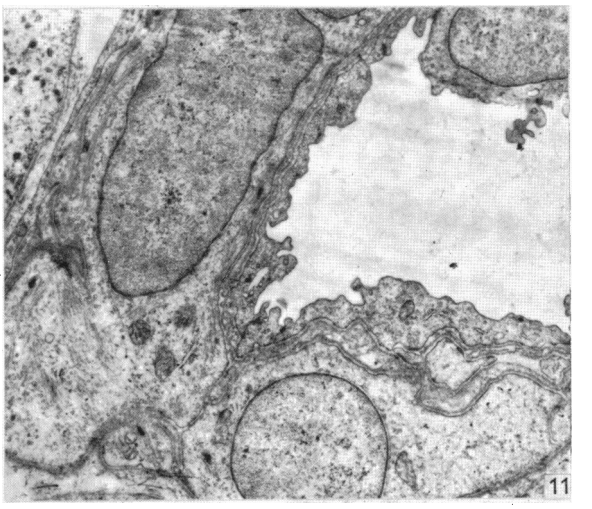
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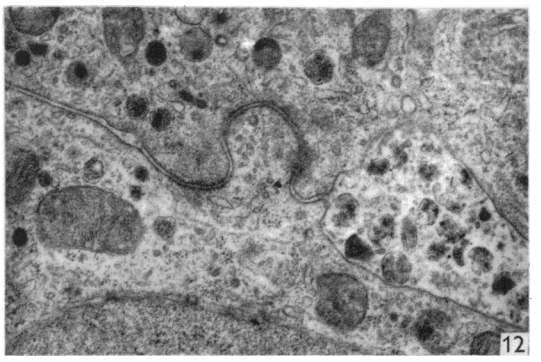
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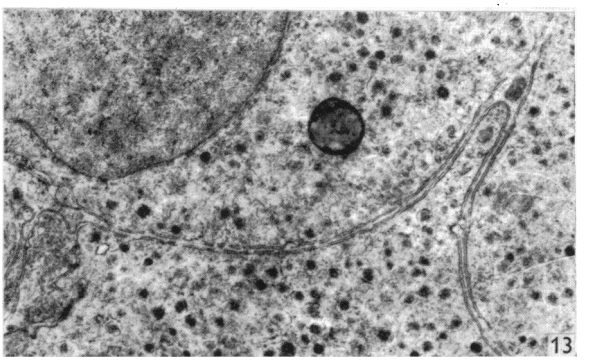
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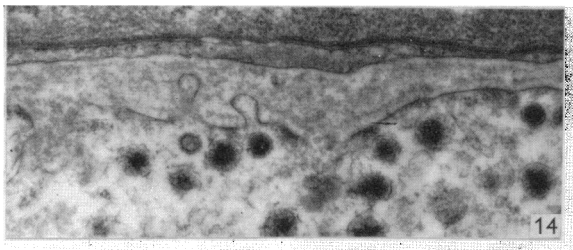
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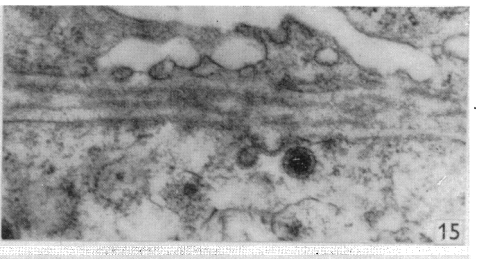
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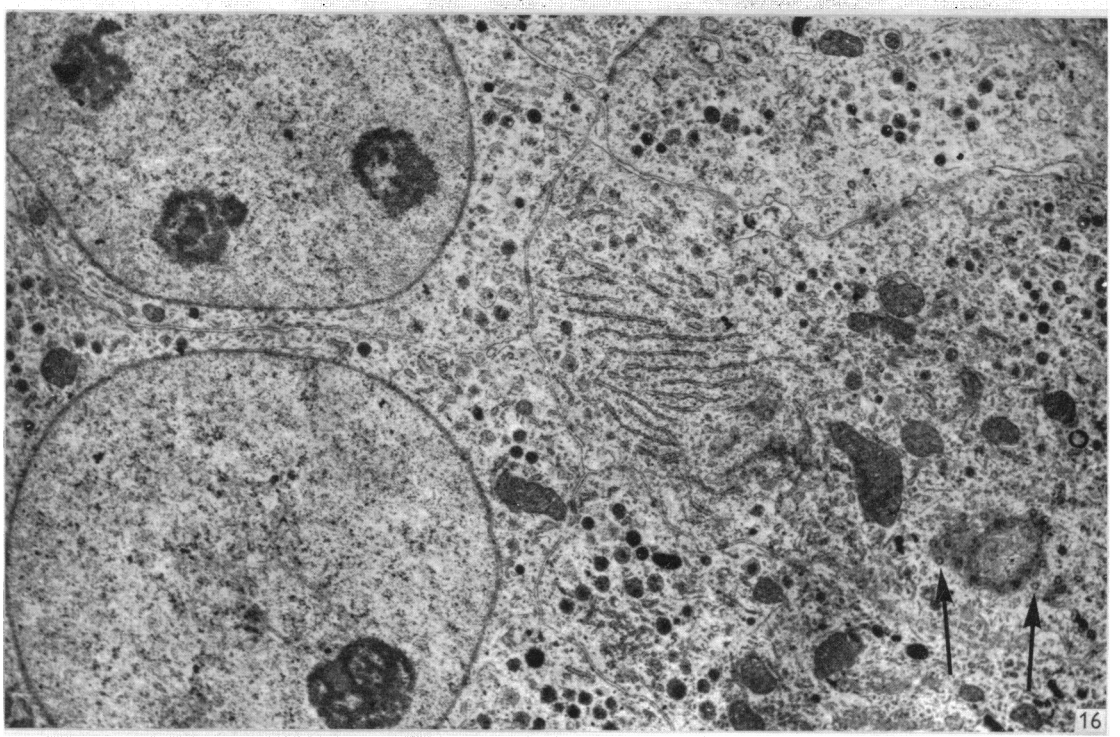
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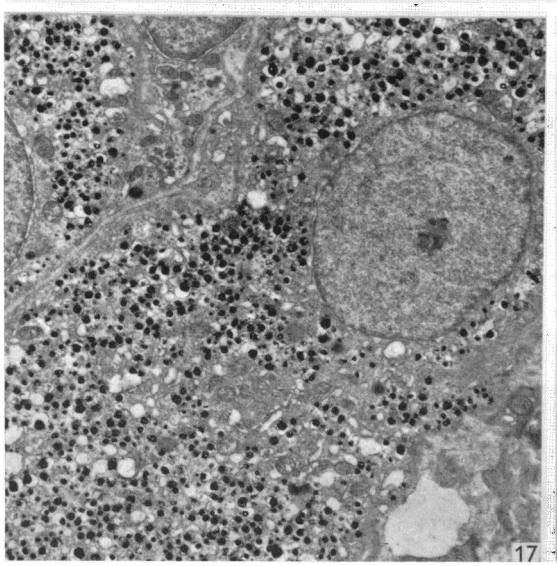
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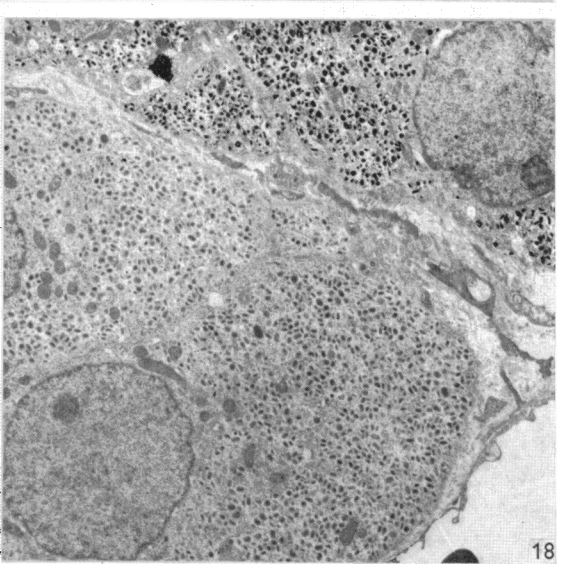
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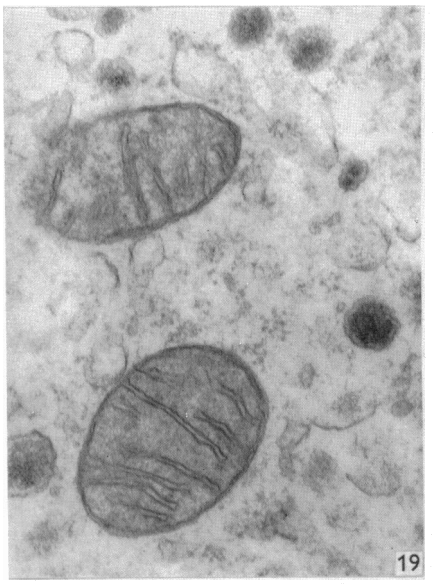
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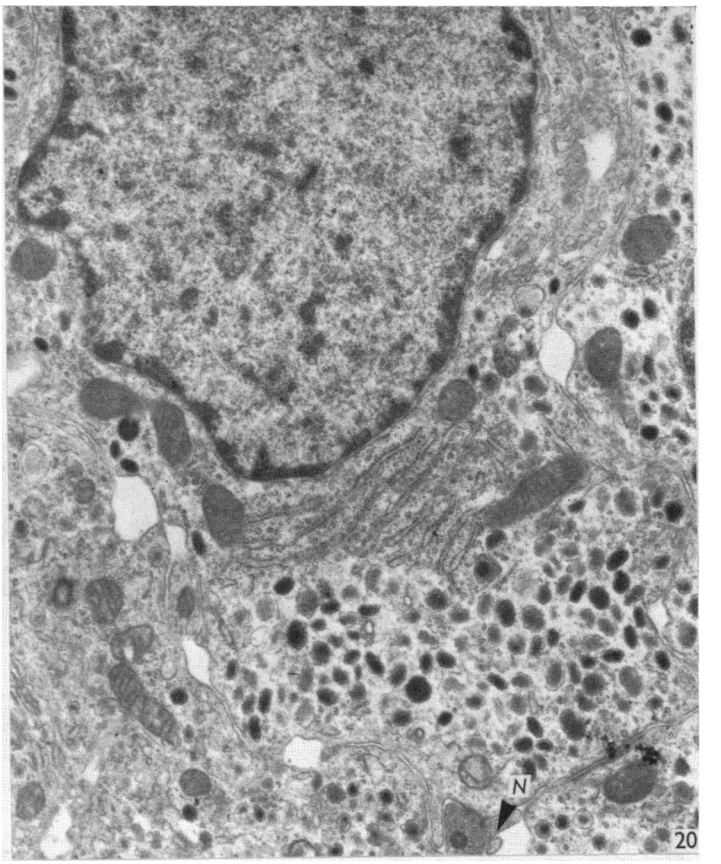
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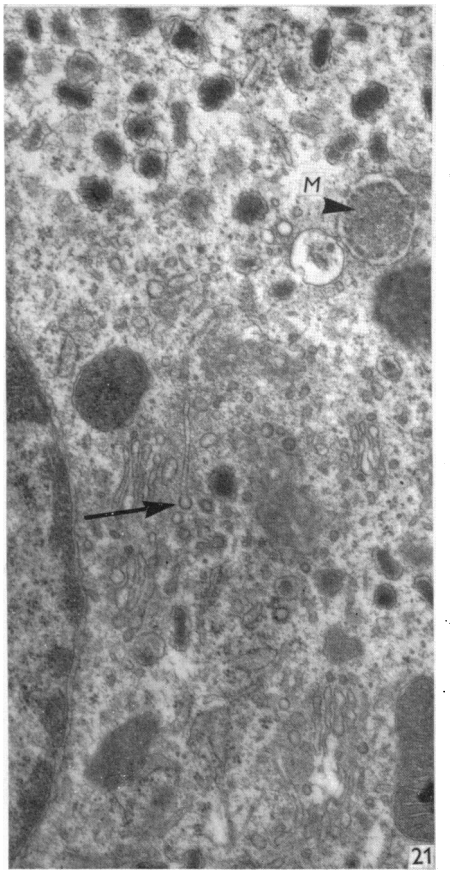
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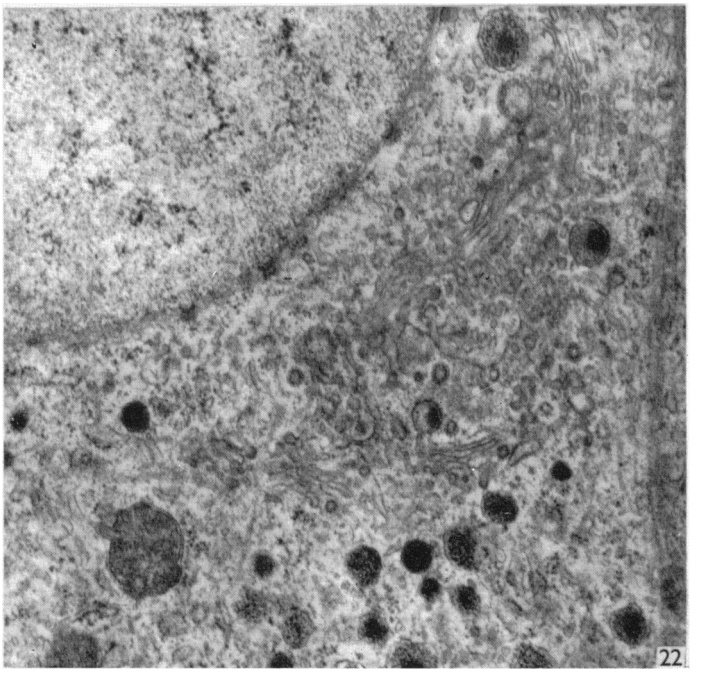
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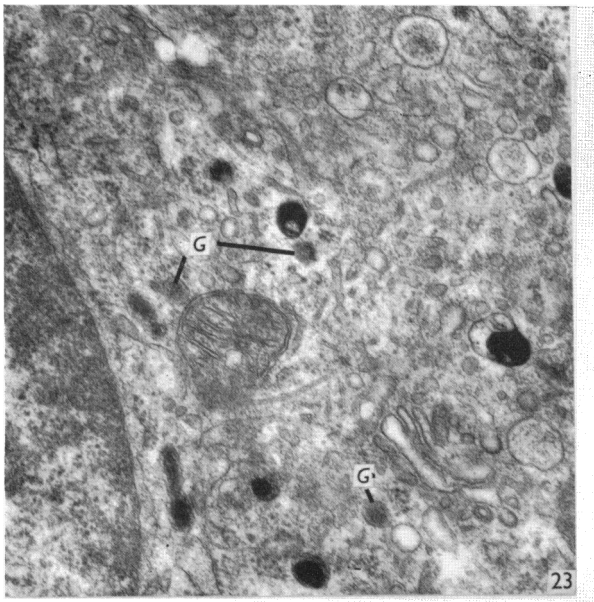
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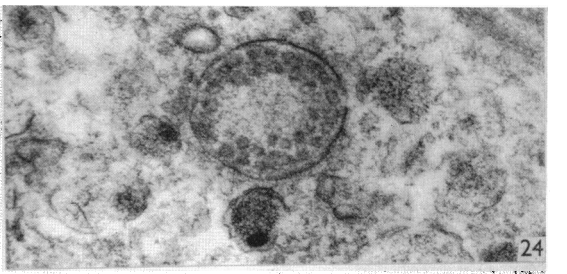
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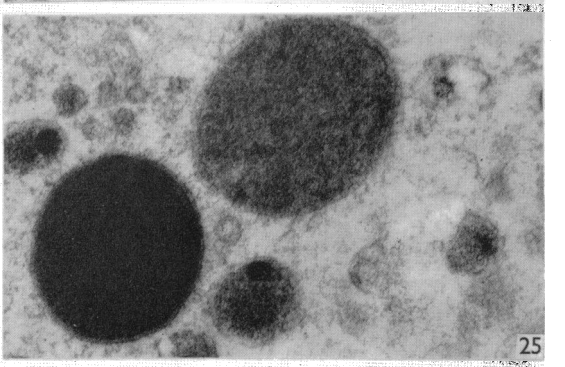
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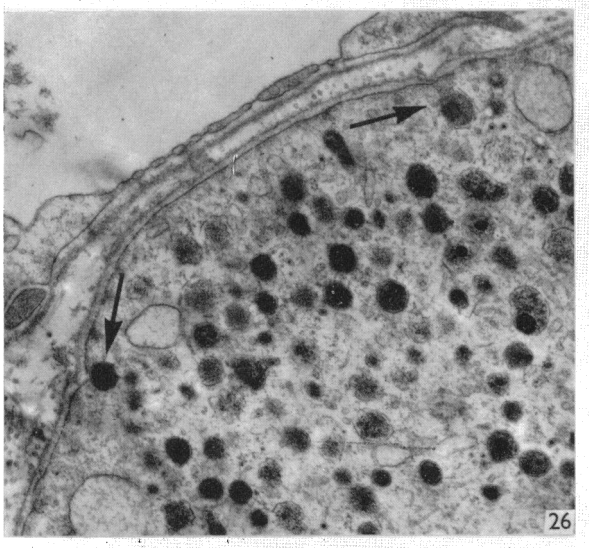
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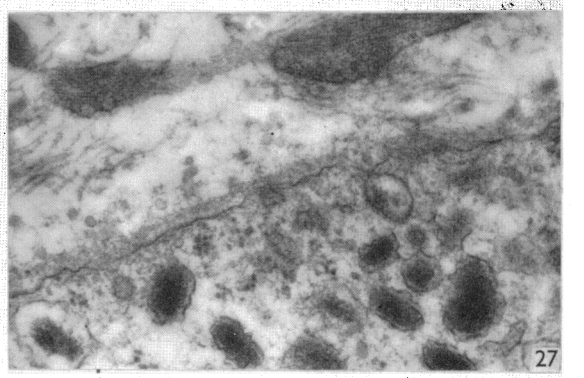
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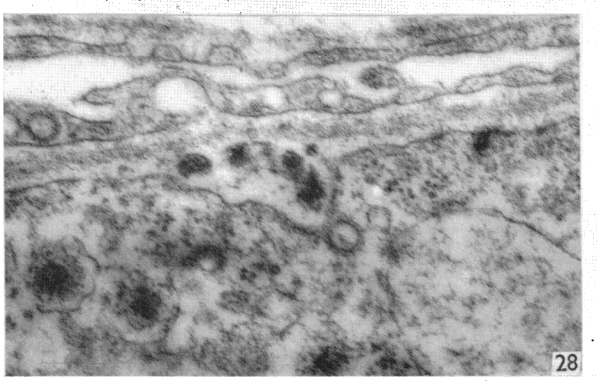
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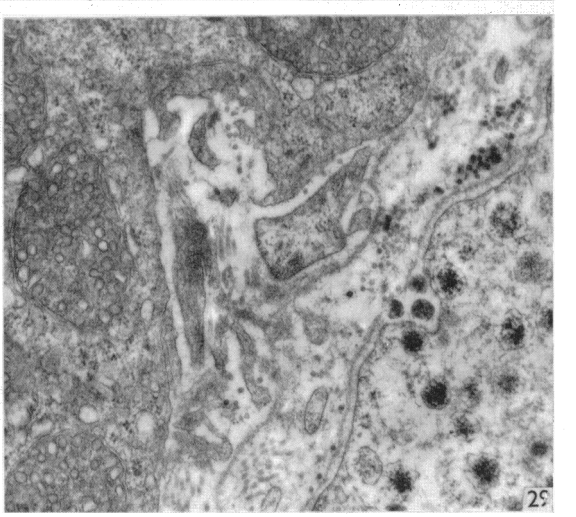
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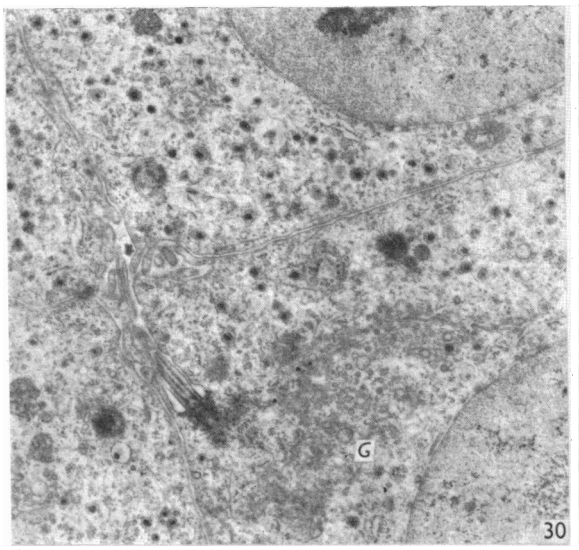
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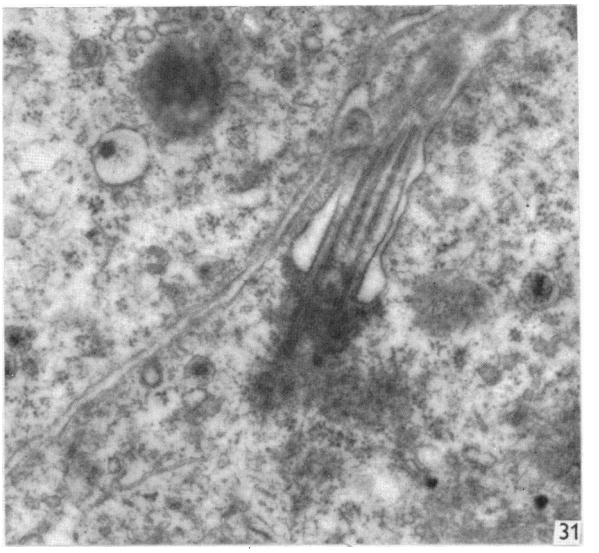
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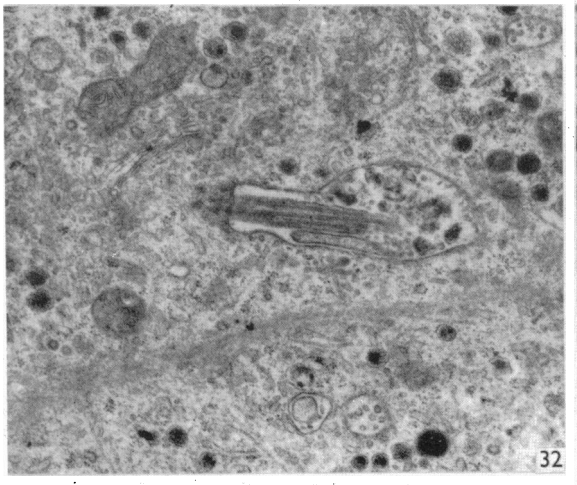
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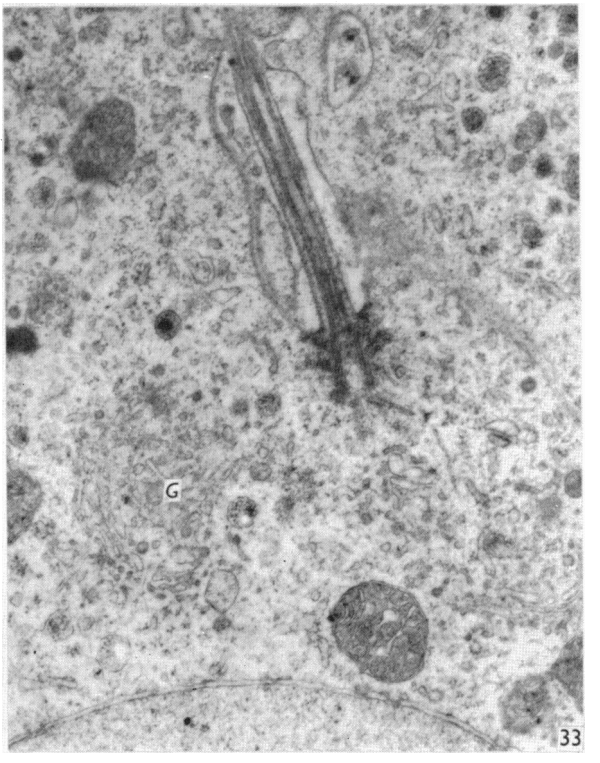
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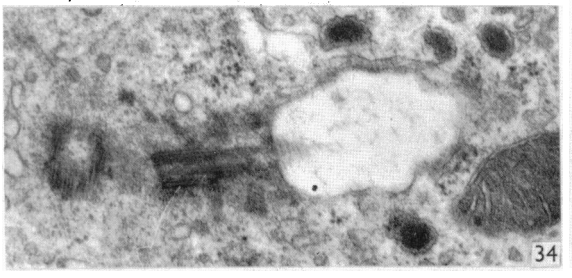
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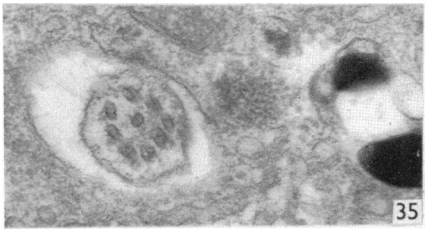
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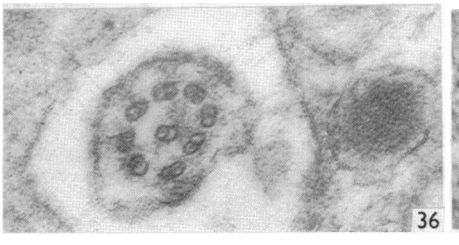
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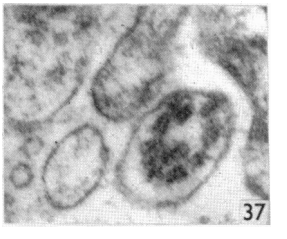
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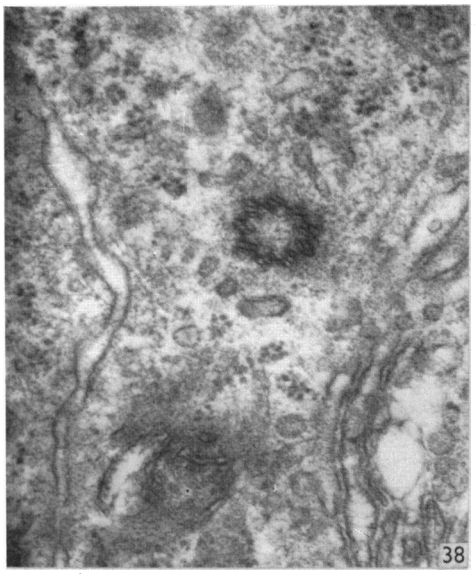
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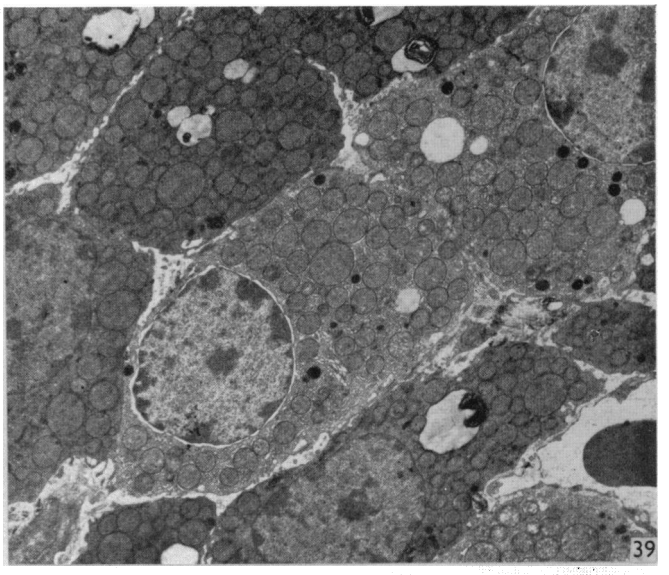
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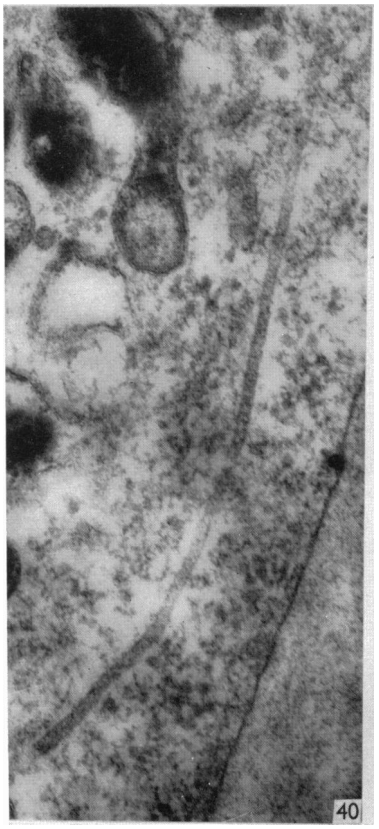
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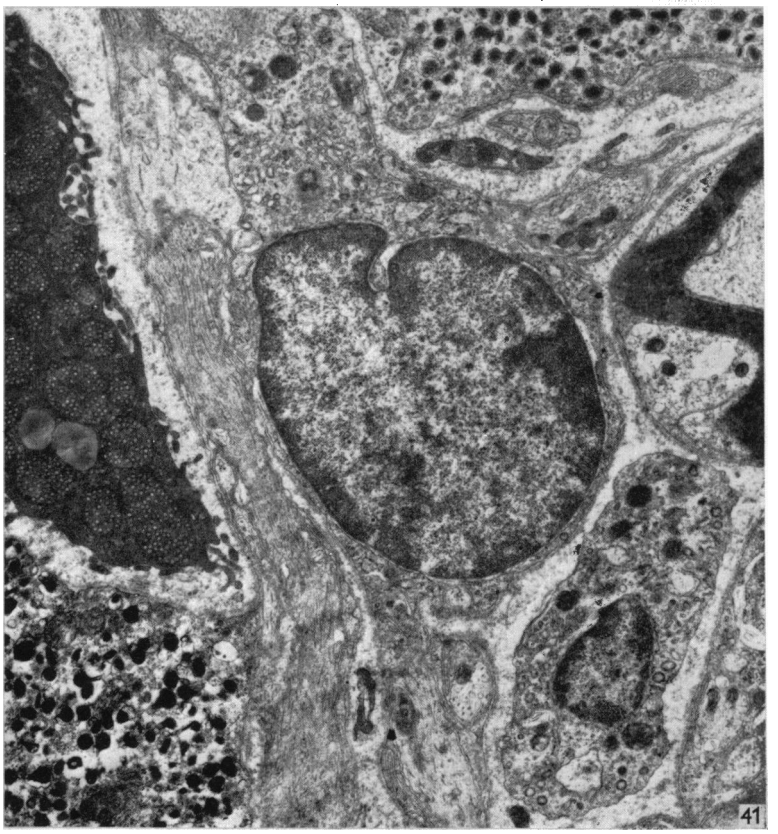
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