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Normal morphology of the adrenal medulla of the Swiss mouse and albino rat and the fine structural changes associated with reserpine induced catecholamine depletion

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THE NORMAL MORPHOLOGY OF THE ADRENAL MEDULLA OF THE SWISS MOUSE AND ALBINO RAT AND THE FINE STRUCTURAL CHANGES ASSOCIATED WITH RESERPINE INDUCED CATECHOLAMINE DEPLETION

by

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PREFACE

Much of the material included in this thesis is the direct result of research done in the fulfillment of requirements for the degree of Master of Science in the Department of Anatomy. In studying tissues with the electron microscope, it became apparent that one great area of confusion results from inadequate correlation between conventional light microscopy and electron microscopy. It also became apparent that further study on medullary innervation might enable better interpretation of experimental results.

This thesis then, contains the experimental results included in the Master's thesis which were obtained by inducing adrenal medullary catecholamine depletion with reserpine as well as a more comprehensive study of the innervation of the adrenal medulla. In view of the necessity for adequate correlation of light and electron microscopy, some time and effort has been devoted to the preparation of serial sections of the same material respectively for light and electron microscopy in an attempt to bridge this frequent gap. The historical material, review of literature, and experimental methods have been greatly condensed, and a more complete discussion and bibliography can be found in the Master's thesis.

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I. INTRODUCTION AND HISTORY

The adrenal medulla has long been noted for its classic chromaffin reaction and for its production of pressor substances. Oliver and Schafer (1895) are generally credited with establishing the endocrine nature of the gland when they showed that the adrenal gland contained a substance, which, when put into the blood stream, caused a general pressor effect. However, long before the identification of the active agent in the adrenal medulla, it was shown that the adrenal medullary cells stained dark brown with chromic acid or potassium dichromate (Henle 1865). Thus, the chromaffin reaction was described. As exemplified by Hartman and Blatz ('19), the chromaffin reaction became used as an indicator of adrenaline in medullary cells and vessels. For a period of time, many workers, as illustrated by Ogata and Ogata ('23), considered the chromaffin reaction to depend upon the action of adrenaline-like compounds but to derive its characteristic color from precipitated chromium compounds. It remained for Gerard et al. ('30) to establish the fact that the brown color was due to the oxidation product of adrenaline which is similar to organic tars; and that this reaction could be produced by any oxidizing salt,

whether chromium or not. Of importance to electron microscopy, is the fact that osmium tetroxide is such an oxidizing agent (Plecnik '02, Cramer '28).

Light microscopic studies of the adrenal medulla are numerous. Early concepts of morphology are typified by Cramer ('28). Using osmium tetroxide as an indicator of adrenaline, he described medullary cells as being in different states of secretory activity and first described the presence of "light" and "black" cells being indicators of unequal glandular activity. The adrenal medulla was comprehensively studied by Bennett and Kilham ('40), and Bennett ('41). They described arterially supplied capillaries of the medulla together with medullary veins formed by the junction of the cortical capillaries, as being distributed in such a way as to give double vascularization to all parts of the medulla - the parenchymal cells thus being characterized by a polarity with one pole bordering on a vein and the other on a capillary. Again, they considered the patchiness of the chromaffin reaction to be due to groups of cells being in different phases of the secretory cycle.

The finding that the adrenal gland contains more than one amine (Holtz, et al. '47; Goodall '51), has resulted in much speculation over the possible

presence of more than one cell type. Eranko ('51) described fluorescence of certain cells in the medulla after formalin fixation. Hillarp and Hokfelt ('55) described an iodate reaction which formed dark insoluble pigments with noradrenaline, hydroxy-tyramine, and DOPA but not with adrenaline. On this basis they described two cell types; noradrenaline containing cells and adrenaline containing cells. The presence of two different cell types using these methods has also been described by many other studies (Hillarp and Hokfelt '53; Eranko '55a, '55b; Eranko and Palkama '59, '62; Falck and Torp '61; Houssay, et al. '62); and Wood ('63) describes simultaneous demonstration of adrenaline and noradrenaline containing cells after fixation and staining in an analine-eosin mixture buffered to a pH of 4.1.

II. REVIEW OF LITERATURE

A. Innervation of the Adrenal Gland

A study of the literature, as reviewed by Alpert ('31), reveals that earlier workers such as Ecker, Virchow, and Holm focused their attention chiefly on the presence of ganglion cells in the adrenal medulla and their relative number and location. Other points of past dispute included the origin and abundance of medullated and nonmedullated fibers, and the intracellular or extracellular location of nerve endings. Using the Spielmeyer technique and human tissue, Alpert found that the majority of the nerves approaching the glands were myelinated and that many continued so through the cortex; however, no myelinated nerves were observed in the medullary portion of the glands. Using the Bielschowsky technique to study the finer distribution of nerve fibers, he described fibers as supplying all layers of the adrenal cortex as well as the medulla. The parenchymal cells of the medulla were described as being enclosed in basket-like networks of fibrils from which tiny fibrillae entered the cells. Swinyard ('37), confirming much of the work of Hollingshead, showed that the nerves to the suprarenal gland of the cat arise mainly from the

last few thoracic spinal nerves and that the vagus nerve contributes no fibers to this gland. He found that fifty to seventy percent of the nerve bundles entering the adrenal glands of the cat contain small myelinated preganglionic fibers. Postganglionic fibers were found to arise from terminal ganglia located at variable distances along the course of the nerves as well as within the gland. No evidence of a cortical innervation of the adrenal gland was found and Alpert's findings were attributed to the use of the Bielschowsky technique which also stains reticular fibers.

Bennett ('41), reaffirmed Hollingshead and Swinyard in the belief that the nerve supply to the medulla merely passes through the cortex without distributing fibers to it and further described the medullary cells as receiving their innervation at the capillary pole. MacFarland and Davenport ('41) carefully studied adrenal innervation in the rat and compared this to innervation in other species of mammals. They found that section of the greater splanchnic nerve caused degeneration of from seventy-five to ninety percent of all fibers in the adrenal gland within five days. In contrast to other species of mammals, they found no sympathetic ganglia within

the rat adrenals, and found that some animals had very few or no ganglion cells within the glands. They too found no evidence of cortical innervation, and described medullary nerve endings as consisting of terminal arborizations about individual chromaffin cells with no evidence of intracellular endings.

B. Metabolism of the Adrenal Medulla

Many workers have contributed to the knowledge of the metabolism of the adrenal medullary catecholamines. The biosynthetic pathways have been suggested by such workers as Kirshner and Goodall ('57) who found that noradrenaline produced adrenaline if methionine and adenosine triphosphate were present in a soluble fraction of homogenates of the adrenal medulla. Even before this, many of the enzymes involved were discovered, isolated, and localized by such workers as Langemann ('51), Blaschko ('52), and Blaschko, et al. ('55).

Further biochemical studies have shown that the sympathomimetic amines exist within a granule fraction which can be isolated from adrenal medullary cells (Hillarp, et al. '53, '54; Blaschko and Welch '53; Schuemann '60), as has the majority of the adrenal medullary adenosine triphosphate (Hillarp, et al. '55). The occurrence of catecholamines and adenine nucleotides in chromaffin granules in approximate molar ratio of four to one has led to the suggestion that these compounds are in a nondiffusable complex in the granules; the acidic nucleotides balancing the basic amines (Hillarp, et al. '53; Blaschko, et al. '55; Hillarp '58b;

Hillarp and Thieme '59; Schuemann '60). It has also been shown that the conditions which produce catecholamine release result in a proportionate release of nucleotides predominantly in the form of adenosine triphosphate (Carlsson and Hillarp '56; Blaschko, et al. '56; Carlsson, et al. '57; Hillarp '58a, '58b, '58c; Schuemann '58; Hillarp and Thieme '59; Kirpekar, et al. '63). Thus, it seems that adenosine triphosphate is only secondarily broken down into adenosine diphosphate and adenosine monophosphate in the cytoplasmic sap; that is, where the adenosine triphosphatase and adenosine diphosphate transphosphorylase are located (Hillarp '58a, '60d). The similar occurrence of soluble protein within the granules and its release (Hillarp '58b; Hillarp and Nilson '54) suggests that this also is involved in the storage mechanism; and the work of Phillipu and Schuemann ('63a, '63b) suggests that ribonucleoprotein and ribonucleic acid specifically are involved in the storage mechanism.

Studies of the transport of catecholamines into and out of the granules by such workers as Hillarp and Nilson ('54), Carlsson and Hillarp ('58), and Hillarp ('59) indicate that the membranes of the granules are freely permeable to adrenaline and to noradrenaline. Hillarp ('59) also showed that though

labeled catecholamines apparently freely cross the membranes, they do not exchange with endogenous storage catecholamines. From these studies, Hillarp ('60d) summarizes the "binding hypothesis" of uptake and storage. He states that from studies on isolated granules one can say that an active transport mechanism plays no essential role; that the amines do not exist as free ions or molecules in the intragranular water; and that the catecholamines must be bound within the granules in a nondiffusable state. He further states that release of the amines is accompanied by release of an equivalent amount of nucleotide and that the amines and adenosine phosphates represent nearly the whole ionic content of water lysates of the granules. However, since adenosine triphosphate and adrenaline don't form a stable complex, a third component must be involved, and this is probably water soluble protein - the catecholamines then being directly bound to specific storage sites.

However, Kirshner ('62) advances an alternate hypothesis - the "active transport hypothesis." He proposes an active transport mechanism of uptake on the basis that the temperature requirements are those of a chemical reaction rather than what one would expect with a freely permeable membrane. The

catecholamines or their analogues then, react with the cell membrane and are transported across the membrane and released into the interior of the cell in a process that is endergonic.

Studies of the uptake of catecholamine precursors by the granules, and the assessment of the resulting products and their locations (Bertler, et al. '60b, '60c; Hall, et al. '61; Kirschner '62; Carlsson, et al. '63a) have resulted in further clarification of the conventional biosynthetic pathway. Their findings are well summarized by Bertler, et al. ('60b):

1) DOPA is formed from tyrosine at an unknown synthesis site. This appears to be the rate limiting reaction.

2) Decarboxylation of DOPA to Dopamine occurs in the cytoplasmic sap.

3) Dopamine goes into the granules and is "bound" to particles.

4) Dopamine is hydroxylated to noradrenaline in the particulate fraction.

5) Noradrenaline then accumulates in the granule fraction and is either released, methylated, secreted, or stored.

6) A certain amount of noradrenaline is methylated to adrenaline in the cytoplasm and then

either stored or released.

Studies on the process of secretion have also been controversial and again two predominant hypotheses have evolved. One concept considers the granules to be storage granules and not secretory granules; the catecholamines then being released from the granules into the cytoplasm and in some unknown way transported out of the cell. Various workers have apparently demonstrated three different amine fractions; the largest fraction being in the granules and bound with adenosine triphosphate and probably with protein (Hillarp, et al. '55; Blaschko, et al. '55; Hillarp '58b; Hillarp and Thieme '59; Schuemann '60); a small fraction in the granules and apparently not bound with adenosine triphosphate (Bertler, et al. '60a; Hillarp '60d); and a third small fraction existing as free amines in the cytoplasmic sap (Burack, et al. '61; Hillarp '60c; Carlsson, et al. '63a). From such findings, Hillarp ('60d) proposes the following hypothesis: an intra-cytoplasmic pool of free amines is continuously supplied from the synthesis and storage sites in proportion as the amines are drawn from this pool by secretion or by incorporation into storage granules, the free amines thus acting as a buffer

pool in participation in an equilibrium reaction with bound amines and the unstimulated cell membrane being impermeable to the free amines. With this theory then, the nerve impulse need only produce a short lasting increase in membrane permeability to allow outflow of the catecholamines.

However, Burack and Draskoczy ('62) found that after giving tritiated DOPA to get labeled catecholamines, the specific activity of the stored hormones was identical to that of the hormones secreted into the adrenal vein. Thus, they say that the proposition that a small extragranular pool which shunts freshly formed catecholamines past the granules into the blood is vitiated, and instead propose a degranulation mechanism; that is, "secretion only by degranulation."

C. The Action of Reserpine

The fact that reserpine induces secretion of medullary amines has been demonstrated by Muscholl and Vogt ('57), Stjarne and Schapiro ('58) and Blanchi, et al. ('62), who demonstrated that reserpine caused increased catecholamine levels in the medullary venous blood. Depletion of medullary catecholamines has been demonstrated in various animals by such authors as Holzbauer and Vogt ('56), Zbinden and Studor ('58), Eranko and Hopsu ('58, '61), Euler and Lishajko ('61), Clementi and Zocche ('63), and many others.

Eranko and Hopsu ('58) suggested that there is a difference in response to reserpine with different species of animals. For example, in rats and mice, a low dose rather than a high dose produces a preferential loss of noradrenaline which can be blocked by denervation (Eranko and Hopsu '61; Camanni, et al. '58), while in dogs there is a preferential loss of adrenaline (DeSchaepdryver '59). There are also species differences as regards direct or indirect action, that is, peripheral or central action. Hillarp ('60b) showed depletion of both amines with and without denervation in sheep; and

Callingham and Mann ('58b) showed a similar peripheral action in rats. However, Camanni, et al. ('60) demonstrated a slight decrease in the amount of depletion with denervation, even in rats. In contrast, the predominant action in cats appears to be central or mediated over the nervous system in that very little depletion occurs in the presence of denervation.

The specific effects of reserpine on catecholamine metabolism have also been investigated by many authors. The fact that iproniazid has been found to block reserpine (Carlsson, et al. '57; Zbinden and Studor '58; Camanni, et al. '60; Clementi and Zocche, '63) suggests that monoamine oxidase is important in reserpine induced release of catecholamines. This was suggested by Abood and Romanchek ('56) who found that reserpine inhibits oxidative phosphorylation probably at the cytochrome oxidase level. Other authors (Schuemann '58; Hillarp '60b; Kirpekar, et al. '63) have shown that reserpine not only causes a release of catecholamines from the medullary granules, but also causes a release of adenosine phosphates.

The predominant action of reserpine thus seems to be involved with the storage mechanism, and apparently blocks the uptake of catecholamines into

the so-called storage granules (Bertler '61; Bertler, et al. '61; Kirshner '62; Lundborg '63; Carlsson, et al. '63b; McLeon and Cohen '63). Support is lended to this in that; reserpine apparently does not interfere with the formation of catecholamines from DOPA (Bertler '61); reserpine does not block beta hydroxylation (Bertler, et al. '61); and reserpine does not block the formation of amines but instead blocks their uptake as shown by studies of catecholamine uptake in platelets (Brodie, et al. '57; Sana, et al. '60).

D. Electron Microscopic Studies

The adrenal medulla was first studied with the electron microscope by Lever ('55). Using osmium tetroxide fixation, he observed small osmiophilic granules surrounded by membranes and described them as spherical and microgranular in nature. Other workers as Sjostrand and Wetzstein ('56) and Kleinschmidt and Schumann ('61) have similarly described the nature of the granules. However, Sjostrand and Wetzstein reported that the granules were surrounded by clear spaces ("Hofe") which they proposed once contained lipid. Eranko and Hanninen ('60) and DeRobertis and Vaz Ferriera ('57b, '57c) subsequently have shown that the "Hofe" are actually due to improper osmotic pressure.

There have been many conflicting reports of morphological types of parenchymal cells. Lever ('55) reported the presence of "light" and "dark" cells similar to those seen with the light microscope by Cramer ('28). Sjostrand and Wetzstein ('56) and Wetzstein ('57) described two distinct parenchymal cell types in the mouse, guinea pig and cat adrenal medulla - "Hauptzellen" and "Nebenzellen." However, Eranko and Hanninen ('60), DeRobertis and Vaz Ferriera

('57b, '57c), Geyer ('59), Burgas ('59), and Kleinschmidt and Schumann ('61) could not demonstrate the Nebenzellen as described by Wetzstein. Yates, et al. ('62), using the findings of the biochemists that noradrenaline was predominantly within a fraction of larger granules, while adrenaline was predominantly within smaller granules; correlated phase and electron microscopic studies of the medullary cells. They found two cell types with the phase microscope; one being at the periphery with distinct granules and clear cytoplasm; the other being centrally located with indistinct granules. With the electron microscope they found that the peripheral cells contained granules with an average diameter of 2000 Angstroms while the diameter of the granules in the central cells averaged 1000 Angstroms. They thus concluded that noradrenaline-containing cells are located at the periphery of the hamster medulla and are distinct and different from the more centrally located adrenaline-containing cells. However, Michel-Bechet and Cotte ('63) and Michel-Bechet ('63) also studied the hamster and reported four cell types. The first two types they considered as being morphofunctional extremes of the same cell type; type one having more granules; while type two

had less granules but a better developed Golgi apparatus and parallel laminations of endoplasmic reticulum. Type three consisted of rare cells with small dark granules of unknown significance, while type four were misplaced cortical cells.

Thus with the electron microscope, the same conflicting hypotheses have developed as did with biochemical and enzymological studies. Are there distinct cell types for the different catecholamines, or are the differences in cells only due to different morpho-functional stages of the same cell type?

The electron microscope has also been used in studying the secretory process of the adrenal medulla. Lever ('55) studying innervated and denervated adrenal medullary tissue from rats, proposed a process of secretion involving intracytoplasmic granular dissolution; that is, a hypothesis consistent with that summarized by Hillarp ('60d) which considers the granules as storage forms rather than secretory granules, and which considers secretion as involving the release of the catecholamines into the cytoplasm. On the other hand, the alternate hypothesis "secretion by degranulation" as proposed by Burack and Draskoczy ('62) is also apparently supported in the electron microscope studies of DeRobertis and Vaz Ferreira

('57b, '57c) DeRobertis and Sabatini ('60), and DeRobertis ('62). These authors, working with rabbit tissue following splanchnic stimulation, describe a secretory process in which the catechol droplets appear to become attached to the cell membrane - secretion then taking place through the attached portion of the membrane leaving an empty vesicle which incorporates into the cell membrane.

Thus, as in the study of cellular morphology, the electron microscope has developed the same conflicting hypotheses as were suggested by the biochemists and histochemists. Is secretion by a process of intracytoplasmic granular dissolution or is secretion by a process of granular expulsion?

Out of better understanding of the physiology of the adrenal medulla, investigators became curious about the effects of neurohumoral and neuropharmacological agents as possible aids in studying the morphological changes associated with secretory activity in the adrenal medulla. Investigation of the neuropharmacological drugs has been somewhat discouraging in that variable results are obtained. As described earlier, such workers as Holzbauer and Vogt ('56) have shown that in cats the depletion

caused by reserpine was predominantly due to a central action and thus indirect. However, workers as Callingham and Mann ('61) have demonstrated that in rats, there is not only an indirect central effect, but also a direct or peripheral effect which predominates. Using these concepts, workers are now beginning to study the morphological changes induced by such depletion, with the electron microscope. Yates ('63) reported that twenty-four hours after the injection of reserpine, changes consisted of a decrease in the number of granules with an increase in the number of vacuoles. After three injections of reserpine he found an even greater decrease in granules and increase in the number of vacuoles. Clementi and Zocche ('63) reported that three days after reserpine in rats, the endoplasmic reticulum and Golgi apparatus was slightly swollen. At four days, the Golgi complex was filled with sacs and vesicles and there were small granules near the Golgi zone; and by nine days the tissue appeared like normal controls. Shionoya ('63) studying human tissue, reported that reserpine treatment, in cases of occlusive arterial disease, did not result in a decrease in the number of granules, but did cause a decreased density of the granules and an increase in microgranular forms. Yates ('64),

correlating the chromaffin reaction as shown by the light microscope with ultrastructural changes associated with reserpine induced depletion of catecholamines, found that smaller doses of reserpine will produce a negative chromaffin reaction associated with granules still being present as shown by electron microscopy. He interprets this as evidence that, after limited reserpine injections, catecholamines can be released from the medullary cells without granule disappearance.

III. MATERIAL AND METHODS

The purposes of this project were as follows: first, to study the general ultrastructural morphology of the adrenal medulla and correlate the electron microscopic appearance with light microscopic appearance; second, to study the innervation of the adrenal medulla as shown by both light and electron microscope; and third, to determine the fine structural changes associated with the secretory process of the medullary cells as induced by reserpine. Three groups of animals were used. The first group consisted of thirteen albino Swiss mice which were used for purposes of studying ultrastructural morphology of the adrenal medullary cells. Tissue was removed from these animals while under ether anesthesia. The second group consisted of five Sprague Dawley rats which were used for purposes of correlating ultrastructure with light microscopy. The third group consisted of twenty-two male albino Sprague Dawley rats which were used in an experiment to determine the fine structural changes associated with reserpine induced depletion of catecholamines. These animals were sacrificed by a blow to the head.

Six of the rats in the third group were untreated

and served as controls for the experimental procedure as well as providing additional sources for study of nervous tissue. The remainder were given 2.5 mg./Kg. reserpine subcutaneously for one, two and three days. Animals were sacrificed every twenty-four hours for eight days. Adrenal medullary tissue was studied twenty-four hours following the first injection, twenty-four hours following the second injection, and every twenty-four hours for six days following the third injection.

Tissues processed for electron microscopy were fixed in phosphate buffered osmium tetroxide, dehydrated in ethyl alcohols, and embedded in either methacrylate or Epon 812 embedding media, heat polymerization being used in all cases. Sectioning was done on a Porter Blum microtome using glass knives; sections being used with a reflected light color of silver to gold. Sections were post stained with uranyl acetate and viewed with Phillips 100B and RCA EMU 3G electron microscopes.

Material obtained for correlation of light and electron microscopic study consisted of serial thin and thick sections of osmium fixed, epon embedded material. After placing a thin section of silver to gold color on a grid for viewing with an electron

microscope, a serial section of approximately one micron thickness was obtained and placed on a glass slide for viewing with a light microscope. The thick sections were then stained with Toluidin blue before viewing.

Material used for conventional light microscopy was fixed in formalin, embedded in paraffin, and variously stained with hematoxylin and eosin, stains for ganglion cells, and Bodian's method for nerve fibers. Serial sections were studied in tracing the innervation of the adrenal gland by light microscopy.

IV. OBSERVATIONS AND DISCUSSION

A. General Morphology

1. Introduction

The characteristic histochemical reactions of the adrenal medulla are due to the strong reducing abilities of adrenaline, noradrenaline, and other substances such as DOPA and dopamine which are located within the cells. As the catecholamines other than adrenaline and noradrenaline are in very minute amounts, and as other reducing substances are not present in great quantities, these reactions are considered by most authors as specific for the catecholamines; that is, for adrenaline and noradrenaline. The rapid reduction of osmium tetroxide thus enables this substance to serve not only as a fixative but also as somewhat of a selective stain. One must remember, however, that it is not possible to specifically demonstrate adrenaline or noradrenaline with osmium tetroxide rather than some other strongly reducing substance.

2. Light Microscopy

As described by previous workers, the

general orientation of the adrenal medulla is that of fairly large, rounded cells arranged in clumps or groups of cells that tend to be discrete. In some areas (fig. 1) groups of cells can be found surrounding a vessel in such a way as to suggest the polarity of cells described by Bennett and Kilham ('40) and by Bennett ('41). In this particular case, the nuclei of the cells surrounding the vessel are smaller and more dense than other nuclei which can be seen throughout the medulla. One thus notices, even on light microscopy, some apparent differences in the nuclei of different parenchymal cells. Examination of photomicrographs at higher magnification (fig. 2) reveals that not only are there nuclear differences, but also apparently differences in cytoplasm which can be seen with conventional light microscopy - some of the cells having distinctly paler cytoplasm than others.

3. Comparison of Light and Electron Microscopy

When one compares osmium fixed tissue with formalin fixed tissue on the light microscope, the most striking feature is that of contrast. Instead of seeing slight differences in darkness of cells as with formalin fixation and conventional

staining, one sees some cells that appear almost totally black and others that appear quite pale. It can also be seen that there are some cells which are intermediate in density (figs. 3, 4). In serial sections alternately prepared for light and electron microscopy, the same cells can be compared for appearance under the light microscope and under the electron microscope (figs. 3-5). One notices first, the marked difference in resolution. In the photomicrographs, one sees a definite but not distinct granularity within the cellular cytoplasm, while in the electron micrographs of the serial sections, the same cytoplasm is seen to contain distinct sharply defined granules throughout. These granules are seen to be quite osmiophilic and thus black in appearance, which is in high agreement with the demonstration by numerous authors as Blaschko and Welch ('53), Hillarp, et al. ('54), and Schumann ('60) that the majority of the catecholamines is within granules.

4. General Cellular Arrangement

The presence of densely osmiophilic granules is thus the primary characteristic of medullary cells. These cells are often separated

from cortical cells by a series of blood vessels and fibrous tissue constituting a vascular bed. However, the extent of this vascular bed varies somewhat from animal to animal and often the corticomedullary junction is relatively sharp (fig. 1). Though hints of the polarity of cells described by Bennett and Kilham ('40) and Bennett ('41) are seen, this does not seem to be a prominent feature of untreated tissue fixed for electron microscopy. When cells are seen in relationship to bloodvessels (fig. 6), there is a prominent subendothelial space which apparently communicates with intercellular spaces. The intercellular spaces are not prominent except near the corticomedullary junction and near blood vessels or nerve trunks where they are seen to contain much fibrous and collagenous material.

Low magnification electron micrographs (figs. 5, 6) show the "light cells" and "dark cells" described by Lever ('55), as do the photomicrographs (figs. 2, 3). The "dark cells" are characterized by having a greater number of granules, a greater density of granules, a greater density of background cytoplasm, and a greater density of the nuclei, than

do the "light cells." Cells intermediate in number and density of granules, density of cytoplasm, and density of nuclei can also be seen. In untreated animals one notices that the granules within any one cell usually tend to be quite similar.

The majority of the time one does not see specialized forms of intercellular attachment, but instead merely parallel cell membranes separated by a potential intercellular space. However, two types of specialized cellular attachments are occasionally seen. When parenchymal medullary cells are adjacent to nonparenchymal cells (fig. 7) the cell membranes can be seen to extensively interdigitate. The second form of specialized attachment is occasionally seen when parenchymal cells are adjacent to one another, and takes the form of a desmosome-like structure (fig. 39).

5. Medullary Parenchymal Cells

As previously mentioned, the primary characteristic of the parenchymal cells is the presence of densely osmiophilic granules generally considered to be chromaffin granules. The granules are characteristically surrounded by a single membrane (figs. 7 - 10), though examples of other

membrane relationships can be seen. DeRobertis and Vaz Ferreira ('57c) described a "multivesicular catechol-containing body," and similar structures can occasionally be seen (figs. 9,10). Other manifestations of granule-membrane relationships include: a granule surrounded by its single membrane apparently within a double membraned structure (fig. 9); and two granules with their single surrounding membranes together with a vesicle within a common single membraned structure, (fig. 10). DeRobertis and Vaz Ferreira ('57c) suggested that the "multivesicular catechol-containing bodies" have two membranes within the cell and pass into the intercellular spaces, leaving the outer membrane attached to the cell membrane. This concept has not been verified in this study; in fact, the presence of similar structures within the cytoplasm with but a single surrounding membrane is inconsistent with the proposal. However, the suggestion that the membranes are dynamically involved in the actions of the medullary cells seems quite probable in view of the many different manifestations of granule-membrane relationships.

Close examination of individual granules in certain cells reveals that they are not all identical

(fig. 8). In some instances, the contents of the membranes consists of a lightly osmiophilic microgranular substance. In other instances, the granules consist of a densely osmiophilic center surrounded by a lighter microgranular substance; and in others, there is a clear nongranular space next to the membranes. There is much apparent gradation in both size and density of the granules. These differences in granules are especially prominent near a well developed Golgi zone (figs. 8, 29, 30).

However, in untreated animals the Golgi zone is usually diffuse and not prominent (fig. 7); and the majority of cells seen do not show evidence of Golgi zones. This can be explained by the fact that the plane of sectioning would more frequently miss the Golgi zone when it is small.

The endoplasmic reticulum in untreated animals is predominantly smooth in form and classical parallel lamellae or flattened cisternae are not frequently seen (figs. 7-10, 29, 30). However, occasionally in control animals one finds lamellae of rough endoplasmic reticulum, just as one occasionally finds well developed Golgi zones (fig. 29).

The mitochondria bear little resemblance to the osmiophilic granules as was once thought

(Hillarp, et al. '54). They are usually spherical or ovoid in form and tend to be evenly dispersed throughout the cells. They usually contain shelf-like cristae but occasionally tubular appearing cristae are seen in mitochondria within the same cell. The cristae are composed of two membranes, the membranes of one crista being continuous with the membranes of the adjacent crista and connecting with the inner mitochondrial membrane. There is an apparent difference in density of the matrix between cristae compared to the matrix between two membranes of any one crista (fig. 11).

The nuclei, as previously mentioned, tend to vary in density from "light cells" to "dark cells." The nucleoplasm is usually fairly homogenous, though often a neighboring cell may show clumping of chromatin as is frequently held to be characteristic of tissue fixed in phosphate buffer systems. The nuclear membrane is typically double and one or more nucleoli are usually present.

B. Adrenal Innervation

1. Introduction

As described earlier, light microscopic studies by such workers as Swinyard ('37), Bennett and Kilham ('40), and MacFarland and Davenport ('41) have resulted in certain generally accepted concepts regarding the innervation of the adrenal glands of the rat. The nerves are thought to arise primarily from the last two thoracic and upper four lumbar segments of the spinal cord and to predominantly pass over the splanchnic nerves. The fibers pass through the celiac ganglion where some synapse to result in a number of postganglionic fibers. From here a mixed nerve trunk passes toward the adrenal gland containing both preganglionic and postganglionic nerve fibers. Periodically, ganglia can occur along the course of the nerve trunk and more and more of the preganglionics are lost. The nerve trunk entering the adrenal gland then consists of both preganglionics and postganglionics. It is currently held by such workers as Bennett ('41) that the preganglionic fibers innervate the parenchymal cells of the medulla as might be expected in view of the suspected origin of the medullary chromaffin cells

from the neural crests. The postganglionic fibers then predominantly if not exclusively innervate blood vessels (MacFarland and Davenport '41).

2. Light Microscopy

The course of the nerve trunk to the adrenal glands can be easily traced (figs. 12-16). The nerve bundle can be seen to leave a ganglion which corresponds to the "celiac ganglion" and begin its course through areolar and fibrous tissue to the adrenal gland. In the rat, the nerve trunks are noted for not necessarily being associated with the vessels. The nerve bundle loses much of the clear staining myelin before penetrating the capsule of the adrenal gland and thus appears much more dense. The bundle of nerve fibers then passes through the cortex without demonstrable synapse and enters the adrenal medulla (figs. 16, 17). In the past there was much controversy as to whether nerve fibers ended extracellularly or intracellularly. This question is only poorly answered with the limited resolution of the light microscope, and one can best describe nerve fibers as ending at the parenchymal cells (figs. 18, 19).

3. Comparison of Light and Electron Microscopy

The use of osmium fixed tissue is ideal for the demonstration of densely osmiophilic myelin sheaths (figs. 20-22), and firmly discounts many of the older studies reporting the lack of myelinated nerves within the adrenal medulla. As shown in the serial thick and thin sections, the myelinated nerves show well even on lower magnification photomicrographs when the tissue is fixed in osmium. However, the unmyelinated nerves are hard to distinguish by light microscopy even with increased magnification. In contrast, the increased resolution of comparable magnification electron micrographs (fig. 22) illustrates with clarity the presence of both myelinated and unmyelinated nerves in a nerve trunk which might be roughly compared to light microscopic materials stained by Bodian's technique (fig. 17).

4. Electron Microscopy

The non-parenchymal portion of the medulla contains nerves as well as vessels and fibrous tissue. The nerves, as earlier shown, are both myelinated and unmyelinated in nature. Even some of the smaller nerve bundles can be seen to contain both myelinated

and unmyelinated fibers (fig. 23). The myelin sheaths are found to consist of classic parallel laminations of myelin (fig. 24). As might be expected, examples of the loss of myelin from myelinated preganglionic fibers can be found. In some instances (fig. 25), all that is seen is a tendency for the myelin to thin out in the particular plane of section. However, examples of myelin actually terminating also can be found (figs. 26, 27). The myelin is seen to thin out two layers at a time, the two laminations spreading to envelop Schwann cell cytoplasm much as in a Node of Ranvier. However, in contrast to a Node of Ranvier, following the loss of the myelin, the subsequent Schwann cell is not associated with a build up of myelin laminations and the nerve fibers continue without myelin. Coupland ('62) describes the fine nerve axis cylinders running between chromaffin cells as being accompanied by an extension of Schwann cell cytoplasm which drops away as the nerve approaches the nerve ending. Examples of nerve fibers between cells without surrounding Schwann cell cytoplasm, and thus apparently close to their terminations occasionally can be seen (figs. 29, 30). Some of the fibers are said to invaginate the plasma membrane; however, throughout

the "intracellular" course, the fibers are surrounded by the plasma membrane and protoplasmic continuity does not occur. Nerve endings are described as being synaptic in nature with thickened presynaptic and postsynaptic membranes (DeRobertis and Vaz Ferreira '57a; Coupland '62). In addition, in nonstimulated nerve endings, small vesicles can be seen (fig. 28). DeRobertis and Vaz Ferreira also report that in stimulated nerve endings there is a loss of the small vesicles.

Very occasionally one finds an "intracellular" structure with synaptic type membrane thickenings that corresponds to a nerve ending without vesicles (figs. 29, 30). One also notices that there is great variation in size and density of the granules; invaginations of the cell membranes next to the intercellular spaces; and parallel lamellae of rough endoplasmic reticulum in adjacent cells.

C. Experimental Results

1. Introduction

As previously stated, there are two predominant theories of secretion; the one as exemplified by Hillarp ('60d) and Lever ('55) proposing secretion by a process of intracellular granular dissolution; the other as exemplified by Burack and Draskoczy ('62) and DeRobertis and Sabatini ('60) proposing secretion by granular expulsion. The fact that any manipulation of the animal can result in physiologic activation of the adrenal medulla is generally recognized, as is the fact that so-called normal tissue can thus contain cells in all states of activity (Cramer '28; Bennett '41). Since reserpine, as previously discussed, causes direct depletion of catecholamines in the adrenal medulla of the rat, animals were treated with reserpine in order to induce a more constant condition which might be meaningfully interpreted.

2. Twenty-four Hours after First Reserpine Injection

When compared to untreated controls, this tissue shows few differences (figs. 31, 32). The granules are relatively evenly dispersed throughout

the cytoplasm and in general are fairly uniform in density. A few apparent vacuoles are seen in most cells as can occasionally be seen in untreated animals; and in addition, very small particles within the general size range of ribosomes are found free within the cytoplasm.

3. Twenty-four Hours after Second Reserpine Injection

Increased vacuole formation is now apparent (fig. 33). There is a great difference in size and density of granules within most cells and they are no longer evenly dispersed, tending to be in decreased numbers in the central portions of the cells. Small particles are again present within the cytoplasm; and in some areas, one finds "vacuoles" that are elongated resembling parallel lamellae of endoplasmic reticulum. Within some of the vacuoles there is a remnant of material suggestive of the plane of sectioning passing through the marked infoldings of the irregular membranes.

4. Twenty-four Hours after Third Reserpine Injection

Vacuolation is still a prominent feature. Not all cells appear equally involved in terms of apparent granule content or granule size (fig. 35); and in some cells there is a near complete lack of granules

while others appear to be nearly normal. Small particles consistent with the general size of ribosomes are still free within the cytoplasm (fig. 36); and in some areas, these particles appear to be lining the membranes of vacuoles.

5. Two Days after Third Reserpine Injection

Marked vacuolation is still present though there appears to be a great difference in the number of vacuoles present from one cell to another (fig. 37). The relative decrease in number of osmiophilic granules is evident. The majority of cells now show the presence of rough endoplasmic reticulum in parallel lamellae studded with ribosomes. There is at the same time, a relative deficit of granules; and those granules present tend to be near the cell margins (figs. 38-40).

6. Three Days after Third Reserpine Injection

Rough endoplasmic reticulum is not a predominant feature in the majority of the cells (fig. 41); and there is a decrease in the number of vacuoles in any one cell. Concurrently, there is apparently a relative increase in the number of

osmiophilic granules seen.

7. Four Days after Third Reserpine Injection

There is an apparent absence of vacuoles and numerous osmiophilic granules are present in most cells (fig. 42). However, the granules are not equally prominent in all cells.

8. Five Days after Third Reserpine Injection

The tissue now has a near normal appearance. Again there are cells differing in the density and number of granules, the density of the cytoplasm, and the density of the nuclei (fig. 43), that is, "light cells" and "dark cells." There is, however, still some relative decrease of granule numbers in the central perinuclear regions of the parenchymal cells, and occasional remnants of organized endoplasmic reticulum can be seen.

9. Six Days after Third Reserpine Injection

One again sees "light cells" and "dark cells" with granules that are evenly dispersed throughout most of the cells as in control tissue (fig. 44). Also as in untreated animals, one can again find cells apparently in different states of secretory activity.

D. Discussion

In the sequence of events in secretion as proposed by DeRobertis and Sabatini ('60), catecholamine formation begins within some of the small Golgi vesicles. There is a clear space between the dense material and the membranes of the vesicles. This space is reduced as the size of the vesicles and the amount of electron density of the contents increases. As the droplets increase in size and density, they move toward peripheral regions of the cytoplasm. Secretion then involves a decrease in the density of the droplets and an accompanying enlargement of the space between the contents and the membranes. The catechol droplets then attach to the cell membrane and there is a further increase in the size and a decrease in the density of the granules. The droplet is then expelled through the attached portion of the membrane and leaves behind a completely empty membrane. Certain findings in this study are consistent with this proposal. In control animals, the described changes could occasionally be seen near Golgi regions. It is interesting to note examples of a nerve ending with presynaptic and postsynaptic membranes but without vesicles (figs. 29, 30). It is this form of nerve

ending that corresponds to the nerve endings of stimulated nerves described by DeRobertis and Vaz Ferreira ('57a). It thus seems not unreasonable to assume that this portion of medullary tissue is in a state of recent stimulation, and one might then interpret the invaginations in the cell membrane and the different sizes and densities of granules near the well developed Golgi apparatus, as being examples of the process of secretion by degranulation. Also consistent with this hypothesis is the tendency towards margination of the granules in test animals.

However, the occurrence of vacuolation and the suggestion of transition of vacuoles and/or smooth endoplasmic reticulum into rough endoplasmic reticulum is more consistent with a process of secretion involving intracytoplasmic granular dissolution as proposed by Lever ('55) and recently again suggested by Yates ('64). It is of interest to note that ribonucleoproteins are apparently released from granules along with the catecholamines (Philippu and Scheumann '63a, '63b), and that labeled catecholamine precursors introduced in vivo are picked up by the microsomal fraction. These findings could account for the increased preponderance of rough endoplasmic reticulum with reserpine induced depletion if one

accepts intracytoplasmic granular dissolution as the secretory process.

In view of the above findings consistent with both proposed mechanisms of secretion, it seems pertinent to keep in mind that reserpine causes both a direct and centrally induced catecholamine depletion in rats (Callingham and Mann '58b; Camanni, et al. '60). In addition, the evidence for secretion by degranulation has been largely derived from studies involving stimulation of the splanchnic nerves (DeRobertis and Sabatini '60). It seems not too improbable then, to propose that the direct action of reserpine causes an intracytoplasmic dissolution of granules while the concomitant indirect action mediated by the nervous system causes secretion by a process of granular expulsion at the cell membranes.

Also of interest are the findings of Camanni, et al.('58) and Eranko and Hopsu ('61) that low doses of reserpine in rats causes preferential secretion of noradrenaline which is blocked by denervation. It is interesting to speculate that possibly noradrenaline is released via granular expulsion and adrenaline via intracytoplasmic dissolution and that cells showing definite margination of granules contain predominantly noradrenaline.

The claim by many authors that there are separate cell types for adrenaline and noradrenaline cannot be supported by this study, even though larger granules have been shown to contain predominantly noradrenaline and to be located in cells within a certain area while a fraction of smaller granules have been shown to contain predominantly adrenaline and to be located in cells within a different area (Yates, et al. '62). The findings in this study; that any one cell can contain many sizes of granules; and that though the granules in any one cell in untreated animals tend to be about the same size and density, there are still cells that appear intermediate in terms of size and density of granules; tend more to support the view that these are morpho-functional variations in the same type of cell and that if differences exist, they are probably in terms of the amount of an enzyme or enzymes present.

V. SUMMARY AND CONCLUSIONS

A brief historical review and a brief review of the literature concerning innervation of the adrenal gland, catecholamine metabolism, the action of reserpine, and electron microscopic studies of medullary cells is presented.

The appearance of normal, that is, untreated adrenal medullary tissue in mice and rats is described with emphasis on correlation between light and electron microscopy. The innervation of the adrenal medulla as shown by light and electron microscopy is described. Experiments involving reserpine-induced depletion of medullary catecholamines were undertaken.

Male albino rats were given 2.5 mg./Kg. reserpine subcutaneously daily for three days in order to study fine structural changes associated with the reserpine induced catecholamine depletion. Animals were sacrificed every twenty-four hours for nine days. Medullary cells were studied following the first injection, twenty-four hours following the second injection, and every twenty-four hours for six days following the third injection. Tissue was fixed in osmium tetroxide, embedded in Epon 812, and stained with uranyl acetate.

Control animals were characterized by marked

abundance of intracellular granules surrounded by membranes and evenly dispersed throughout the cytoplasm. Endoplasmic reticulum was found to be predominantly smooth and evenly dispersed with little evidence of parallel orientation. Mitochondria were numerous and the Golgi apparatus was usually diffuse.

Occasional examples of invaginations of cell membranes, or empty vesicles attached to the cell membranes, were seen in control animals. In these cells, Golgi complexes are well developed and much discrepancy in granule size and density exists. These apparently active cells are associated with nerve endings which do not have visible synaptic vesicles. Other cells with nerve endings which have visible synaptic vesicles are not associated with as marked changes.

Test animals show a progressive decrease in number of granules associated with an increasing variation in size of granules up to forty-eight hours after the third injection. At the same time, there is a progressive increase in vacuolation; localization and definition of the Golgi zone; and increasing predominance of rough endoplasmic reticulum in the form of parallel flattened cisternae. These changes do not occur to the same degree in all cells;

and between two and six days after the third injection, the cells revert to a form practically identical to control animals.

The possible significance of these findings in relation to the leading theories of secretion is discussed.

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Figure 1

Normal Rat: Low magnification general view of rat adrenal medulla - photomicrograph.

Tissue has been fixed in formalin and stained for ganglion cells. The zona reticularis of the adrenal cortex (R) is seen ending abruptly at the corticomedullary junction. The medullary cells tend to be in "clumps" and "whorls," though in places a distinct orientation can be seen as in those cells surrounding the vessel (V). The nuclei of those cells surrounding the vessel are smaller and more dense than other nuclei as designated by the arrows.

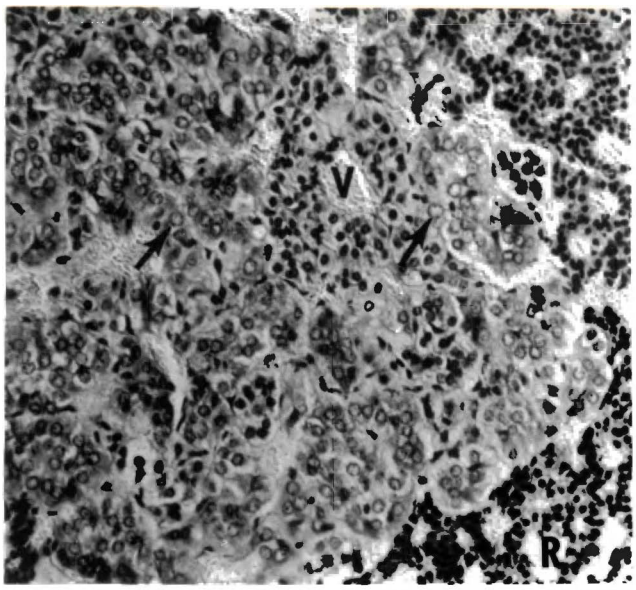
150 X

Figure 2

Normal Rat: Higher magnification general view of rat adrenal medulla - photomicrograph.

The conventional light microscopic appearance of the adrenal medulla when stained with hematoxylin and eosin is illustrated. As can be seen, some cells (arrow) appear to have lighter cytoplasm than others.

380 X



2

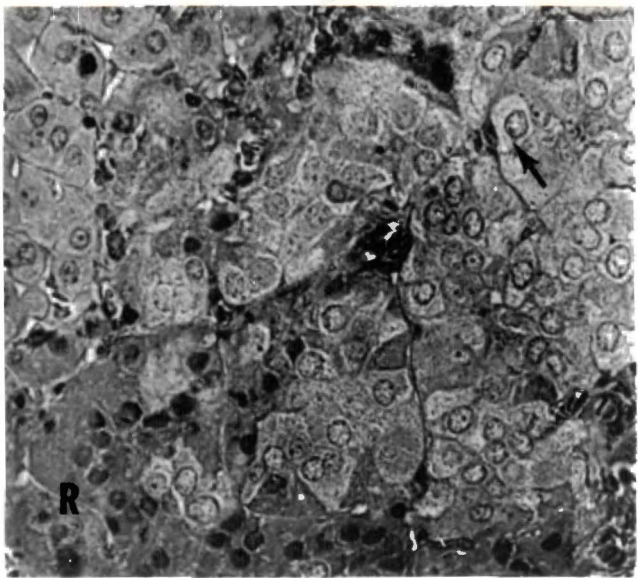


Figure 3

Normal Rat: Low magnification general view of rat adrenal medulla - photomicrograph.

This micrograph is of a thick section of epon embedded osmium fixed tissue stained with Toluidin blue and can be compared to figures 1 and 2.

570 X

Figure 4

Normal Rat: Higher magnification of figure 3 - photomicrograph.

Some cells can be seen to be darker in appearance than others, and many appear to be granular in nature. Also labeled are vessel (V) and nucleus (N).

1300 X

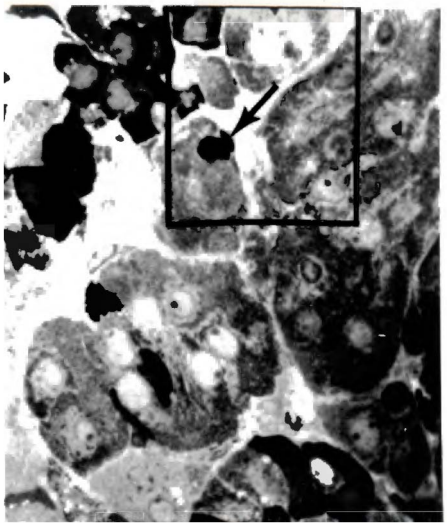
Figure 5

Normal Rat: Serial section following figures 3 and 4 - electron micrograph.

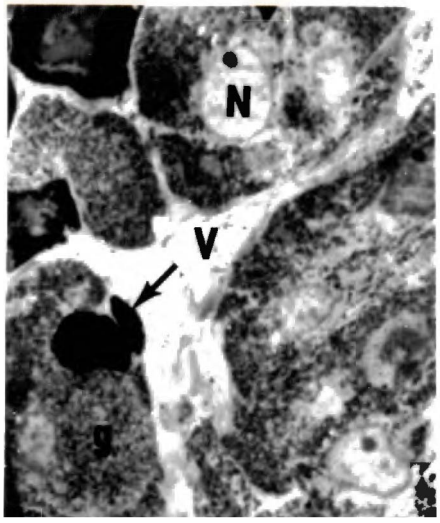
The dark cell (arrow in all figures) can now be seen to be distinctly granular in nature. The difference in resolution of the light and electron microscope at comparable magnification is obvious.

1300 X

3



4



5

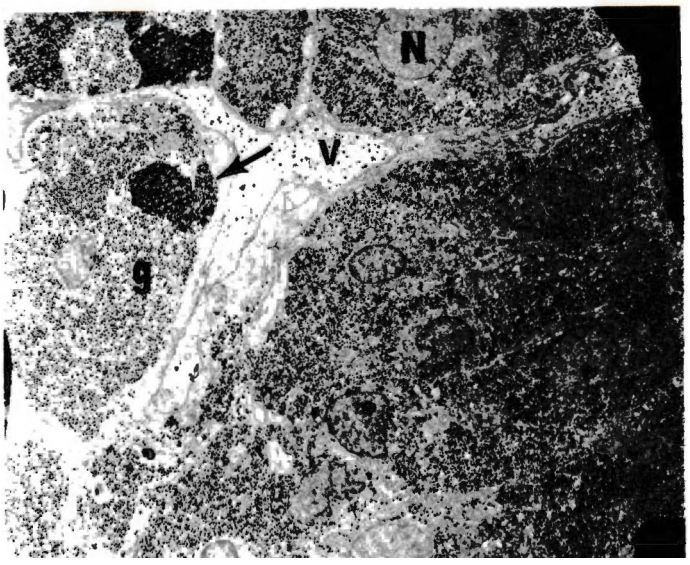


Figure 6

Normal Rat: Low magnification general view of rat adrenal medulla - electron micrograph.

"Light cells" (C1), "dark cells" (C3), and intermediate cells (C2) are evident. The background cytoplasm and nuclei (N1, N3) also vary in density as they do in mouse tissue. Other labeled structures include blood vessels (V), fibrous tissue (F), endothelium (e), and endothelial cell nucleus (Ne).

6,340 X

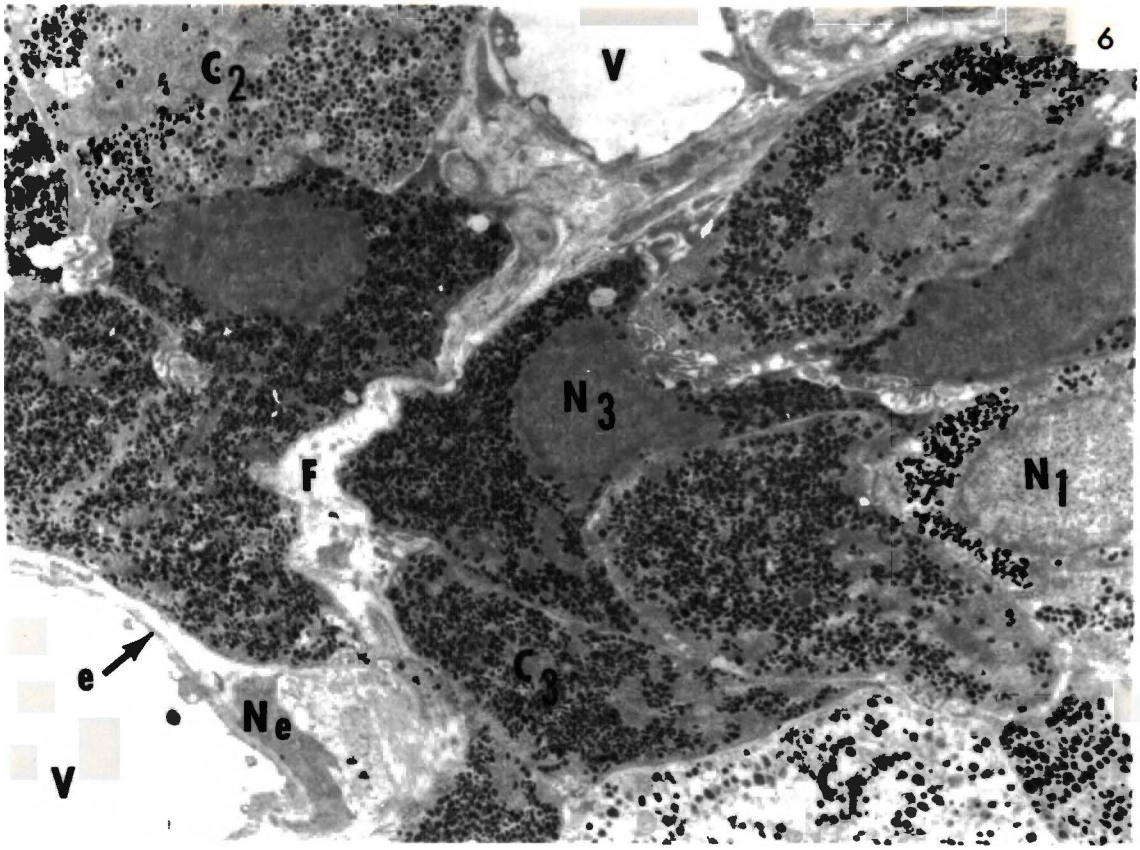

C2
V
N3
F
N1
e
C3
Ne
V

Figure 7

Normal Mouse: Composite of a single adrenal medullary parenchymal cell - electron micrographs.

As illustrated in this composite, when parenchymal medullary cells are adjacent to cortical cells, the cell membranes often interdigitate (Mc). Numerous mitochondria (M) are present. Many of the osmiophilic granules are surrounded by membranes (Mg). Also labeled is the cell nucleus (N). Finer detail is shown in figures 8, 9, and 10, which correspond to the areas so designated in the composite.

16,800 X

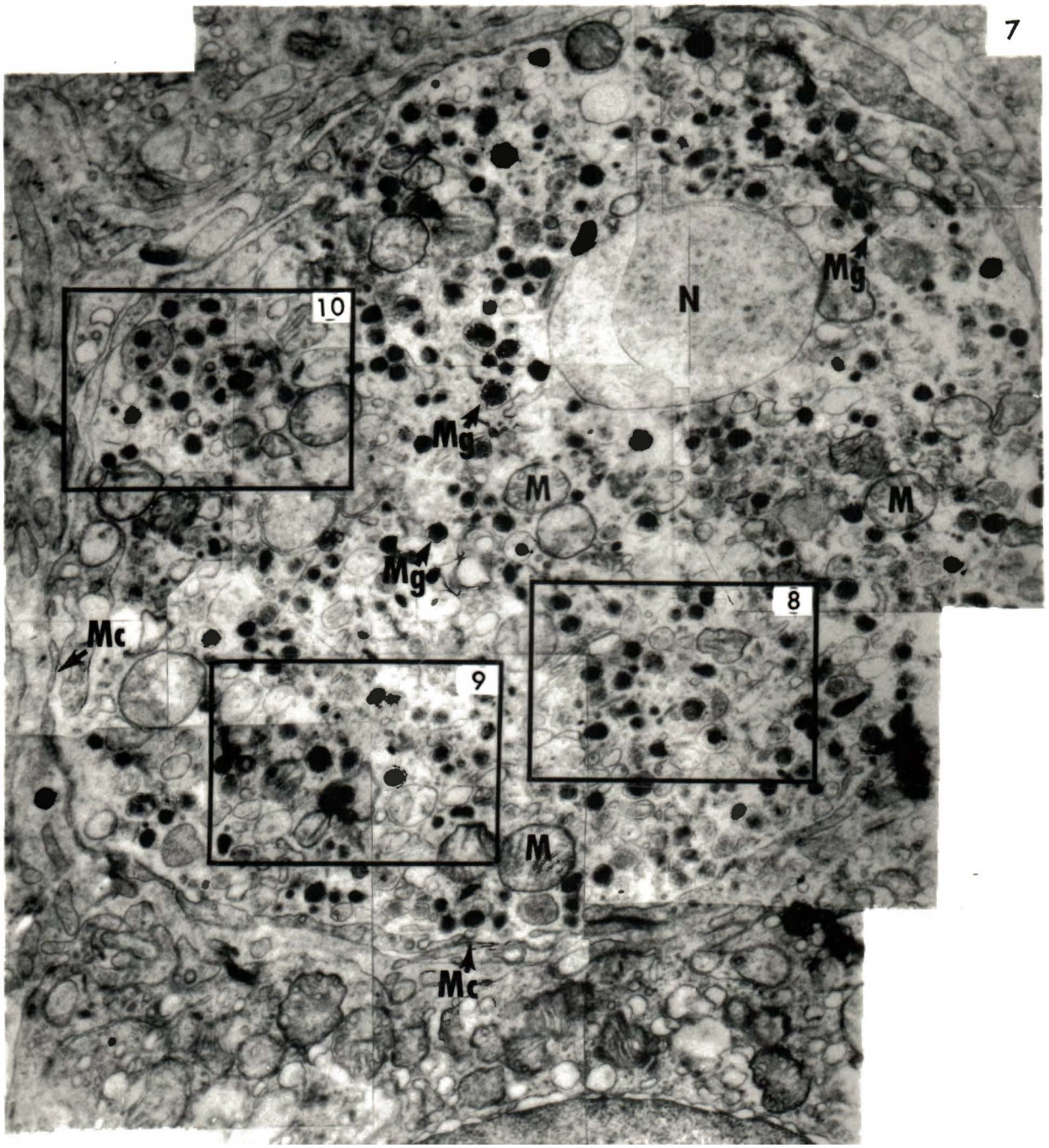

7
N
Mg
Mg
Mg
10
M
M
8
Mc
9
M
Mc

Figure 8

ormal Mouse: High magnification of a portion of figure 7 - electron micrograph.

The granules are usually surrounded by a single membrane (Mg). Some are densely osmiophilic and membranes are difficult to see (ga) while in others there is a peripheral microgranular area (gb) or a clear area (gc) next to the membrane. A few granules tend to be entirely microgranular in form (gd); and in general there is gradation in both size and density (g1, g2, g3, g4). These differences are especially prominent near a Golgi zone (G).

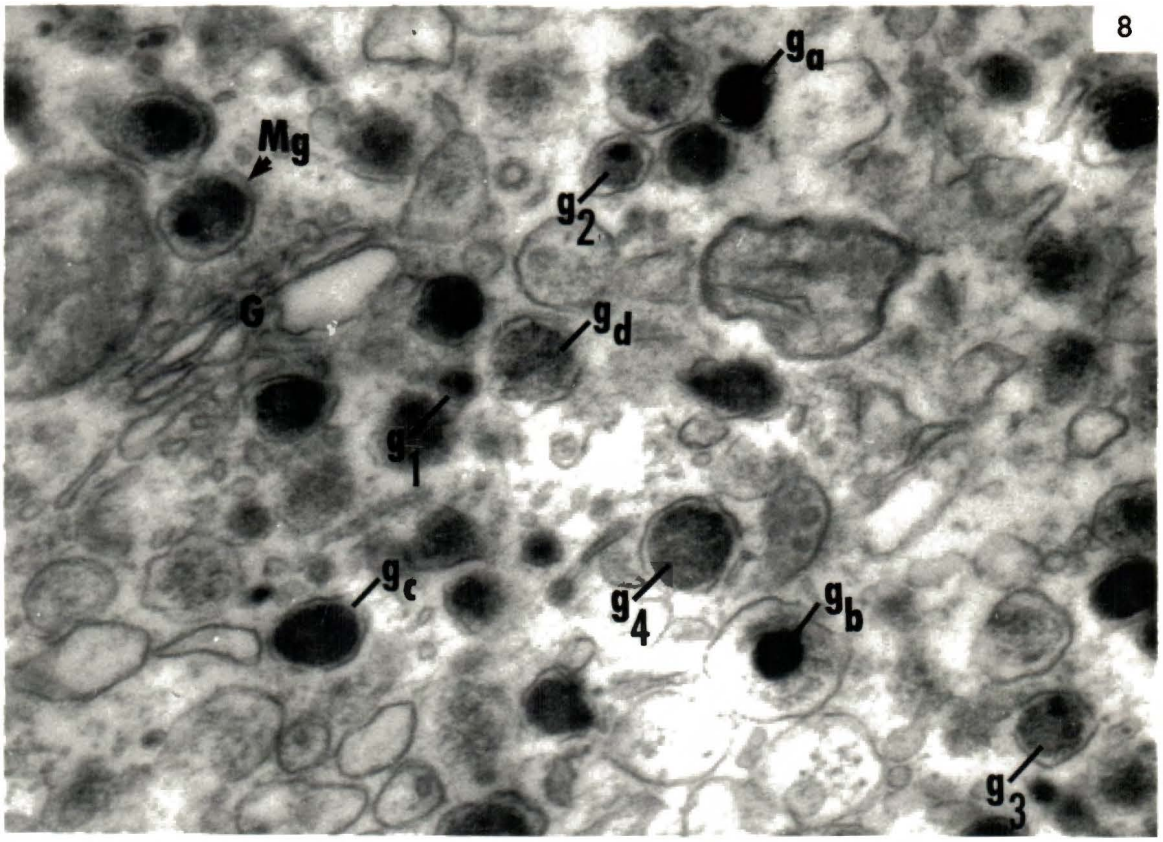
59,080 X

Figure 9

Normal Mouse: High magnification of a portion of figure 7 - electron micrograph.

A granule (g) is surrounded by its membrane (Mg) and apparently within a double membraned structure (MM). Endoplasmic reticulum (er1) is primarily smooth. Occasionally one sees multi-membraned structures as at area X.

59,080 X


8
g_a
Mg
g_2
G
g_d
g_1
g_c
g_4
g_b
g_3

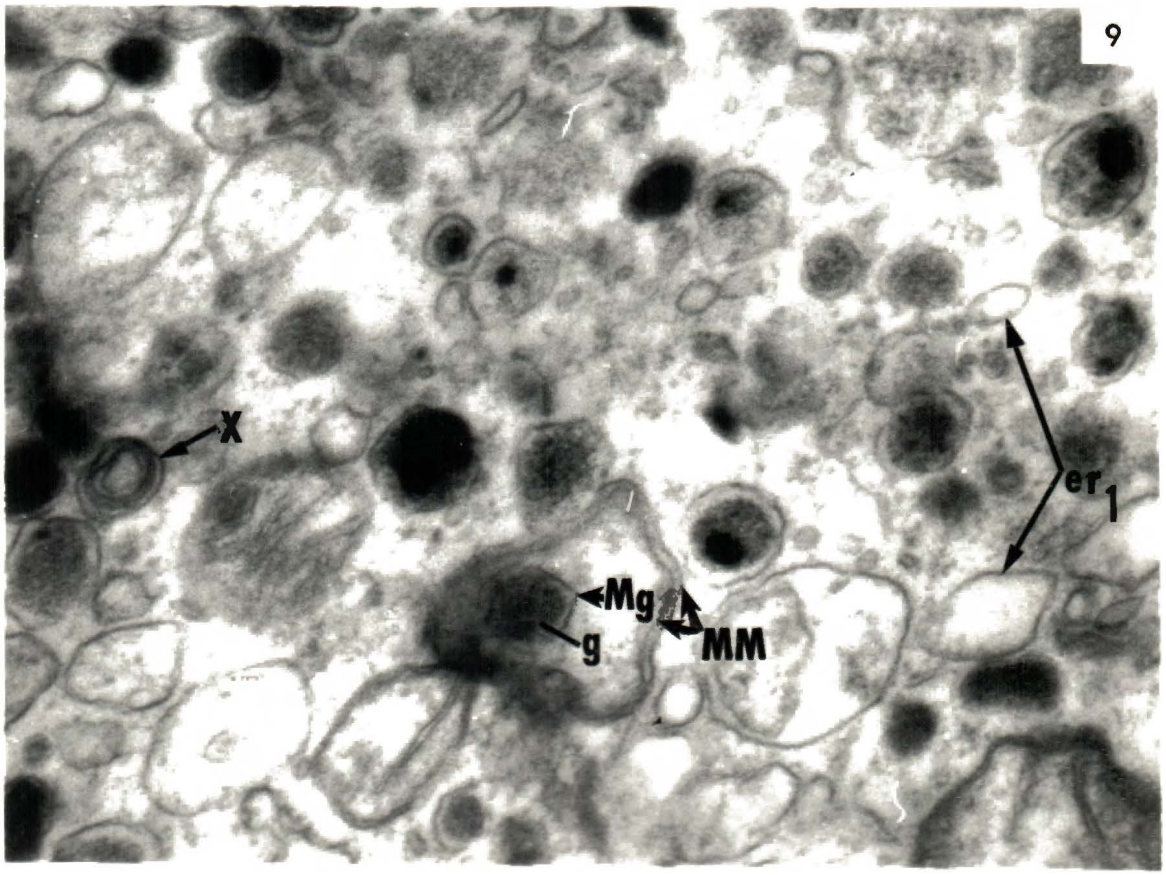

9
X
er_1
Mg
MM
g

Figure 10

Normal Mouse: High magnification of a portion of figure 7 - electron micrograph.

This micrograph demonstrates a structure consisting of two granules with their membranes (g) and a vesicle (v) within a common single membrane (arrow). This structure contains material which is more dense than the general cytoplasm.

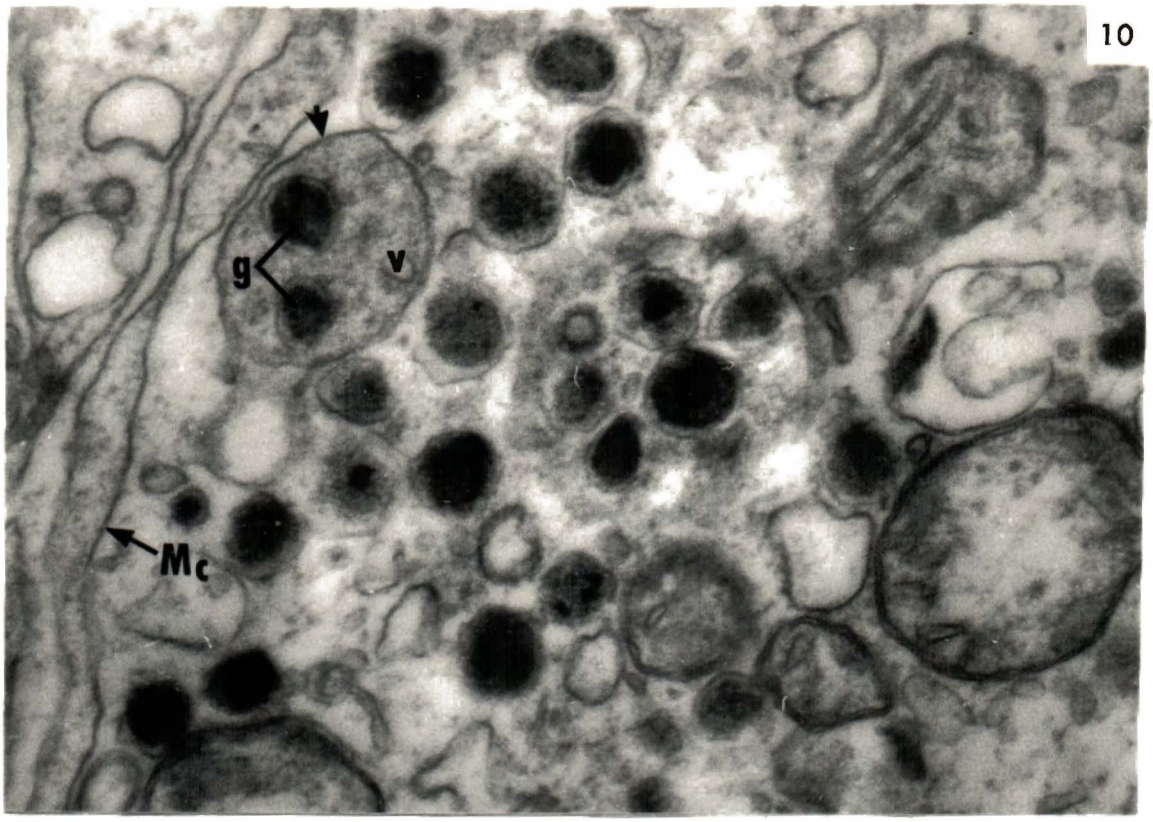
59,080 X

Figure 11

Normal Mouse: Mitochondrial detail - electron micrograph.

Cristae (C) are composed of two membranes (arrows), the membranes of one crista being continuous with the membranes of the adjacent crista resulting in a series of structures that appear shelf-like on cross section. The area between the membranes of any one crista (a1) and between the cristae and the outer membrane of the mitochondrion is more dense than the area between adjacent cristae (a2).

104,000 X


10
g
v
Mc

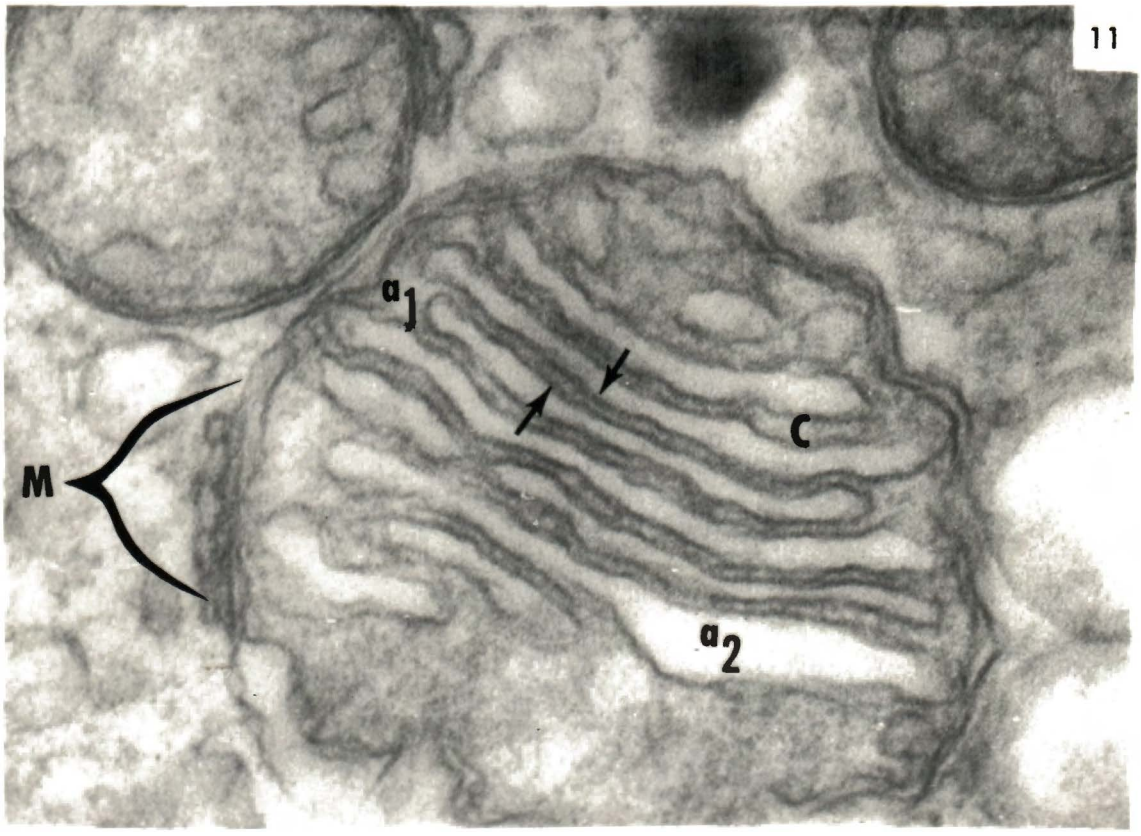

11
a1
C
M
a2

Figure 12

Normal Rat: Ganglion stained with Bodian's technique - photomicrograph.

A large sympathetic ganglion (Ga) and small artery (A).

150 X

Figure 13

Normal Rat: Adrenal nerve stained with Bodian's technique - photomicrograph.

A bundle of nerve fibers (Nt) leaving the sympathetic ganglion (Ga).

150 X

Figure 14

Normal Rat: Adrenal nerve stained with Bodian's technique - photomicrograph.

Nerve fibers (n) piercing the capsule of the adrenal gland.

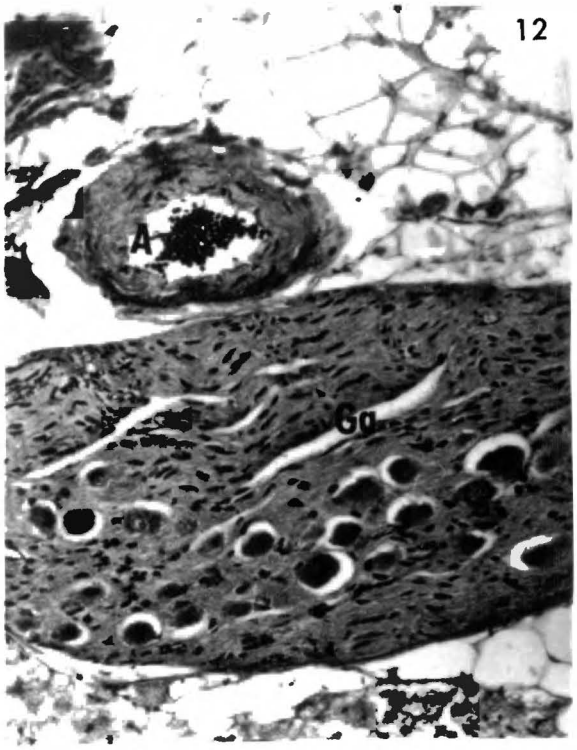
380 X

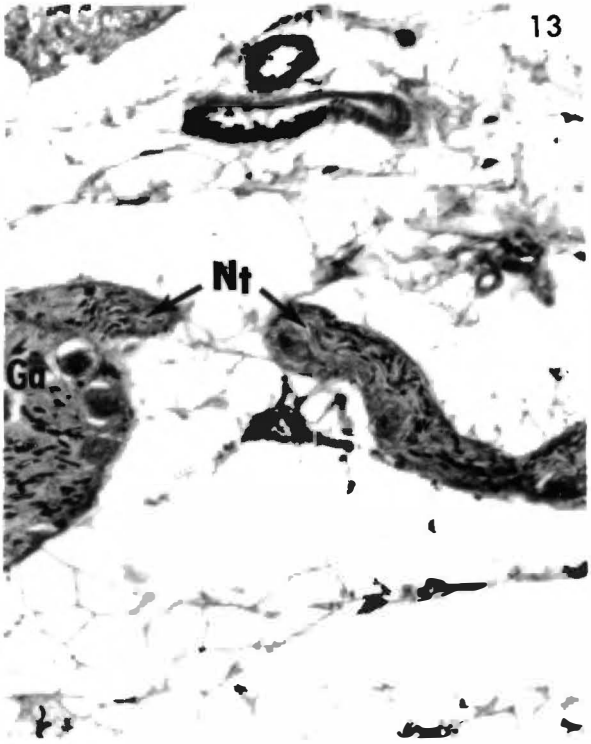
Figure 15

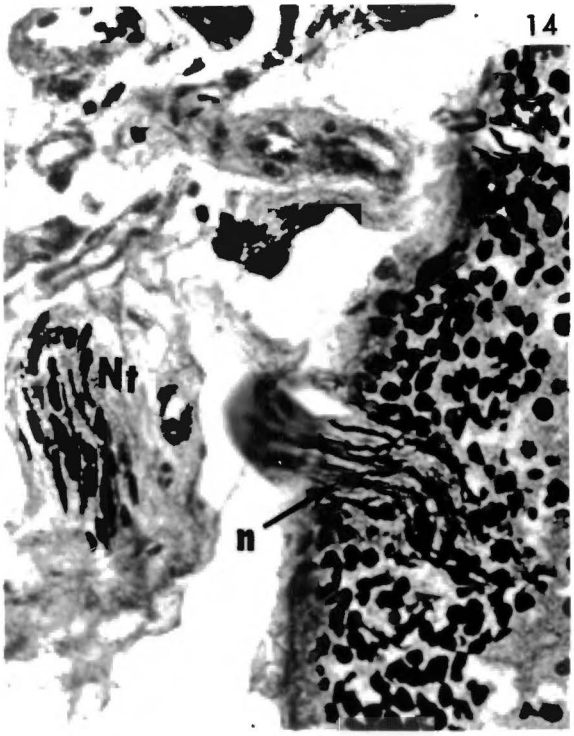
Normal Rat: Adrenal nerve stained with Bodian's technique - photomicrograph.

Nerve fibers (n) passing through the adrenal cortex.

380 X


12
A
Gα


13
Nt
Gα


14
Nt
n

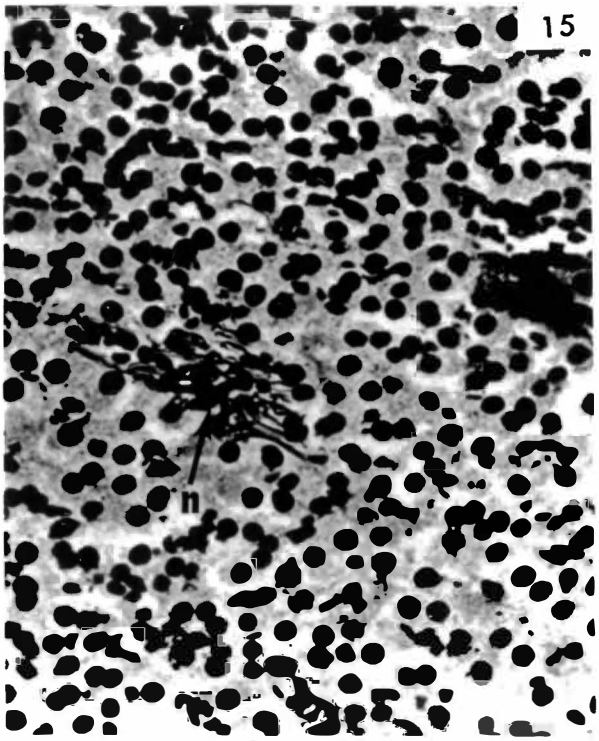

15
n

Figure 16

Normal Rat: Low magnification of adrenal medulla stained with Bodian's technique - photomicrograph.

The cortico-medullary junction delineated by edulla (M) and zona reticularis (R).

150 X

Figure 17

Normal Rat: Higher magnification of figure 16 - photomicrograph.

A large nerve bundle (n) within the medulla.

380 X

Figure 18

Normal Rat: Nerve ending - photomicrograph.

Nerve fiber (arrow) ending at a parenchymal cell.

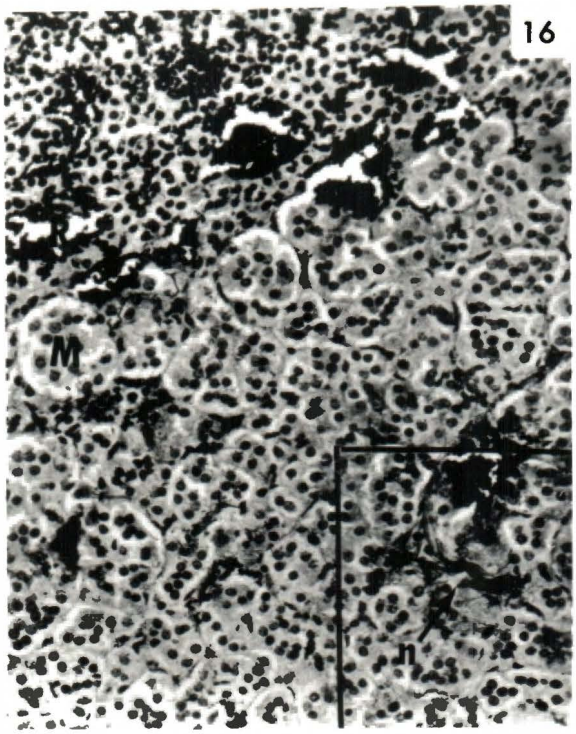
380 X

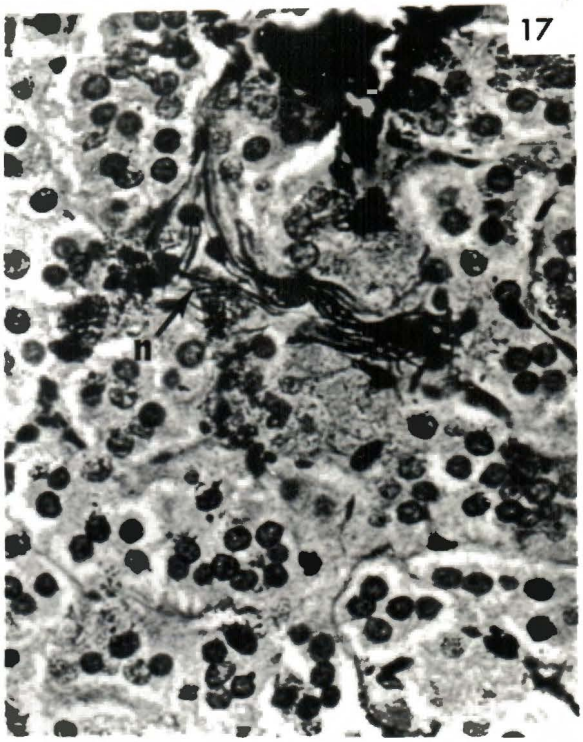
Figure 19

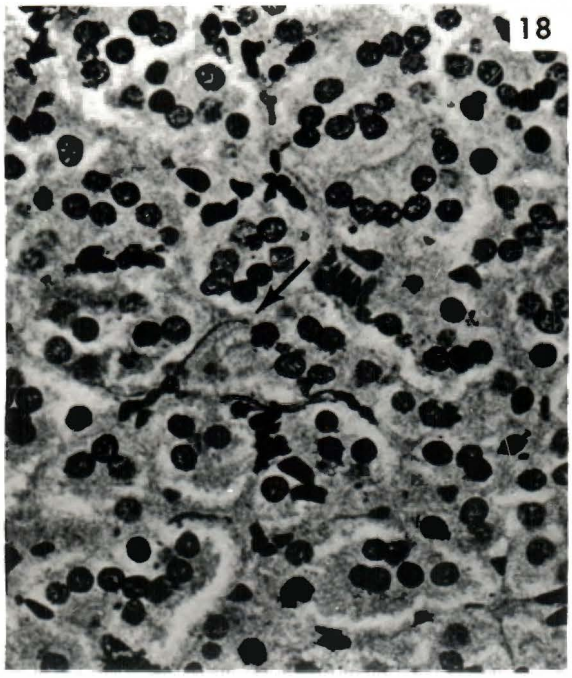
Normal Rat: Nerve ending - photomicrograph.

Another example of a nerve fiber (arrow) ending at a parenchymal cell.

380 X


16
M
n


17
n


18

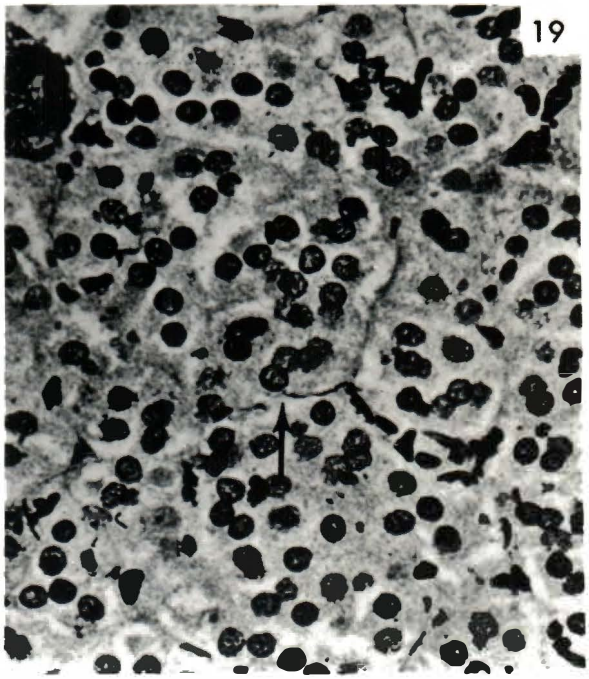

19

Figure 20

Normal Rat: Low magnification general view of medullary nerve trunk - photomicrograph.

This photomicrograph is of a nerve trunk as seen in a thick section of osmium fixed, epon embedded material stained with Toluidin blue. Dark myelin (arrow) is evident surrounding some nerves. Other unmyelinated fibers (n) are not distinct on light microscopy.

570 X

Figure 21

Normal Rat: Higher magnification of figure 20 - photomicrograph.

At this magnification, unmyelinated nervous tissue is still difficult to delineate.

1300 X

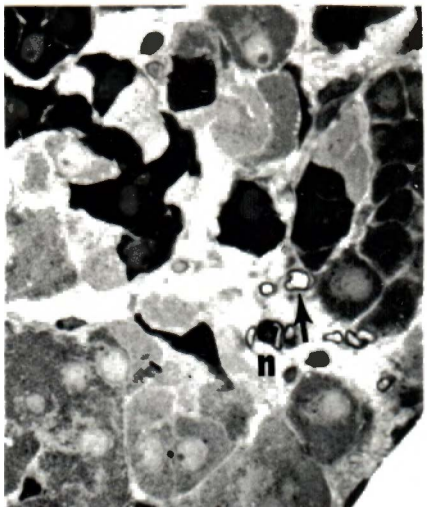
Figure 22

Normal Rat: Serial section following figures 20 and 21 - electron micrograph.

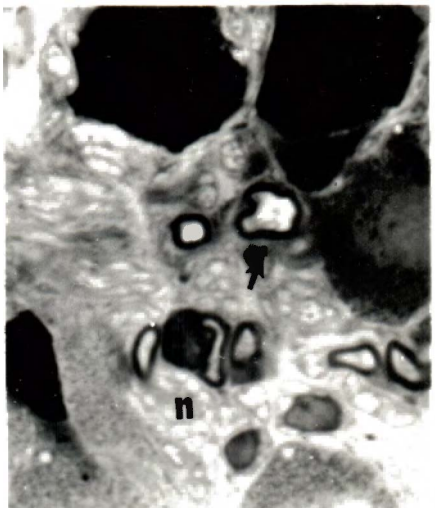
The increased resolution demonstrates the presence of unmyelinated as well as myelinated nerves within the adrenal medullary tissue.

1300 X

20



21



22

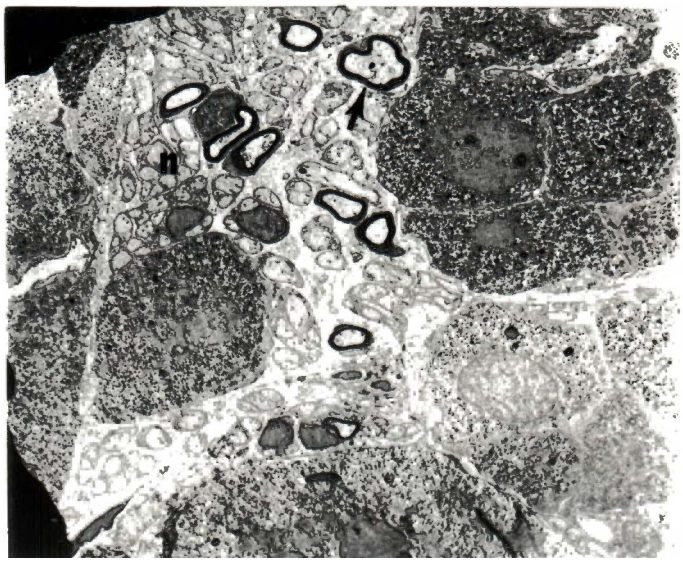


Figure 23

Normal Rat: Adrenal medullary nervous tissue - electron micrograph.

Both myelinated (nm) and unmyelinated (nu) nerve fibers are present. Laminations in the myelin are visible (arrow). Supporting Schwann cells (Cs) are frequent. Both light and dark cell types are present (C1, C3).

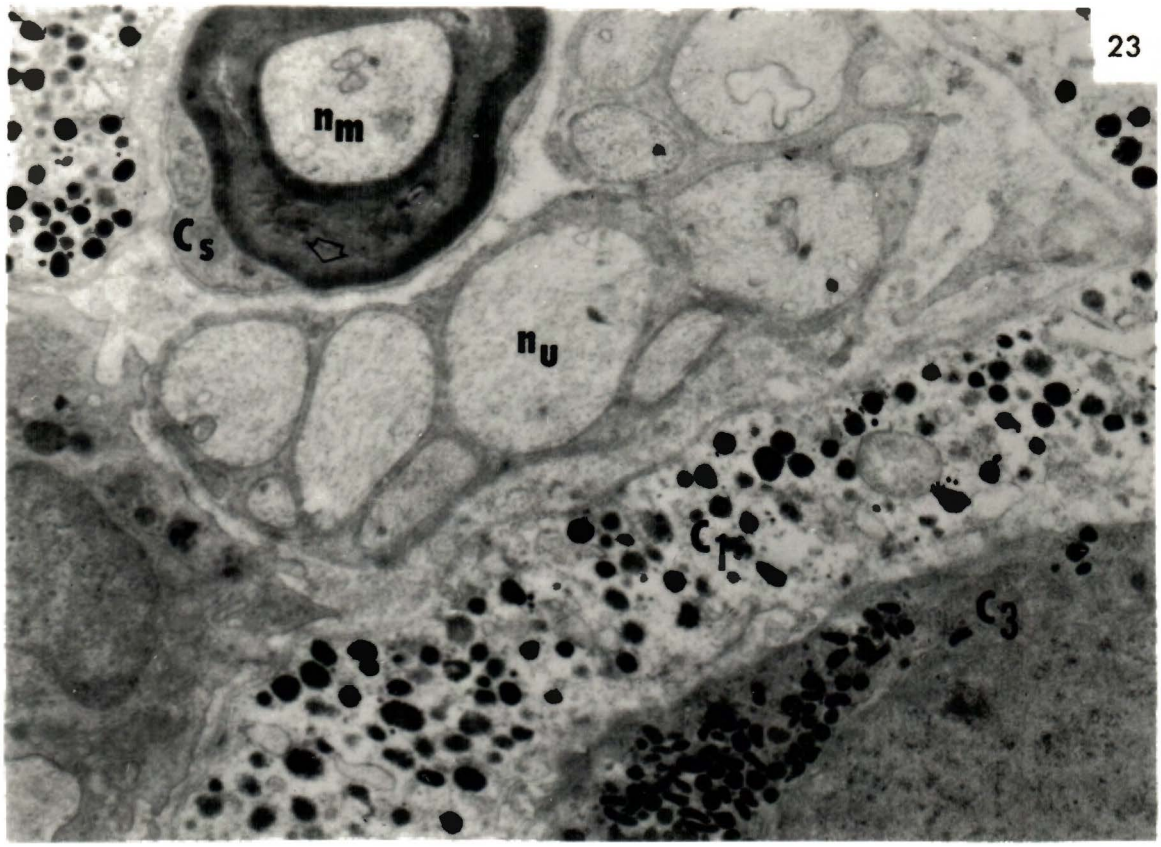
19,630 X

Figure 24

Normal Rat: High magnification of a portion of myelin sheath - electron micrograph.

At this magnification, the laminations in the myelin (arrows) are quite distinct.

117,900 X


23
n_m
C_s
n_u
C_1
C_3

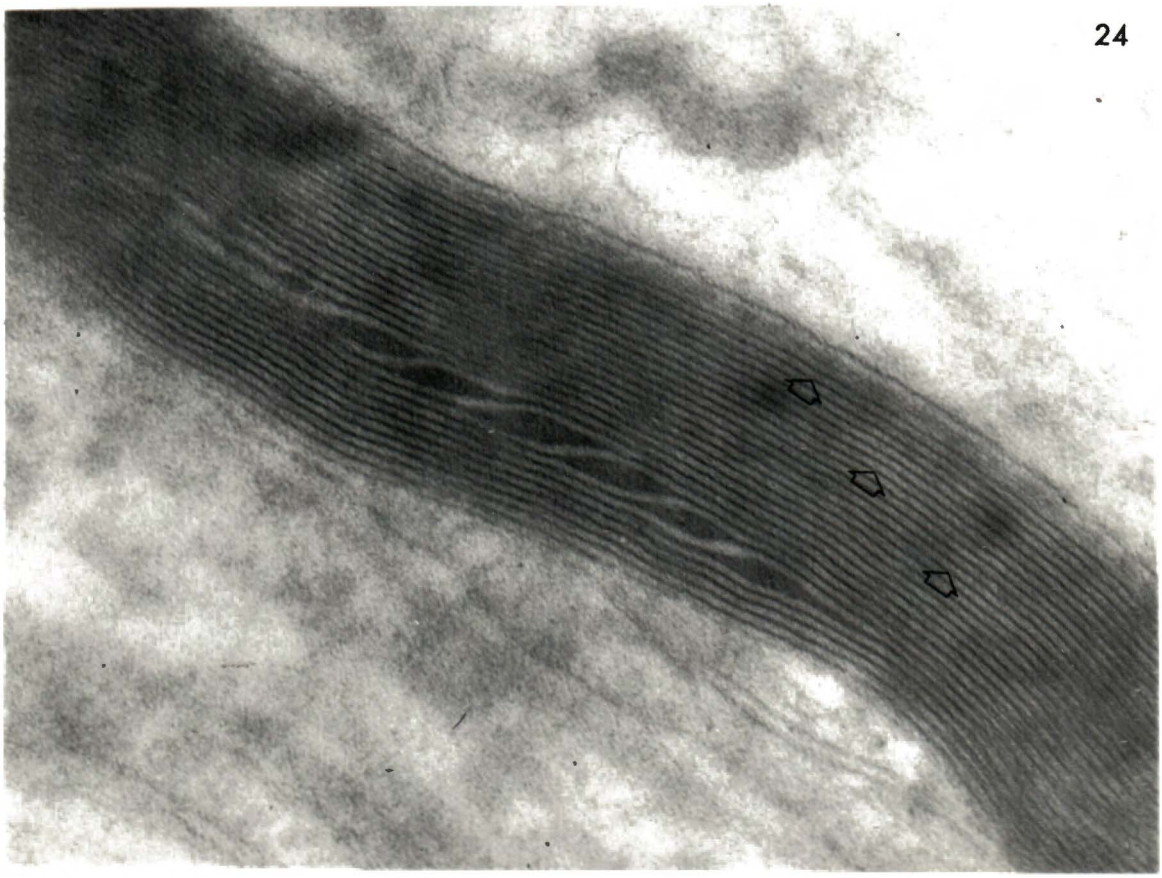

24

Figure 25

Normal Rat: Nerve trunk within the adrenal medulla - electron micrograph.

Both myelinated (nm) and unmyelinated (nu) nerves are present. Between them can be seen much fibrous tissue and collagen (Co). The myelin around the labeled myelinated nerve can be seen to apparently thin out in the vicinity of the arrow.

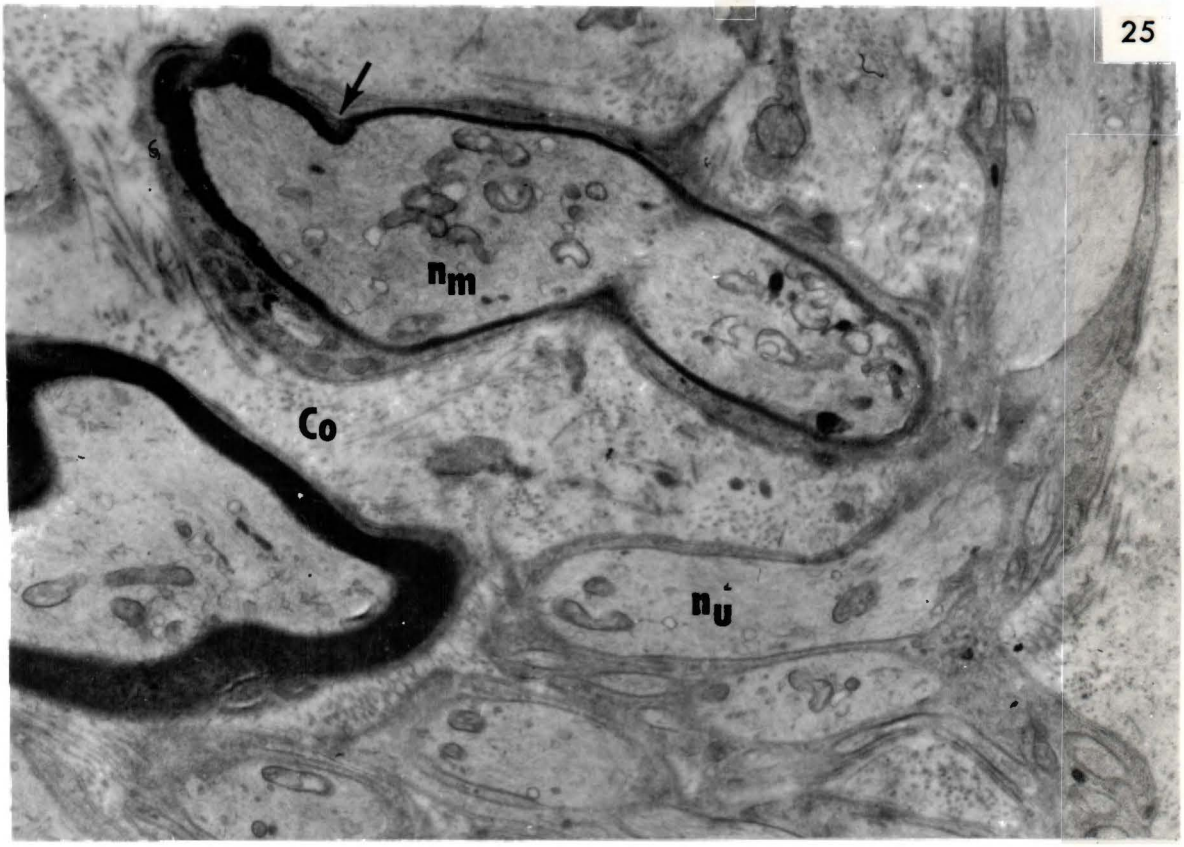
13,860 X

Figure 26

Normal Rat: Nerve trunk within the adrenal medulla - electron micrograph.

Again both myelinated and unmyelinated nerves are present. Also evident are Schwann cells (Cs). The myelin appears to terminate in the area of the arrow and the nerve proceeds from this point without myelin.

13,860 X


25
nm
Co
nu

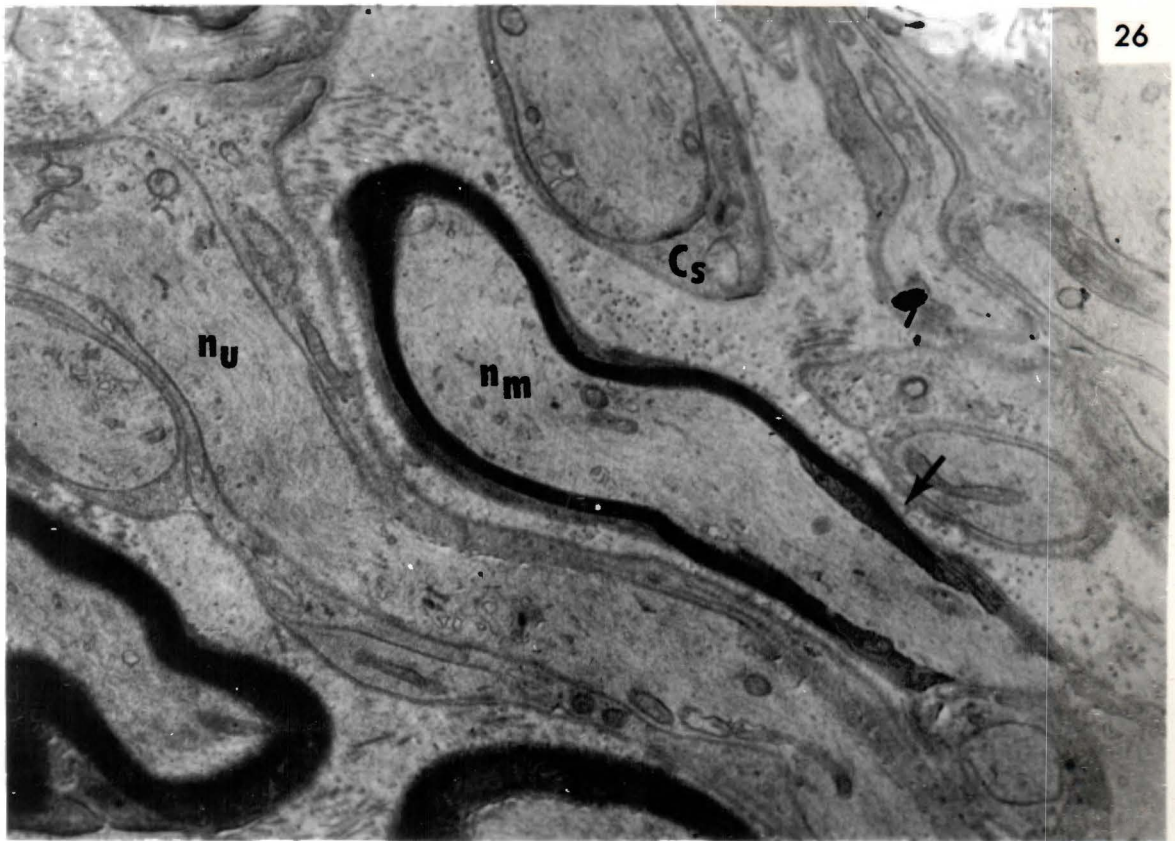

26
Cs
nu
nm

Figure 27

Normal Rat: High magnification of figure 26 - electron micrograph.

This micrograph shows the termination of myelin. The characteristic laminations of the myelin come off two at a time (arrows) to surround the Schwann cell cytoplasm.

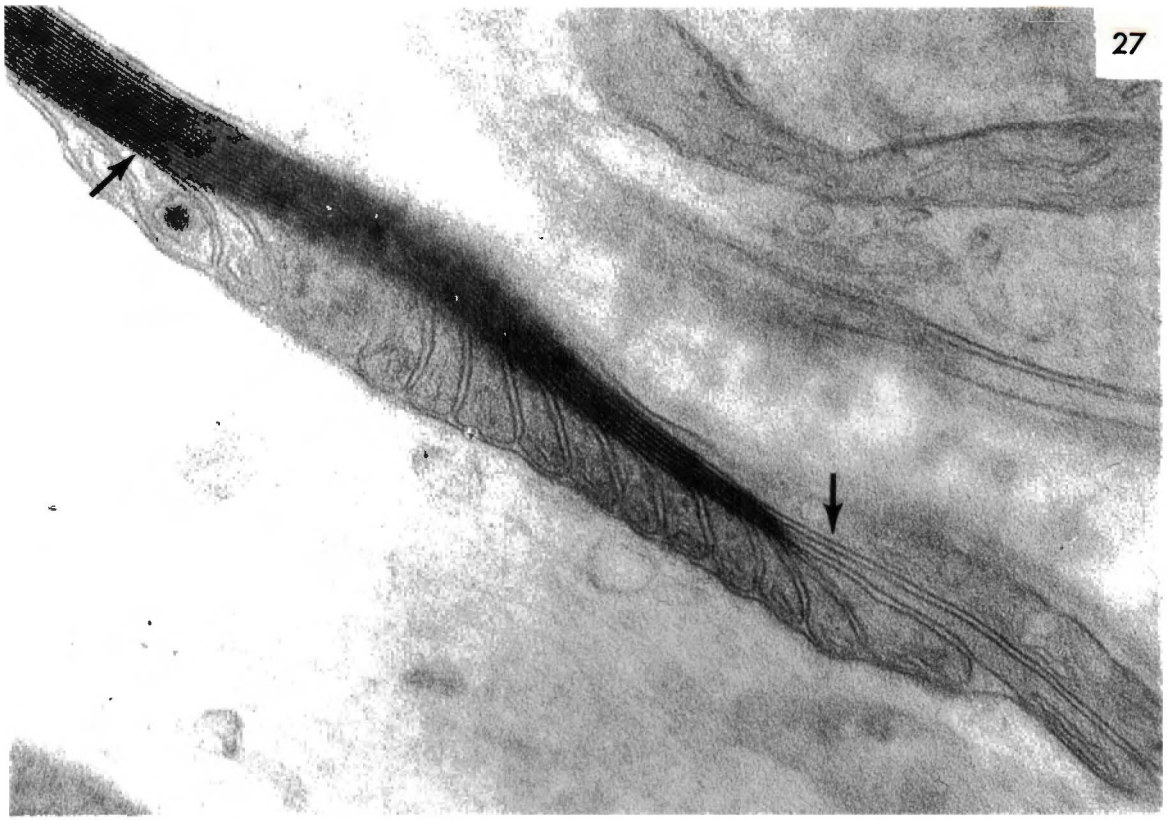
80,720 X

Figure 28

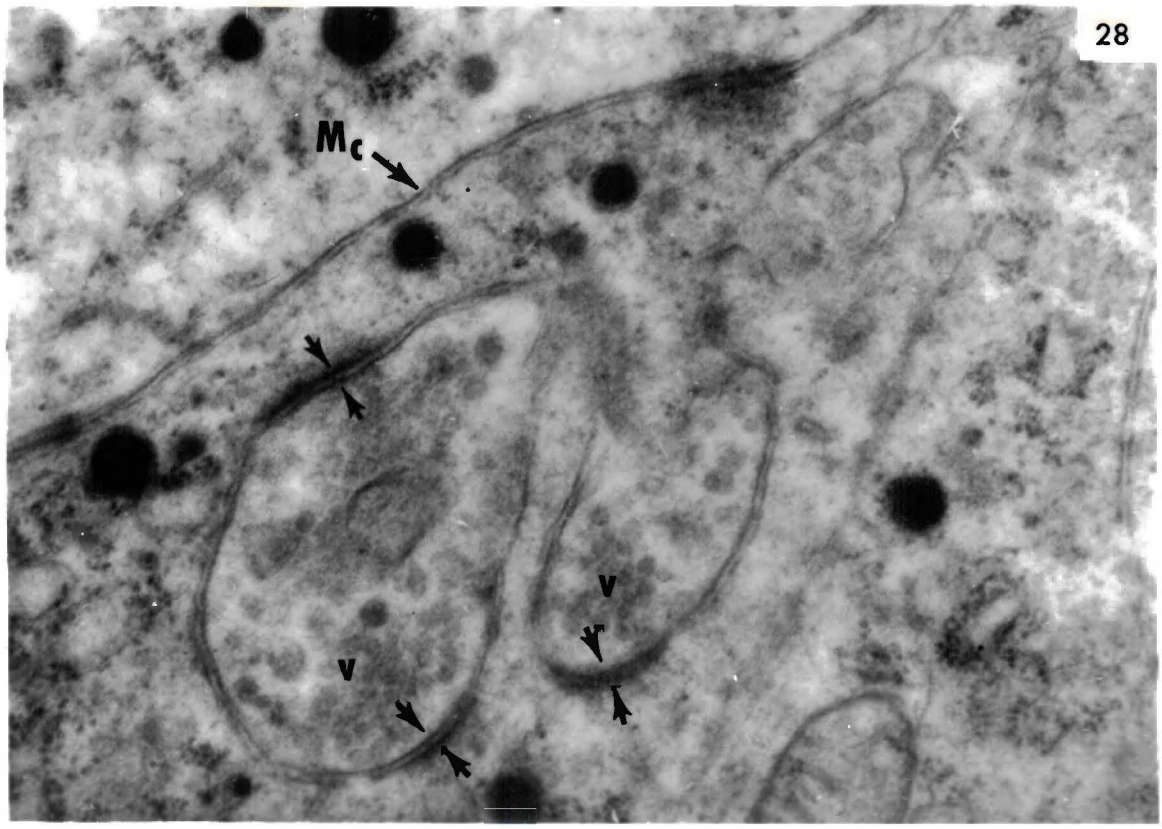
Normal Rat: "Intracellular" nerve ending - electron micrograph.

The characteristic appearance of nerve endings on the parenchymal cells of the adrenal medulla is illustrated. Synaptic thickenings can be seen (arrows) and small vesicles (v) are within the nerve ending. Also labeled is the cell membrane (Mc).

59,080 X



27



28

Figure 29

Normal Rat: An area of apparently active medullary tissue - electron micrograph.

Golgi zones (G) are prominent. There is great variation in size and density of granules (g1, g2, g3, g4). Endoplasmic reticulum is predominantly smooth and in the form of small vacuoles. Invaginations of the cell membrane are present next to intercellular spaces (i). An adjacent cell shows parallel lamellae of rough endoplasmic reticulum (er2). Note the presence of a small nerve (n) and the thickening of cell membranes where parallel (Mc).

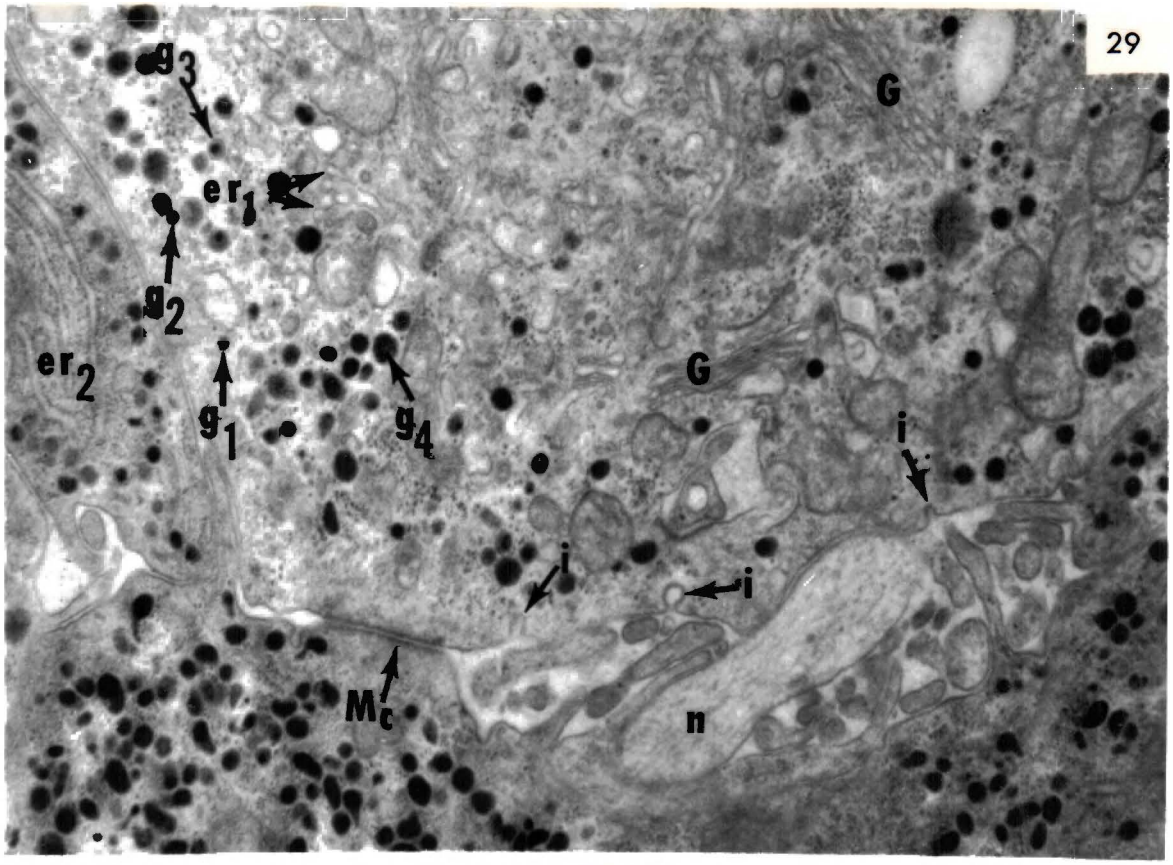
22,300 X

Figure 30

Normal Rat: Higher magnification of an area of figure 29 - electron micrograph.

This micrograph illustrates the presence of numerous smaller granules within the cytoplasm of the cell (arrows). The tissue at n2 corresponds to descriptions of nerve endings within medullary cells with thickened synaptic membranes (s). Note the absence of synaptic vesicles.

45,450 X


29
g3
er1
g2
er2
g1
g4
G
G
i
i
i
Mc
n

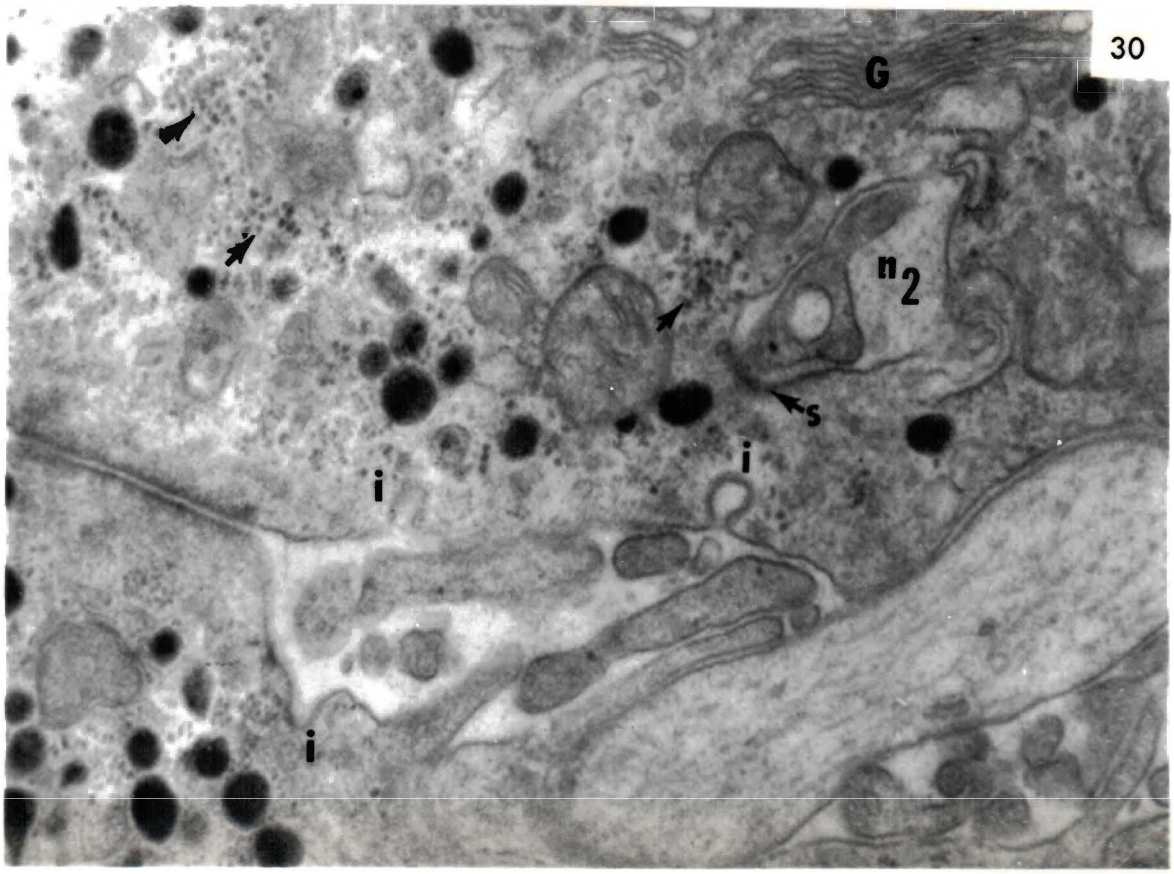

30
G
n2
s
i
i
i

Figure 31

Experimental Rat: Twenty-four hours after first reserpine injection - electron micrograph.

When compared to normal controls there are few differences. A few apparent vacuoles (v) are present. The osmiophilic granules are relatively evenly dispersed throughout the cytoplasm. One does notice a more definite lack in uniformity of size and density of the granules in areas which are apparently not closely involved with Golgi zones (g1, g2, g3, g4).

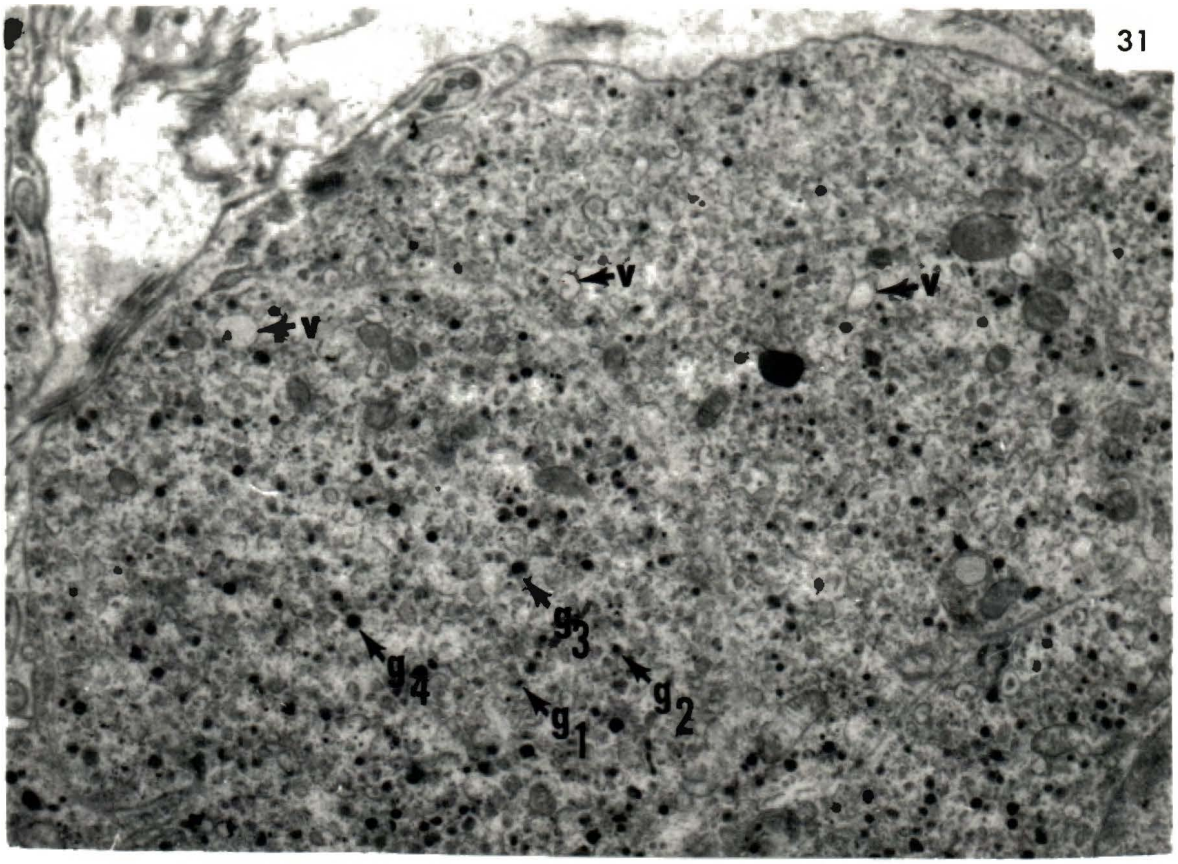
19,630 X

Figure 32

Experimental Rat: Twenty-four hours after first reserpine injection - electron micrograph.

This slightly higher magnification better illustrates the differences in size and density of the osmiophilic granules. There are very small particles (arrows) free in the cytoplasm as were seen in active control tissue.

22,300 X


31
v
v
v
g3
g4
g2
g1

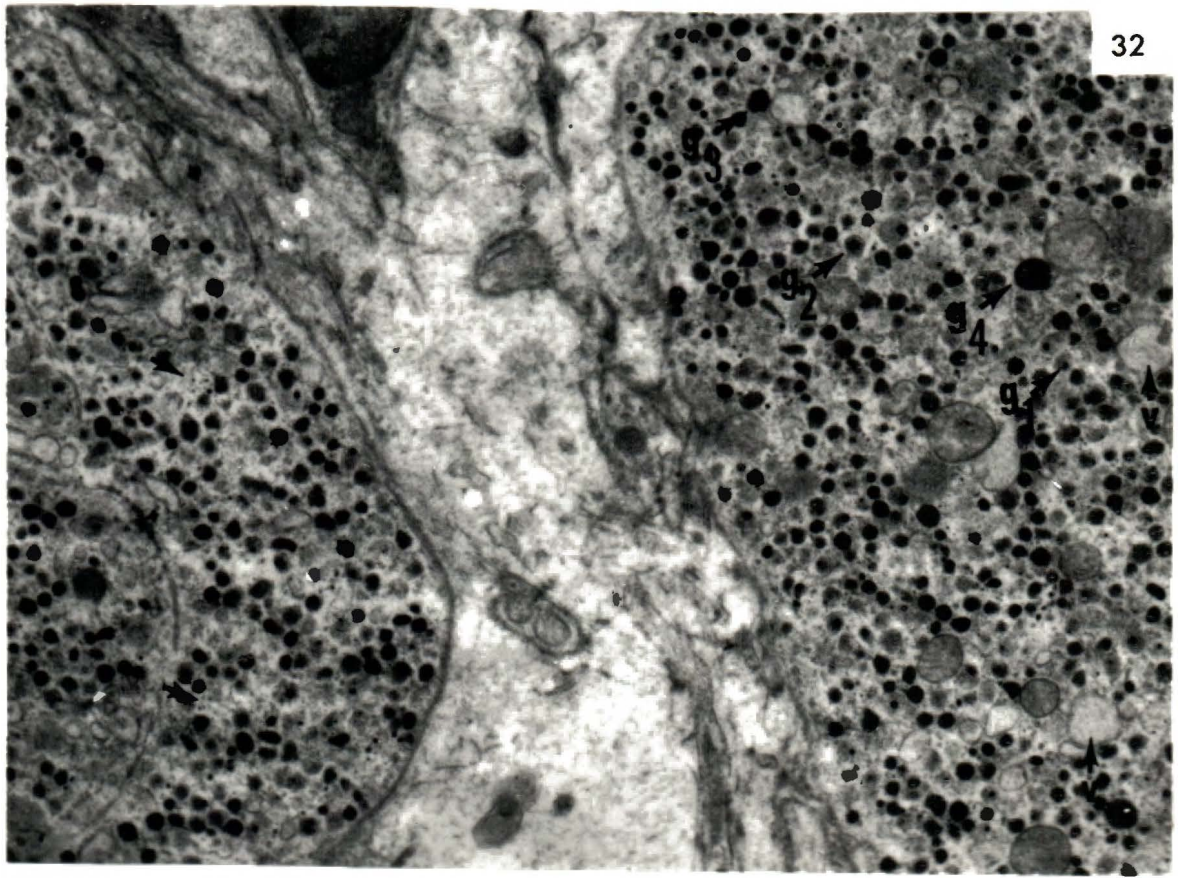

32
g3
g2
g4
g1
v

Figure 33

xperimental Rat: Twenty-four hours after second reserpine injection - electron micrograph.

Increased vesicle formation (v) is obvious. There is now a great difference in size and density of different osmiophilic granules (g), and a relative lack of granules towards the center of the cells. Also labeled are cell membranes (Mc) and a blood vessel (V). Note that in area X, some of the vacuoles or vesicles are elongated resembling dilated lamellae of endoplasmic reticulum.

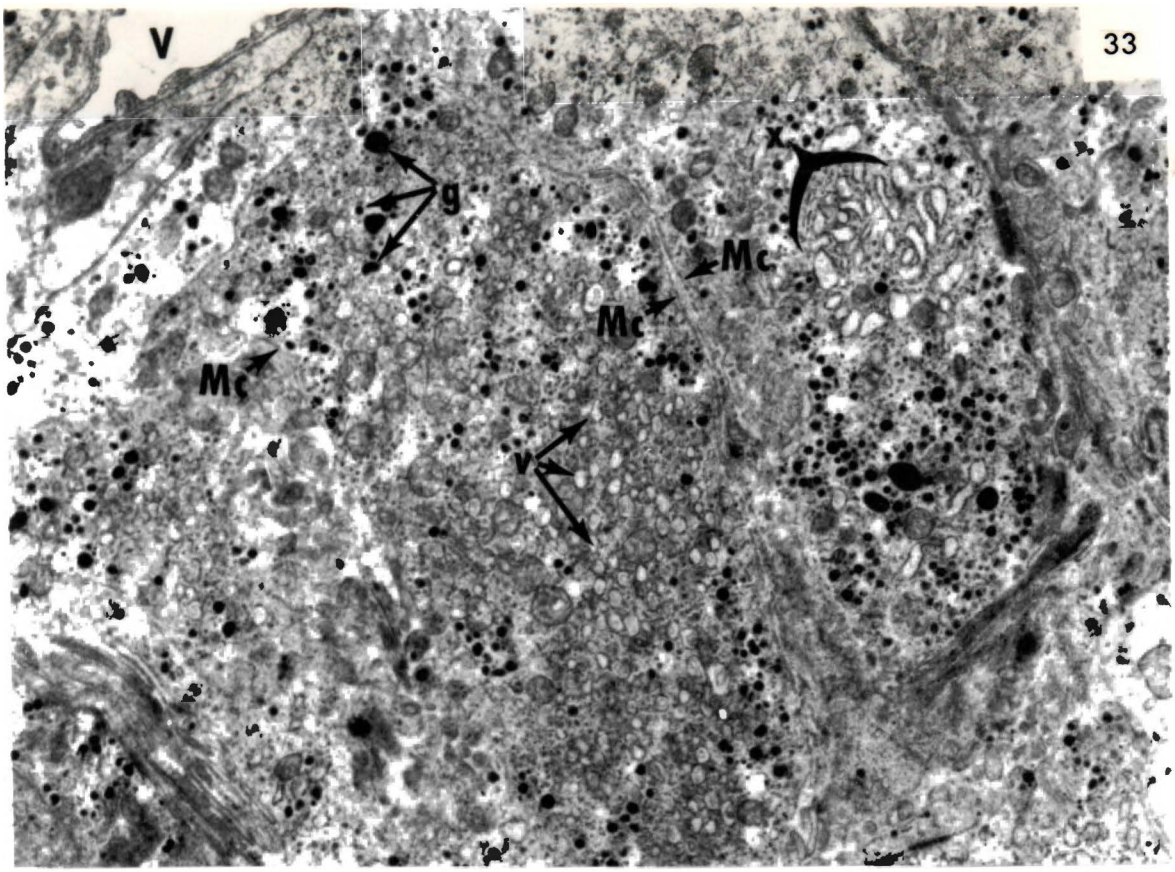
7,000 X

Figure 34

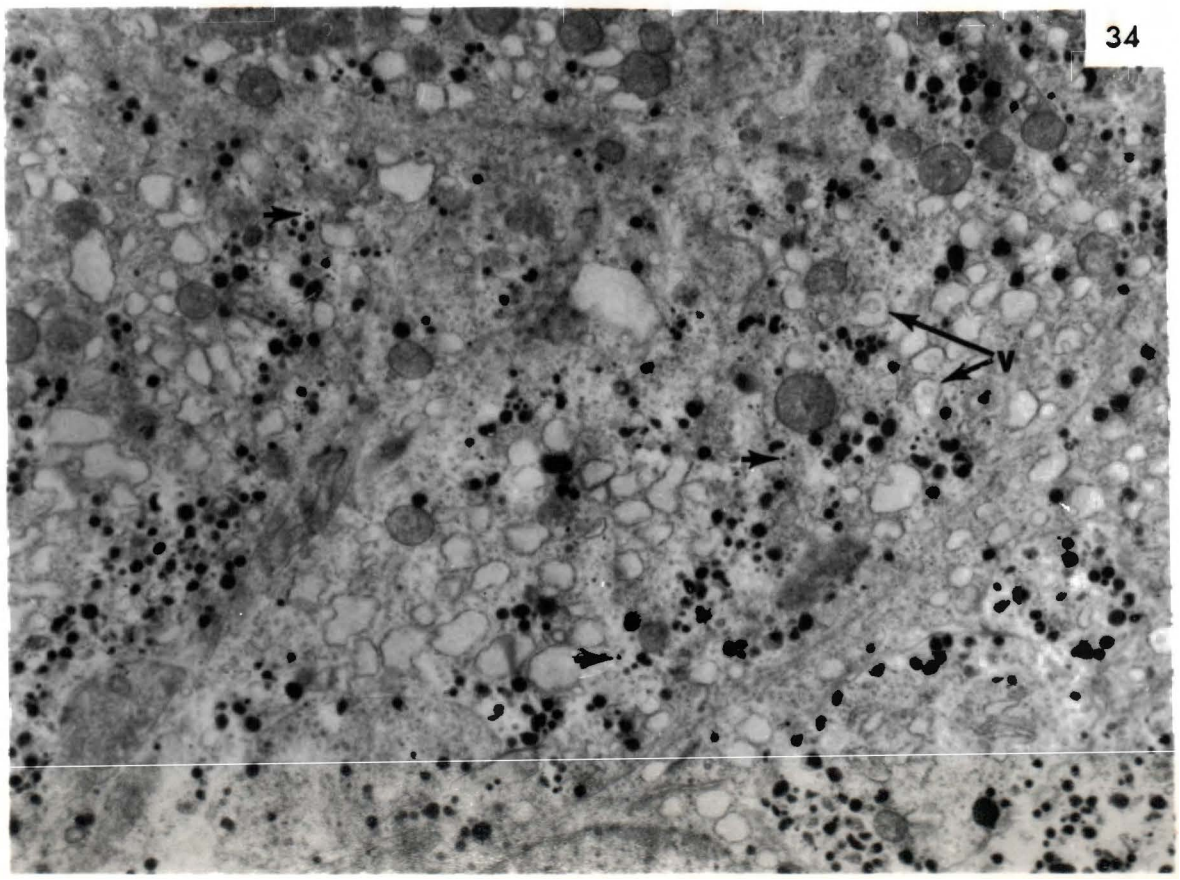
Experimental Rat: Twenty-four hours after second reserpine injection - electron micrograph.

Small densely osmiophilic granules (arrows) are present within the cytoplasm. Within some of the vacuoles there is material suggestive of sectioning of infoldings of the irregular membranes.

8,600 X



33



34

Figure 35

Experimental Rat: Twenty-four hours after third reserpine injection - electron micrograph.

Portions of four different parenchymal cells are illustrated (C1, C2, C3, C4). There is a definite difference in granule size between cells C1 and C4, illustrating the fact that not all cells are identical in appearance even in treated animals. There is a relative lack of granules in C1. Some vacuolation (v) is present.

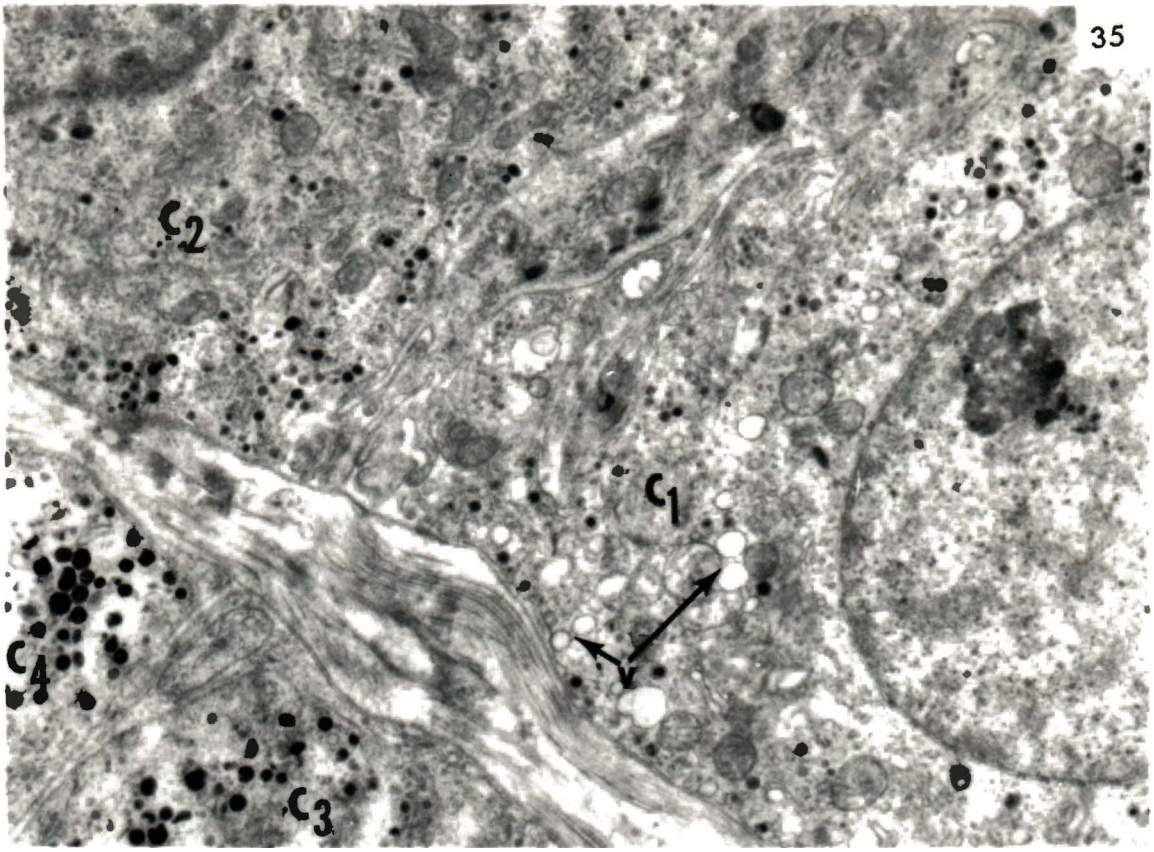
10,200 X

Figure 36

Experimental Rat: Twenty-four hours after third reserpine injection - electron micrograph.

Small granules (arrows) the approximate size of ribosomes are again found free in the cytoplasm. In some areas, these particles appear to be lining the membranes of some vacuoles (v). The intercellular space containing collagen fibers (Co) is separated from the parenchymal cell membranes (Mc) by another membranous structure (Ms) resulting in a potential space.

15,100 X


35
C2
C1
C4
C3
V

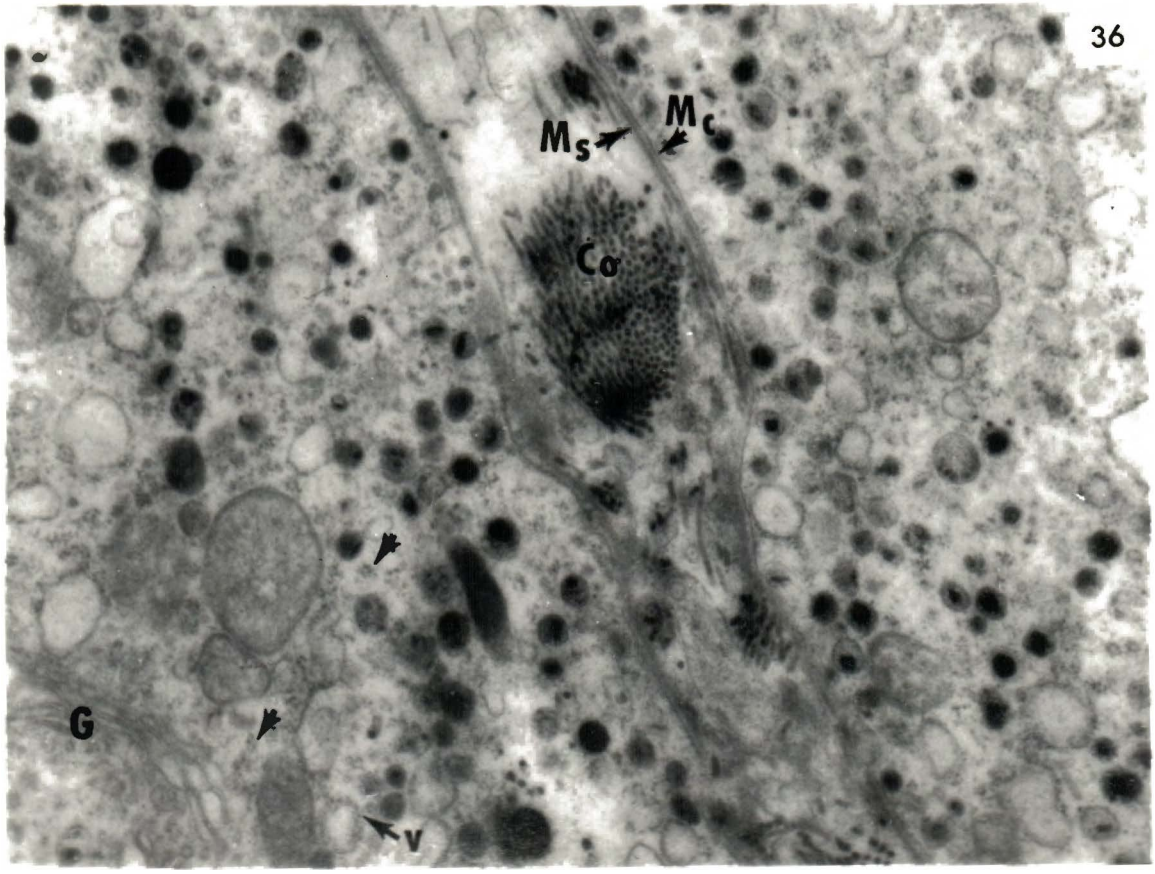

36
Ms
Mc
Co
G
V

Figure 37

Experimental Rat: Two days after third reserpine injection - electron micrograph.

In some areas marked vacuolation is still present. The relative decrease in number of osmiophilic granules is evident. Though cells as C1 contain many vacuoles (v), other cells as C2 contain few if any vacuoles.

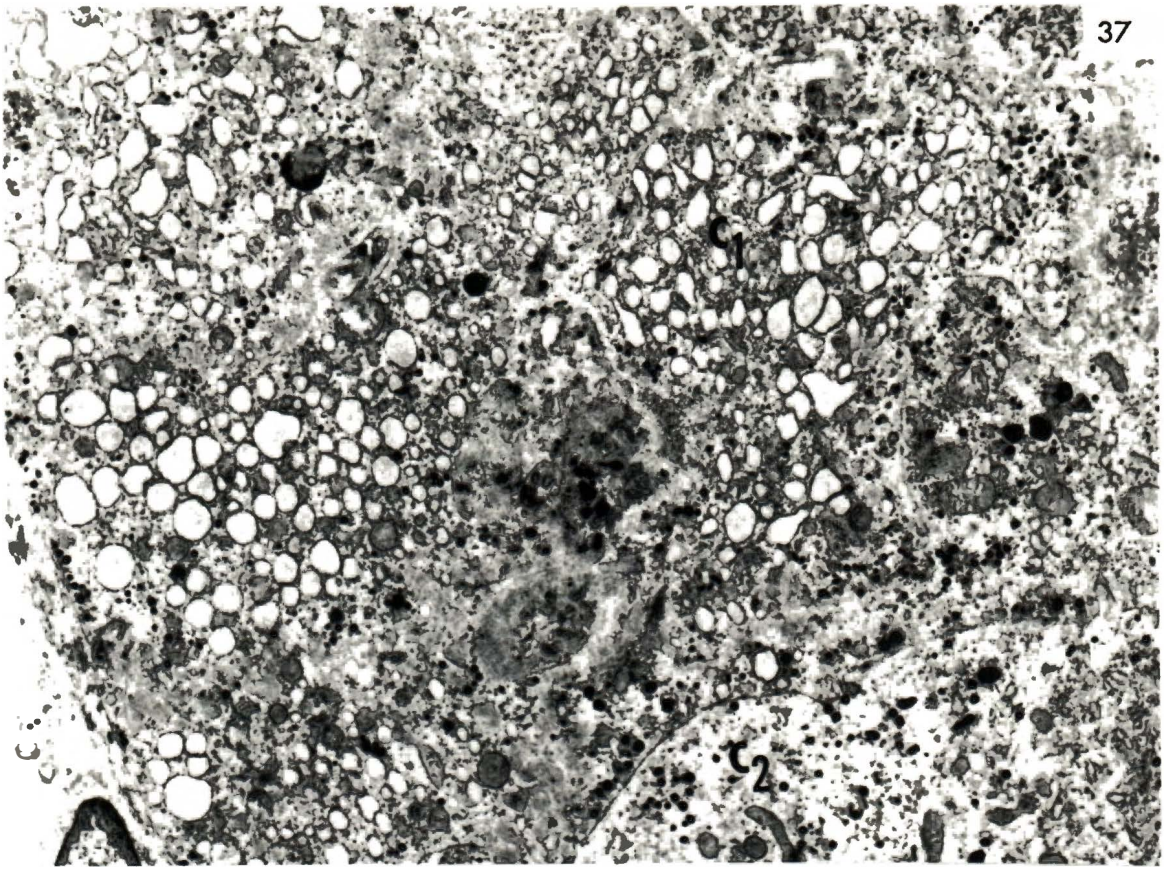
7,000 X

Figure 38

Experimental Rat: Two days after third reserpine injection - electron micrograph.

Endoplasmic reticulum is present in the form of parallel lamellae and is studded with ribosomes (er2). There is a relative deficit of osmiophilic granules, and those present tend to be near the cell margins. Also labeled is a nerve (n).

11,900 X


37
C1
C2

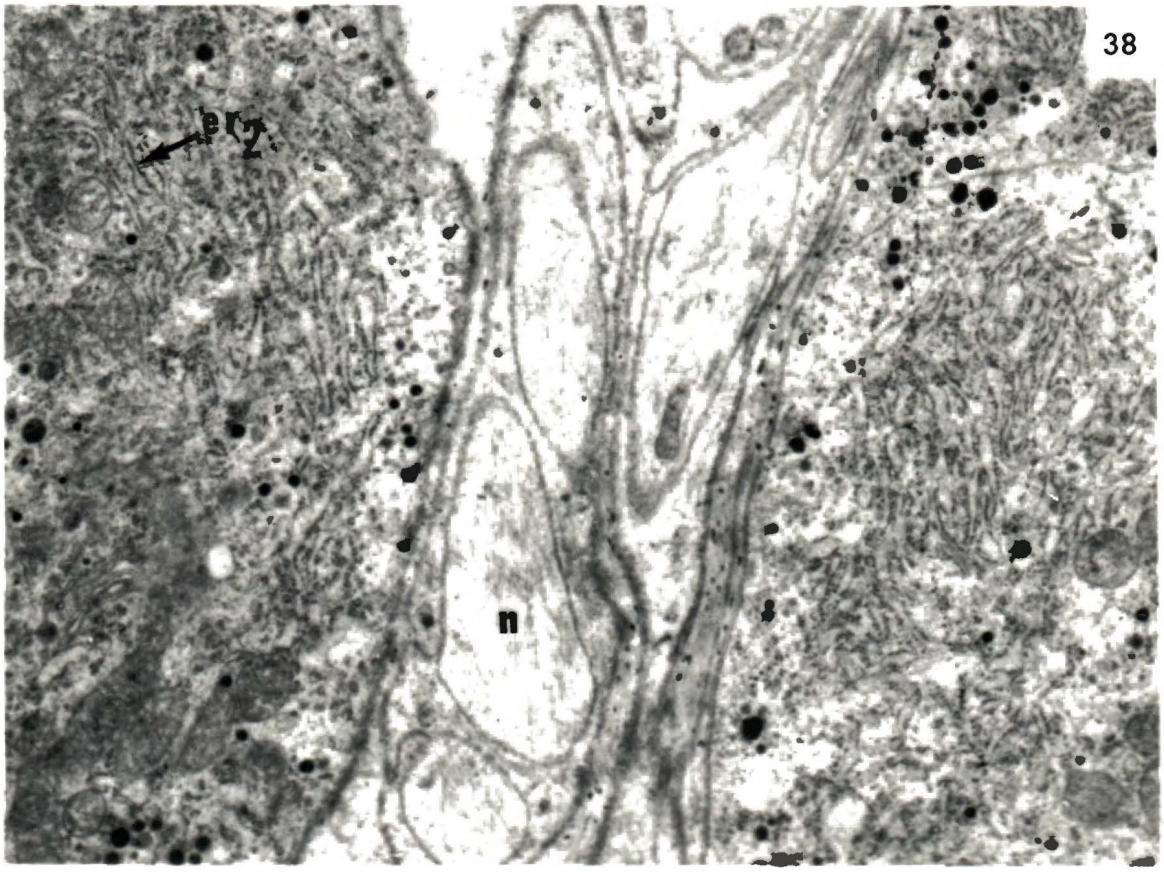

38
er
n

Figure 39

Experimental Rat: Two days after third reserpine injection - electron micrograph.

Rough endoplasmic reticulum is again predominant (er2). The osmiophilic granules are located primarily at the margins of the cells. Also labeled are nucleus (N), desmosome-like thickenings (d), and intercellular space (S).

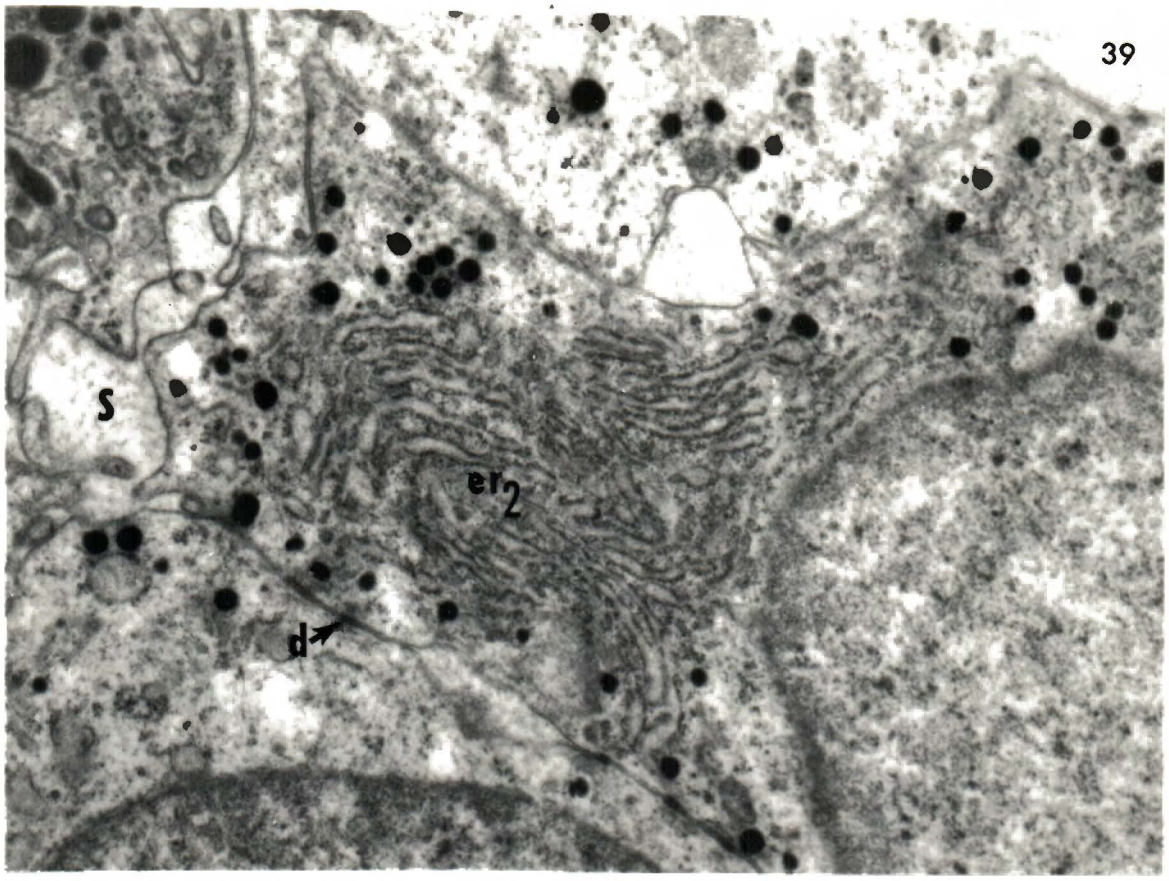
19,630 X

Figure 40

Experimental Rat: Two days after third reserpine injection - electron micrograph.

This micrograph further demonstrates the margination of the osmiophilic granules and the presence of rough endoplasmic reticulum (er2). Also labeled are a nucleus (N), nerve (n), and blood vessel (V).

15,100 X


39
S
er2
d

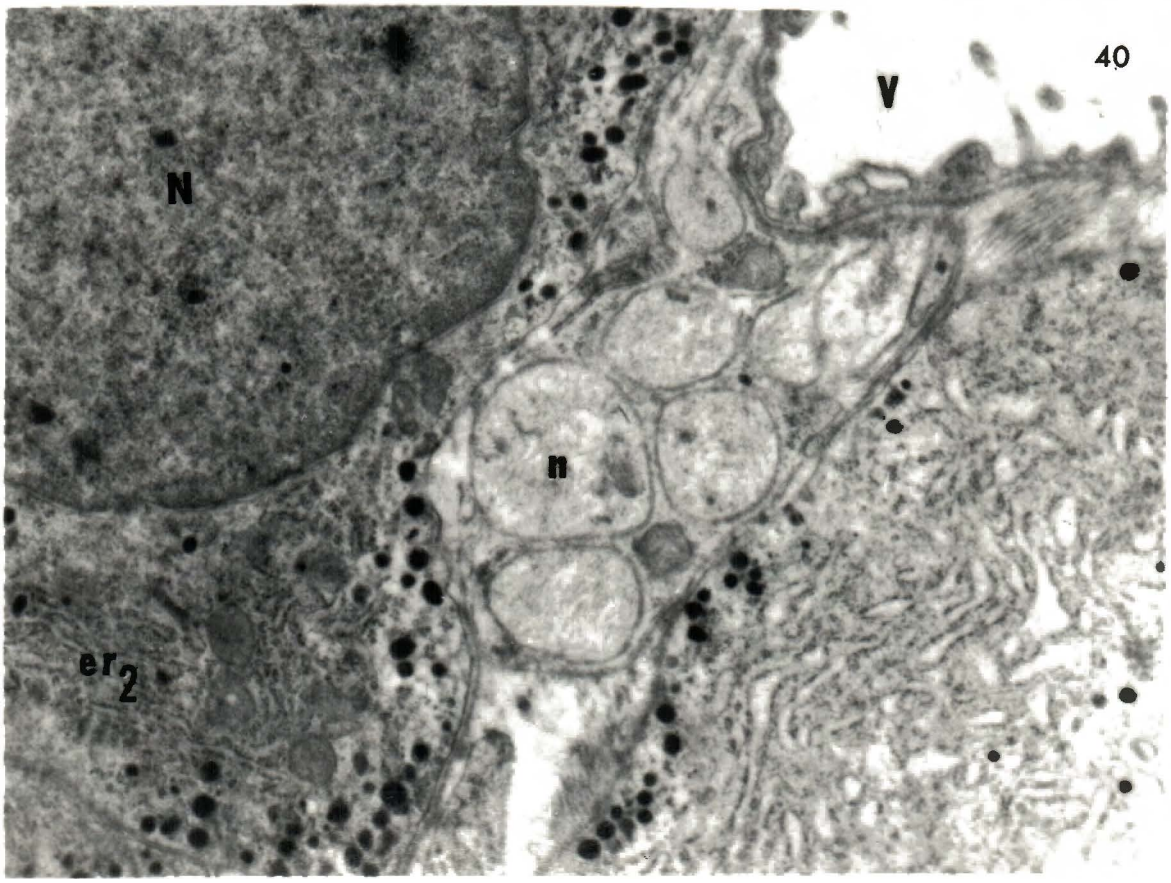

40
V
N
n
er2

Figure 41

Experimental Rat: Three days after third reserpine injection - electron micrograph.

Labeled structures include collagen fibers (Co), nucleus (N), and vacuoles (v). Compared to two days after the third reserpine injection, there is a loss of preponderance of rough endoplasmic reticulum, a relative decrease in degree of vacuolation in any one cell, and a relative increase in the number of osmiophilic granules seen.

7,000 X

Figure 42

Experimental Rat: Four days after third reserpine injection - electron micrograph.

Labeled structures include nucleus (N), nerve (n), and mitochondria (M). This micrograph illustrates the apparent absence of vacuolation. There are numerous osmiophilic granules (g) present, but they are less predominant in cell C1 than in cell C2.

9,900 X


41
V
V
V
N
Co

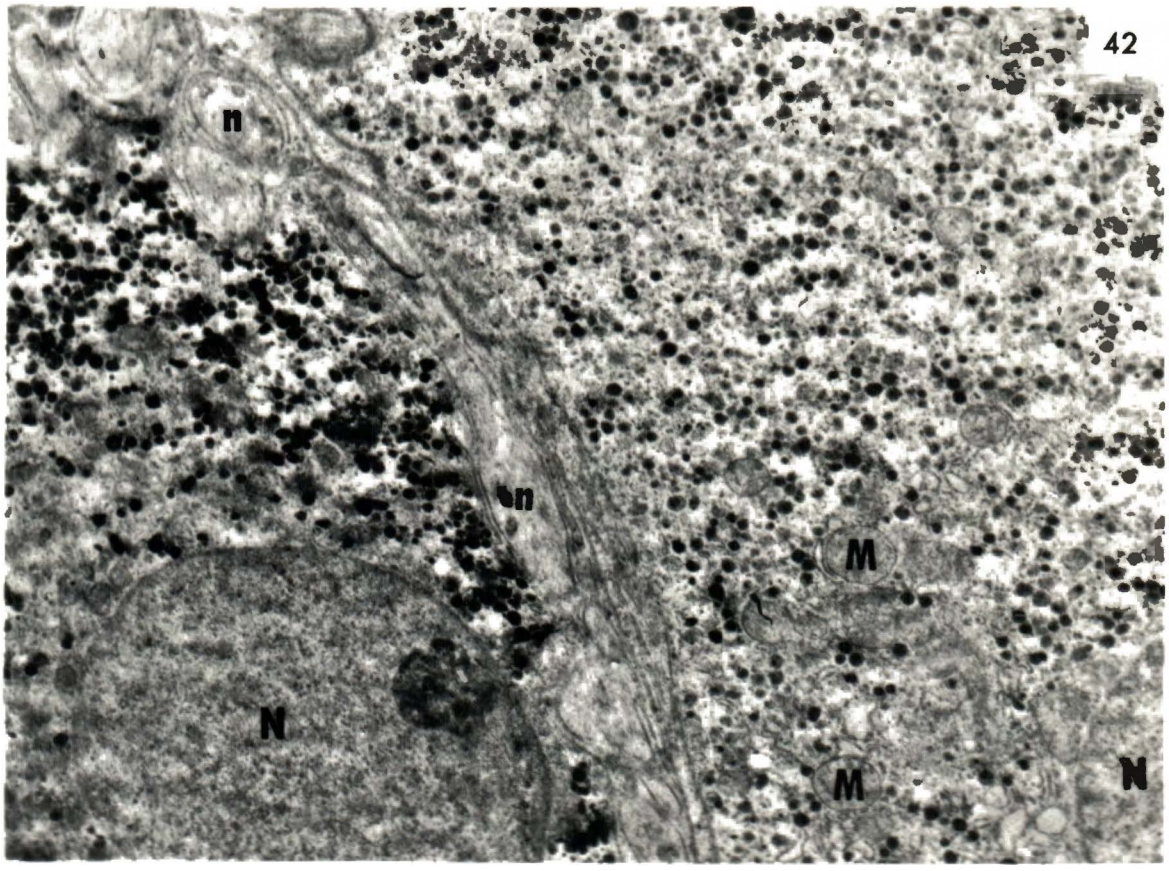

42
n
n
M
M
N
N

Figure 43

Experimental Rat: Five days after third reserpine injection - electron micrograph.

The recovery of treated adrenal medullary tissue to near normal appearance is illustrated. There are a ain cells differing in the density and number of granules (C1, C3), in the density of the nuclei (N1, N3), and in the density of the background cyto-plasm. There is still an apparent decrease in the number of granules in the central perinuclear regions of the parenchymal cells.

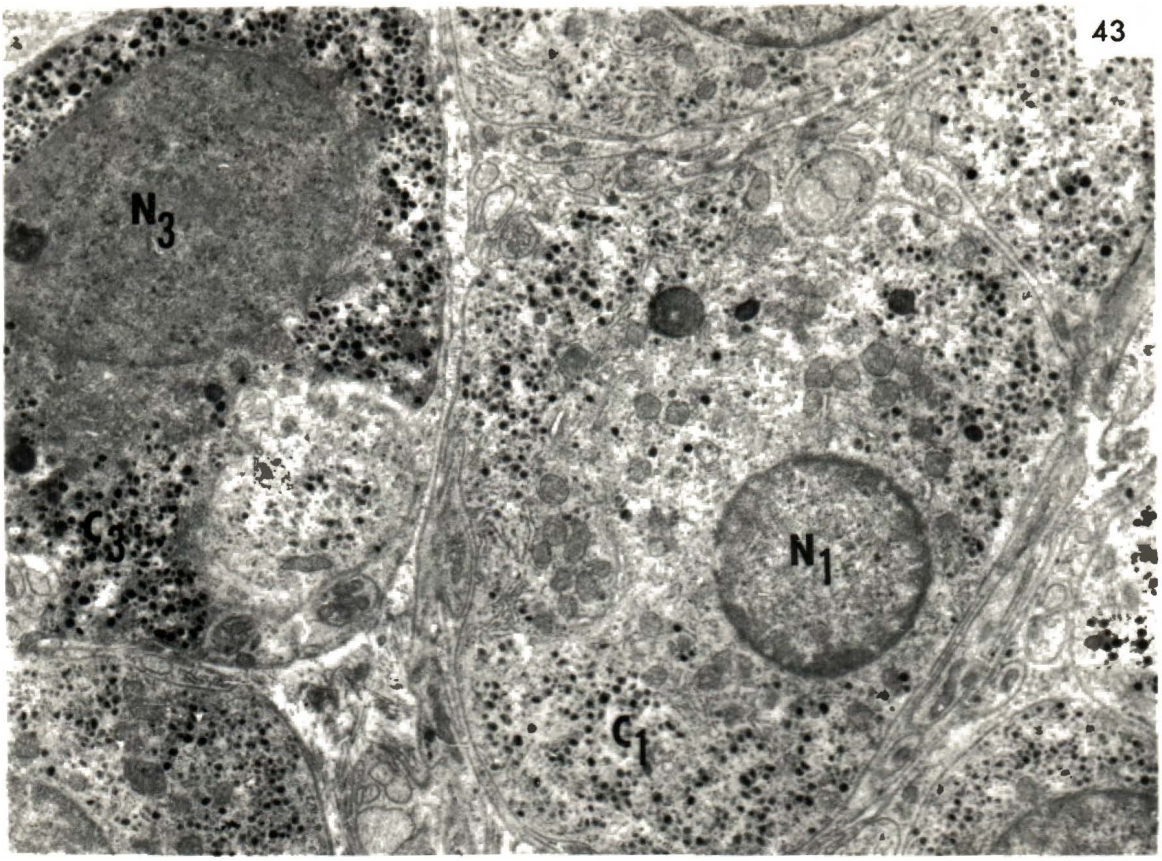
7,000 X

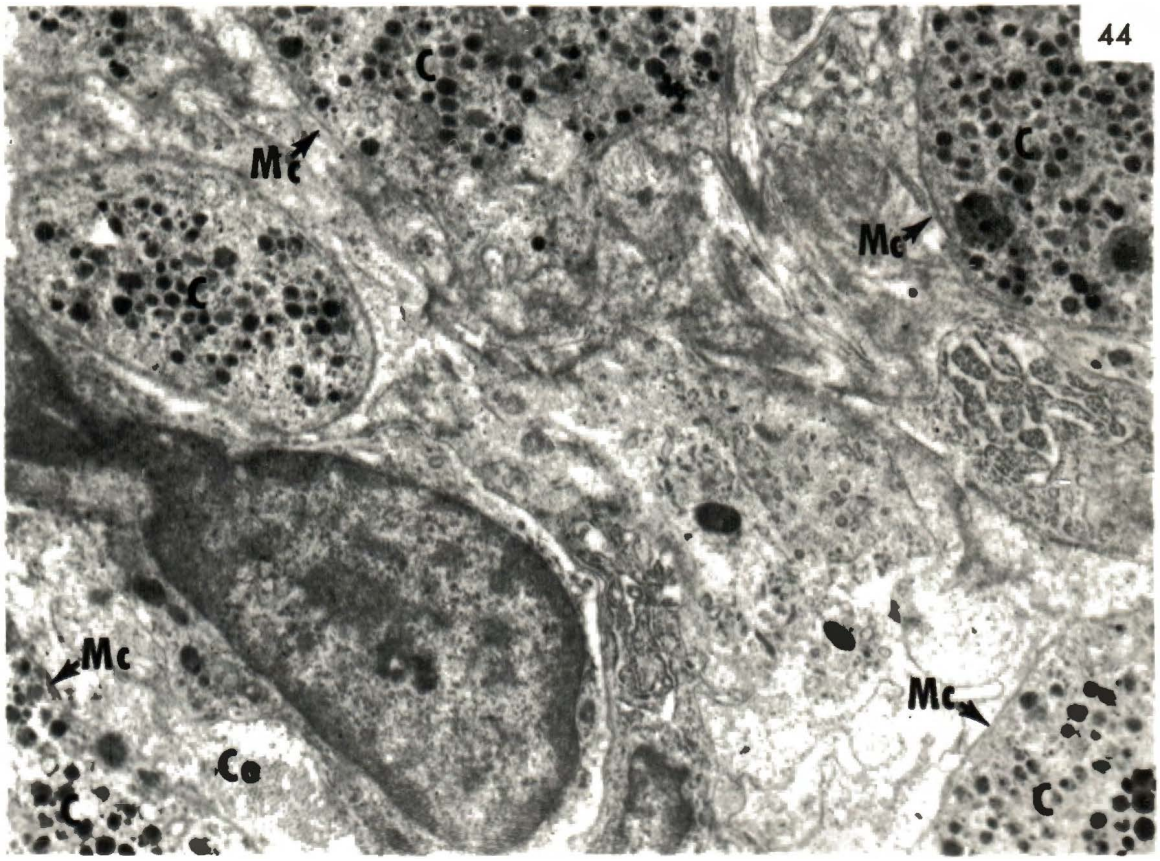
Figure 44

Experimental Rat: Six days after third reserpine injection - electron micrograph.

This micrograph further illustrates the apparently normal appearance of the tissue. Labeled structures include collagen fibers (Co), cell membranes (Mc), and parenchymal cells (C).

9,900 X


43
N3
C3
N1
C1


44
C
Mc
C
Mc
C
Mc
Co
Mc
C
C