An Electron Microscopic Study of the Regenerating Adrenal Gland During the Development of Adrenal Regeneration Hypertension

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THE DEVELOPMENT of a severe form of hypertensive disease (adrenal regeneration hypertension) in the rat during restitution of the adrenal cortex is one of the most interesting yet perplexing problems in the biology of tissue regeneration.[1] Although hypertension can occur in young rats bearing regenerating adrenals without uninephrectomy,[2] severe lesions in the heart, kidney, brain and blood vessels characteristic of hypertensive vascular disease occur to a greater extent when renal mass has been reduced.[1,3] The essentiality of regenerating adrenal cortical tissue for the development of this syndrome has been conclusively shown by the observation that hypertensive disease does not occur when adrenal regeneration is inhibited.[3,4] The mechanism by which the regenerating adrenal initiates the hypertension is not known, although some functional abnormality of the newly formed cells has been implicated.[5]

Study of regenerating adrenal cortical tissue by various cytochemical techniques also has suggested that the newly replicated cells are not entirely normal.[6] Furthermore, electron microscopy of adrenal glands regenerating in situ[7] and intramuscularly[8] has depicted certain cytologic abnormalities of the regenerating cortical cells. However, no study using both light and electron microscopy, has been undertaken to document the sequential morphologic events of adrenal cortical restitution under conditions which lead to the development of hypertensive disease. This has been the major objective of the experiment reported here.

Materials and Methods

Young female Holtzman rats aged 44 days at operation were used. In control and experimental groups, the right kidney and adrenal were removed and in the

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experimental groups the left adrenal was enucleated also, according to the method of Ingle and Higgins.[9] All rats were given Purina Lab Chow and a drinking solution of 1% sodium chloride ad libitum. Eight experimental and 6 control animals were killed by decapitation at zero time and at 2, 4, 7, and 10 days and at 2, 3, 5, 8, and 10 weeks after adrenal enucleation.

After decapitation, the adrenals were removed, freed of adherent fat and connective tissue and slices approximately 1 mm in thickness were cut through the entire glandular mass. These slices were fixed for 4 hr in 3% glutaraldehyde buffered to pH 7.2 with 0.1 M phosphate buffer.[10] After fixation, the tissues were washed in ice cold buffer to remove excess glutaraldehyde and stored in cold buffer overnight.

In the case of the adrenals from control rats, the glands were divided into the three cortical zones by means of a fine scalpel under a dissecting microscope. The zona glomerulosa was isolated by taking thin tangential sections near the surface of the adrenal, the zona fasciculata was identified by the white color imparted by the abundance of lipid and the zona reticularis was identified by the reddish brown color due to the absence of lipid. Ten blocks of tissue were taken from each of these identified zones from 3 of the rats killed at each of the periods indicated above.

In the case of the regenerating adrenal, the cortical tissue was freed from the central hemorrhagic mass or fibrous tissue by means of a fine scalpel under a dissecting microscope. At zero time after enucleation, the tissue samples consisted solely of zona glomerulosa cells since these cells were the only ones remaining attached to the capsule of the gland. After 2, 4 and 7 days of regeneration, it was not possible to identify clearly any cortical zonation under the dissecting microscope or in thick sections examined with a light microscope. Nevertheless, tissue blocks were taken from sites immediately adjacent to the capsule, from an intermediate depth from the capsule and from the edge of the cortex which adjoined the central hemorrhagic mass. By 10 days after enucleation, a recognizable zona glomerulosa was apparent in the light microscope so that tissue sections from this zone were taken by tangential sections of the adrenal in a fashion identical to that used for adrenal glands of control rats. In addition tissue blocks were taken from an intermediate location in the regenerated cortex and from the area of the regenerate adjacent to the central coagulum or fibrous tissue mass. In this way we have attempted to assure adequate sampling of all regions of the regenerating cortical tissue at all time periods throughout the experiment. In all cases, 10 tissue blocks from all locations were taken for further processing from each of 3 rats killed at all time periods.

Tissues were postfixed in 1% osmic acid buffered with 0.1 M phosphate to pH 7.2. After dehydration in a series of chilled ethanol solutions followed by propylene oxide, the tissues were embedded in a mixture of Epon 812 and Araldite according to the method described by Mollenhauer.[11] The blocks were sectioned with glass knives on a Porter-Blum ultramicrotome. Sections approximately one micron in thickness first were cut from each block, stained with toluidine blue, and examined in a light microscope. Before thin sections were cut, the blocks were trimmed to include adrenocortical cells whose zonal position in the adrenal had been identified by light microscopy insofar as possible. Ultrathin sections were doubly stained with methanolic uranyl acetate [12] and lead citrate.[13] Electron micrographs were taken with a Siemens Elmiskop I electron microscope.

For demonstration of lipid, adrenal tissue from the other 3 rats in the control and experimental groups killed at each time interval were fixed in 10% neutral formalin. Frozen sections, cut on a cryostat, were stained with either oil red 0 or Sudan black B and mounted in glycerine jelly.

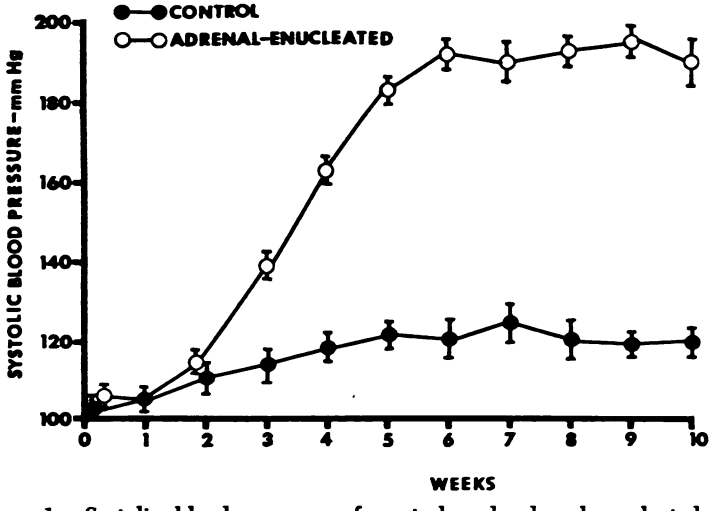
Systolic blood pressure of lightly anesthetized animals was determined in the tail at weekly intervals throughout the experiment using a Physiograph Four (E and M Instrument Co.). Values in excess of 150 mm Hg were regarded as being in the hypertensive range.

Results

General Observations

As documented previously [9] and stated above, only a narrow rim of tissue composed of zona glomerulosa cells remained adherent to the capsule after adrenal enucleation. Within 24 hr, enlargement of the collapsed adrenal was caused by hemorrhage into the center of the gland so that red blood cells were present among the remaining cortical cells, many of which had become necrotic. Cell proliferation began within 4 days and by 7 days regeneration was clearly evidenced by the mitotic figures and increased cortical mass. During regeneration, the connective tissue of the capsule became thickened and edematous but returned to normal width by approximately 3 weeks. Cortical zonation appeared to begin by about 10 days and was virtually normal after 4 weeks as seen by light microscopy. The central coagulum was reabsorbed gradually so that only a small fibrous remnant remained at the end of 5 weeks.

Changes in the systolic blood pressure of rats bearing regenerating adrenal glands (Text-fig 1) were similar to those which have been reported.[1] The mean systolic blood pressure of animals bearing re-



TEXT-FIG 1. Systolic blood pressure of control and adrenal-enucleated rats. The vertical lines on either side of the points represent the standard error of the mean.

generating adrenals was significantly higher than that of controls by 3 weeks after enucleation. The experimental animals were clearly hypertensive at 4 weeks (BP 163 ± 3 mm Hg); a significant difference in blood pressure between control and experimental rats actually existed throughout the last 7 weeks of the experiment.

All controls survived the entire experimental period, whereas approximately 50% of the animals bearing regenerating adrenals died before the end of the tenth week. The mean body weights of the two groups did not differ significantly during the period of observation. Gross pathological changes, as previously described,[1] occurred in the kidney, heart and mesenteric vessels of many animals bearing regenerating adrenals. No gross lesions were seen in any of the organs of control rats, although the kidney and adrenals were enlarged.

Fine Structure of Control Adrenals

The fine structure of adrenocortical cells in uninephroadrenalectomized control rats at all time intervals was similar to that reported by others for unoperated intact animals.[14] Since our interest in this study was particularly directed toward the mitochondria, it is worth recalling the characteristics of these cell organelles in the different zones of the rat adrenal cortex. Mitochondrial cristae in the zona glomerulosa consist of short tubules which may appear as vesicles when cut in cross section. Cristae in zona fasciculata and zona reticularis cells are composed of numerous, closely packed vesicles which virtually fill the entire mitochondrial matrix.

At 2 days after operation, numerous cells with an electron lucid cytoplasm were observed throughout the zona fasciculata and zona reticularis (Fig 1). The mitochondria of these cells were round or oval and contained closely packed vesicular cristae. The light cytoplasm was similar in appearance to that seen in the gerbil adrenal during early periods of ACTH stimulation.[15] The cells of the zona glomerulosa were of normal appearance. By 7 days the cytoplasmic matrix of most adrenocortical cells had returned to a normal appearance despite the fact that the adrenal was undergoing hypertrophy. The mitochondria of zona fasciculata cells appeared somewhat less densely packed with vesicular cristae some of which were connected such that a short tubule was formed (Fig 2); lipid droplets were seen in large numbers at all times (Fig 2). Throughout the remaining time periods no abnormalities in fine structure were observed in either fasciculata or reticularis cells.

The ultrastructure of zona glomerulosa cells throughout the experiment also was similar to that described by others for unoperated intact

animals.[13] The tubular cristae of mitochondria were similar to those in normal animals, in spite of the fact that the control rats in this experiment had received 1% saline to drink (Fig 3). Many glomerulosa cells, however, had increased numbers of lipid droplets while adjacent cells contained none. Moreover, numerous lysosomes were observed in some glomerulosa cells.

Fine Structure of Regenerating Adrenals from 0 to 7 Days

After enucleation, the adrenal capsule became edematous and the evacuated center of the gland was filled with blood. At 2 days the cortical cells in the zona glomerulosa region no longer possessed the appearance of normal glomerulosa cells although the cells which had remained attached to the adrenal capsule according to all previous studies and verified by our own zero time observations, had been zona glomerulosa cells. It was surprising to find, therefore, that the mitochondria of these cells now possessed cristae which were more vesicular in nature than tubular, although some simple tubular characteristics persisted (Fig 4). In some mitochondria the vesicular cristae were located adjacent to the inner membrane, whereas in others they were present within the matrix, the cristae sometimes uniting with other vesicles to form short tubular segments. The matrix of all of the mitochondria was electron lucid. The smooth surfaced endoplasmic reticulum in many cells was vesicular and noticeably dilated, whereas in others the appearance was virtually normal (Fig 4). Numerous free ribosomes were present in the cytoplasm of the cortical cells.

Four days after enucleation, lipid droplets were almost entirely absent from regenerating adrenocortical cells but a small number of nonmembrane limited osmiophilic bodies with an especially dark peripheral ring and a less dense central core were observed (Fig 5). These structures were distinct from lysosomes (Fig 5). Again, mitochondrial cristae in cells from all levels through the cortical tissue were predominantly vesicular and were not as numerous as those seen in mitochondria from control animals.

By 7 days, cortical cells had increased considerably in number and typically possessed an increased amount of smooth endoplasmic reticulum and free ribosomes (Fig 6). The predominant cell type in the newly forming cells comprising the middle and inner portions of the regenerate had mitochondria with reduced numbers of vesicular cristae dispersed in an electron lucid matrix. No cells possessed the typical ultrastructural appearance of normal zona fasciculata-reticularis cells.

It should be noted that recognizable zona glomerulosa cells reap-

peared at 7 days in the outer zone of the regenerate (Fig 7). The mitochondria of these cells were elongated and contained tubular cristae which were frequently connected to the inner mitochondrial membrane but did not form a complex network of anastomosing tubules: these tubules appeared as vesicles when cut in cross section. The matrix of mitochondria was relatively electron dense, sometimes appearing as an irregular mass between the cristae. Numerous lysosomes were a unique feature of such cells. The matrix of the lysosomes was granular and crystalline material was found at the periphery of these bodies. The Golgi apparatus was prominent and vesicles were observed in association with the peripheral cisternae.

Fine Structure of Regenerating Adrenals from 10 to 21 Days

By this time the regenerating adrenal can be divided into an outer zona glomerulosa and an inner region of cells extending from the glomerulosa to the central fibrous tissue mass. In the inner region, light cortical cells were first identified at 10 days in 1 μ sections of regenerating adrenals stained with toluidine blue (Fig 8), and were present in even greater number at 14 and 21 days. The cells appeared light due to the presence of numerous vacuoles observed by light microscopy which, in adjacent ultrathin sections, corresponded to enlarged mitochondria with few cristae and an electron lucid matrix (Fig 9). Such cells also had other distinctive characteristics which included dilated, vesicular endoplasmic reticulum, an irregularly shaped nucleus and numerous electron dense cytoplasmic bodies (Fig 9).

Dark cells seen in the light microscope contrasted markedly with the light cells (Fig 8) and in the electron microscope were characterized by round nuclei, a somewhat electron dense cytoplasmic matrix and numerous tubules and vesicles of smooth endoplasmic reticulum (Fig 10). Lipid droplets were observed in dark cells after 14 days of regeneration and numerous free ribosomes were seen, although others were attached to endoplasmic reticulum. At 10 and 14 days, many cristae in mitochondria of dark cells were located peripherally and apparently were continuous with the inner mitochondrial membrane (Fig 11). By 21 days the number of mitochondrial cristae in dark cells was greater than at earlier periods but did not equal the number of cristae in mitochondria of fasciculata cells of control rats killed at the same time interval. The vesicular appearance of cristae was re-established in many mitochondria of regenerating cells, but in others they formed a system of interconnected, short tubular segments (Fig 10).

Fine Structure of Regenerating Adrenals from 35 to 70 Days

During the late period, the inner region of regenerated adrenals became differentiated into a zona fasciculata and reticularis similar in the light microscope to that of controls; a zona glomerulosa was seen adjacent to the capsule of such adrenal regenerates. After 35 days of regeneration dark cells outnumbered light cells. The changes to be described in the following section occurred in dark cortical cells. Whereas, many mitochondria exhibited tubulo-vesicular cristae at 21 days, virtually all mitochondrial cristae were vesicular after 35 days of regeneration (Fig 12). Almost all mitochondria contained a nearly normal number of cristae but even yet some mitochondria were small and had a few cristae which formed interconnecting tubules. The endoplasmic reticulum was predominantly smooth-surfaced. There was a further increase in the number of lipid droplets of cortical cells as regeneration progressed. Lysosomes also were seen in many of these cells. By 56 and 70 days the number and appearance of vesicular cristae in many mitochondria (Fig 13, 14) could not be distinguished from those of control adrenal cortical cells. Occasionally giant spherical mitochondria packed with vesicular cristae were observed in fasciculata cells (Fig 13). Tubules of smooth endoplasmic reticulum were more prominent in the cytoplasm of many cells at 70 days (Fig 14) than at 56 days (Fig 13). In contrast to the abundant agranular reticulum, small patches of rough endoplasmic reticulum were scattered throughout the cytoplasm of these cells at both times, although numerous ribosomes were unattached to membranes.

Few ultrastructural changes occurred in zona glomerulosa cells of animals receiving saline for 56 and 70 days, although it is well-known that this treatment produces a small zona glomerulosa in adrenals of intact rats.[16] Similar to the results at 35 days, zona glomerulosa cells for the most part contained numerous lipid droplets at 56 (Fig 15) and 70 days. The mitochondrial cristae appeared similar to those in zona glomerulosa cells of control adrenals.

Discussion

The reports of Fortier and DeGroot,[17] Skelton [18] and Gaunt *et al* [19] and the results of the present study clearly indicate that the natural course of adrenal cortical regeneration may be divided into three stages—early, intermediate and late. Although somewhat artificial, such temporal compartmentalization provides a convenient framework within which the diversity and dynamics of the ultrastructural and

functional characteristics of the regenerating adrenal may be considered. The cortical cells which remain attached to the fibrous capsule of the gland after enucleation are believed to be of zona glomerulosal origin and to serve as the "stem" cells from which a new adrenal cortex is derived by successive cell divisions and differentiations. Although this regenerative process appears to be under the direct control of ACTH, the factor(s) concerned with the reestablishment of zones in the regenerated cortex are less well understood. It was surprising, therefore, to find as early as 2 days after enucleation that the mitochondria of the residual cortical cells possessed largely vesicular rather than the tubular cristae so characteristic of normal zona glomerulosa cells.[14] This seems to represent some kind of dedifferentiation and raises important questions concerning the mechanism of this mitochondrial transformation and its functional significance.

In this regard it should be borne in mind that the nature of the enucleation procedure is such that the feedback control of ACTH secretion by the adenohypophysis is interrupted which, together with the stress of the surgery itself, brings about a massive discharge of ACTH. This latter fact has been convincingly documented by Fortier and DeGroot [17] who measured the pituitary content of ACTH before and after adrenal enucleation and by Nakayama, Nickerson and Skelton [20] who have described the virtual disappearance of granules from the ACTH-secreting cells of the anterior pituitary within 12–24 hr postenucleation. Thus, there is concurrence between ACTH release and mitochondrial transformation which suggests a cause and effect relationship. This hypothesis receives strong support from the finding of Kahri [21] that adding ACTH to adrenal cortical cells maintained in tissue culture transforms their mitochondria from tubular zona glomerulosa-cell type to vesicular zona fasciculata cell type.

Gaunt *et al* [19] have observed during the immediate postenucleation period that rats exhibit a transient aversion to salt and are relatively unable to excrete a sodium load. Despite the central blood clot and generally disorganized structure of the newly enucleated adrenal, their studies indicate that the residual cortical tissue is highly functional and that this function is not easily blocked by diuretic substances. These authors postulate that the substance(s) produced has to be primarily a mineralocorticoid, possibly associated with a substance having glucocorticoid activity. The dependence of this postenucleation sodium retention on the presence of the hypophysis and its partial restoration in hypophysectomized animals by administration of β-corticotrophin $^{1-24}$ indicates the essentiality of adequate amounts of ACTH. This would

conform very well with the massive discharge of ACTH known to occur in the immediate postenucleation period.[17] Thus, whatever may be the nature of the secretion product of the early postenucleation adrenal, it is interesting to speculate that it may have some direct relationship with or dependence upon the mitochondrial transformation which occurs at the same time.

By the end of the early period of adrenocortical regeneration (7 days), cells possessing mitochondria with tubular cristae had reappeared beneath the capsule of the gland in a narrow band or zone, presumably a re-established zona glomerulosa. This sequence of events suggests that the restitution of cortical zonation begins only after a period when all residual cortical cells come to possess the morphologic characteristics of zona fasciculata cells. Beginning at this time and extending throughout the intermediate period (10–21 days), the innermost regenerated cells were characterized by mitochondria which possessed decreased numbers of vesicular cristae dispersed in an electron lucid matrix (zona fasciculata cells). The noteworthy aspects of this mitochondrial appearance are that the cells containing such mitochondria correspond to the light cells observed in the light microscope and are observed most prominently during that interval when the rats bearing regenerating adrenals are becoming hypertensive. It is interesting that neither light nor dark cells were described by Penney, Patt and Dixon [8] in regenerating adrenals, although Giacomelli, Wiener and Spiro [22] found increased numbers of light cells in the zona glomerulosa of rats fed a sodium deficient diet and suggested that these cells might be responsible for secreting aldosterone. It is somewhat difficult, however, to reconcile this suggestion with Symington's [23] concept that it is the dark or compact cells which are most active in corticosteroid biosynthesis.

Such ultrastructural mitochondrial changes as observed in the present experiment have not been reported in previous studies of regenerating adrenal cortical tissue either in situ [7] or intramuscularly.[8] At the present time no explanation is apparent for the disparity between these findings and our own. It should be borne in mind that the conditions under which the cortex regenerated were quite different, inasmuch as halved adrenals transplanted intramuscularly undergo considerable tissue necrosis before a blood supply is established and in situ enucleation is followed by the formation of a central coagulum as a result of the continued integrity of the glandular blood supply. Another difference between the studies is the internal environment in which the adrenal regeneration occurred, since neither Sabatini, Bleichmar and De-Robertis [7] nor Penney, Patt and Dixon [8] used uninephrectomized rats

allowed to drink 1% saline ad libitum. The likelihood of these experimental conditions accounting for the differences in the mitochondrial structure reported here seems remote, in view of the fact that no adrenal mitochondrial changes were seen in the uninephrectomized, uniadrenalectomized control animals also drinking 1% saline. Although adrenal hypertrophy occurred in these latter rats, it can hardly be considered comparable to regeneration.

In addition to abnormalities in mitochondrial fine structure, biochemical evidence suggests that the pattern of steroids formed by the regenerating adrenal during the intermediate period and beyond is altered. Adrenal regenerates have an impaired ability to synthesize aldosterone and 18-hydroxycorticosterone [24] and 18-hydroxydeoxycorticosterone.[25] Macchi and Wyman [26] also observed a decreased capacity for corticosterone synthesis in response to ACTH by adrenal regenerates and Masson, Koritz and Peron [27] have reported reduced amounts of corticosterone in adrenal vein blood of animals with adrenal regeneration hypertension. Mitochondria isolated from regenerating tissue have a reduced capacity for converting exogenous 11-deoxycorticosterone to corticosterone.[28] Deficiency in 11β-hydroxylation has been demonstrated further by the accumulation of 11-deoxycorticosterone in the medium when homogenates of regenerating adrenal cortical tissue have been incubated in vitro with added progesterone as substrate.[5] It has been postulated that a defect in the NADPH generating system of mitochondria is responsible for this abnormality, since addition of NADPH to the incubation system corrects the abnormal metabolism and brings about normalization of the end products.[28,29]

In view of these observations, the suggestion has been made [30] that the mitochondrial block in 11β-hydroxylation may result in increased production of 11-deoxycorticosterone in vivo sufficient to initiate the hypertensive process. Indeed, administration of exogenous deoxycorticosterone is known to produce a hypertensive syndrome in uninephrectomized rats drinking 1% saline which is indistinguishable from that which develops in rats bearing regenerating adrenals.[31]

Alternatively, some of the changes in mitochondria of regenerates are similar to those reported following cell injury. Viragh and Bartok [32] observed small numbers of short cristae in liver mitochondria at 24 hr after partial hepatectomy. Autolysis in vitro for 1 hr also produced decreased numbers of cristae in liver mitochondria.[33,34] Furthermore, the reduction in number of cristae may occur by incorporation of cristae into the inner membrane of swollen mitochondria. Malamed [35] has suggested a similar mechanism for loss of cristae during swelling of isolated

mitochondria. Mechanical injury during the enucleation procedure and the subsequent anoxia induced by disturbance of the blood supply may well have caused swelling of mitochondria in the newly regenerating cortical tissue but this mechanism hardly can be invoked as an explanation of mitochondrial changes during the intermediate period of regeneration.

Vesicular and dilated endoplasmic reticulum observed at early times during regeneration are thought to be a reaction of the adrenocortical cells to injury sustained during the procedure of enucleation and subsequent conditions of anoxia before revascularization of the adrenal is completed. Vesiculation is not regarded as an artifact of fixation since the endoplasmic reticulum in control adrenal tissue processed simultaneously was not dilated. Similar changes in the endoplasmic reticulum of rat liver have been produced by X-irradiation,[36] carbon tetrachloride,[37] autolysis,[33,34] hypoxia,[38] and partial hepatectomy.[32] The importance of this alteration with regard to the steroid producing function of the regenerating adrenal glands is most difficult to assess since dilated endoplasmic reticulum is seen infrequently after one week.

Another ultrastructural change at early periods of regeneration was the replacement of lipid droplets by large, highly osmiophilic structures. Penney, Patt and Dixon [8] also observed such osmiophilic structures in intramuscular adrenal regenerates. However, these investigators employed osmic acid fixation in which lipid appears dark. In contrast, the glutaraldehyde used in the present study leaches out some of the lipid making the subsequent staining of the lipid droplets by osmium tetroxide less intense. The droplets observed in the present study are thought to be lipid since frozen sections stained with Sudan Black B showed spherical structures in these cells. The persistence of dense material at the periphery of these structures probably indicates that lipoidal material has resisted extraction by the glutaraldehyde and other preparative procedures. Reappearance of small lipid droplets which replaced the large osmiophilic structures may indicate a decreased functional activity of these cells, since an accumulation of lipid presumably signifies an inactivity of secretory processes along with a greater accumulation of precursors for steroid synthesis.[39]

Five weeks after enucleation the number of vesicular cristae in most mitochondria of regenerating adrenal cortical cells appeared similar to those of control glands. Concomitant with this process, homogenates of regenerating adrenals and preparations of isolated mitochondria showed better conversion of exogenous progesterone or deoxycorticosterone to corticosterone.[5,29]

The presence of increased numbers of dark cells after 5 weeks of regeneration also suggests that the function of the new-formed adrenal is being normalized. Eight weeks after enucleation the fine structure of mitochondria in the zona fasciculata was similar to controls, these mitochondria containing tightly packed vesicular cristae. At this time period it has been shown that the concentration of cytochrome P-450 in regenerating adrenal mitochondria is normal [29,30] whereas at earlier time periods the level of this cytochrome, which is involved in steroid hydroxylations,[40] is reduced. The concentration of cytochrome P-450 found in regenerating adrenal mitochondrial appears to be related to the number of cristae, supporting the concept that this cytochrome and the steroid hydroxylation system are associated with these inner mitochondrial membranes.

It is noteworthy that after the early period zona glomerulosa cells in experimental groups appeared similar to those in unoperated animals in spite of the consumption of 1% saline for as long as 70 days. This observation is surprising in view of the report by Deane and Masson [16] that a high level of salt ingestion reduces the width of the zona glomerulosa in uninephrectomized animals. However, it should be noted that no electron microscopic studies have appeared on the structure of the zona glomerulosa of animals on high salt diets, although such studies have been reported after salt restriction.[22]

Summary

Changes in fine structure were observed in regenerating adrenals of rats under conditions conducive to the development of experimental hypertension. In the early periods of regeneration before blood pressure had increased the simple tubular cristae of zona glomerulosa cell mitochondria changed into vesicular cristae, perhaps due to the massive discharge of ACTH which follows uniadrenalectomy and contralateral adrenal enucleation. Ultrastructural abnormalities were observed in the regenerating rat adrenal during intermediate periods of regeneration when the systolic blood pressure was increasing rapidly to above normal levels. Such abnormalities included reduction in the number of vesicular cristae in zona fasciculata cell mitochondria and arrangement of these cristae in a simplified network of interconnecting tubules. Also at this time, large numbers of light cells were seen in toluidine blue stained sections. These light cells were characterized by dilated vesicular endoplasmic reticulum, irregular nuclei and enlarged abnormal mitochondria. The abnormal ultrastructure of the regenerating adrenal is thought to be related directly to the biochemical abnormality which

manifests itself as a decreased ability to form corticosterone and an increased production of 11-deoxycorticosterone in vitro. In later periods of regeneration the ultrastructure of the adrenal appeared essentially normal, concomitant with the return of more normal corticosteroid biosynthesis.

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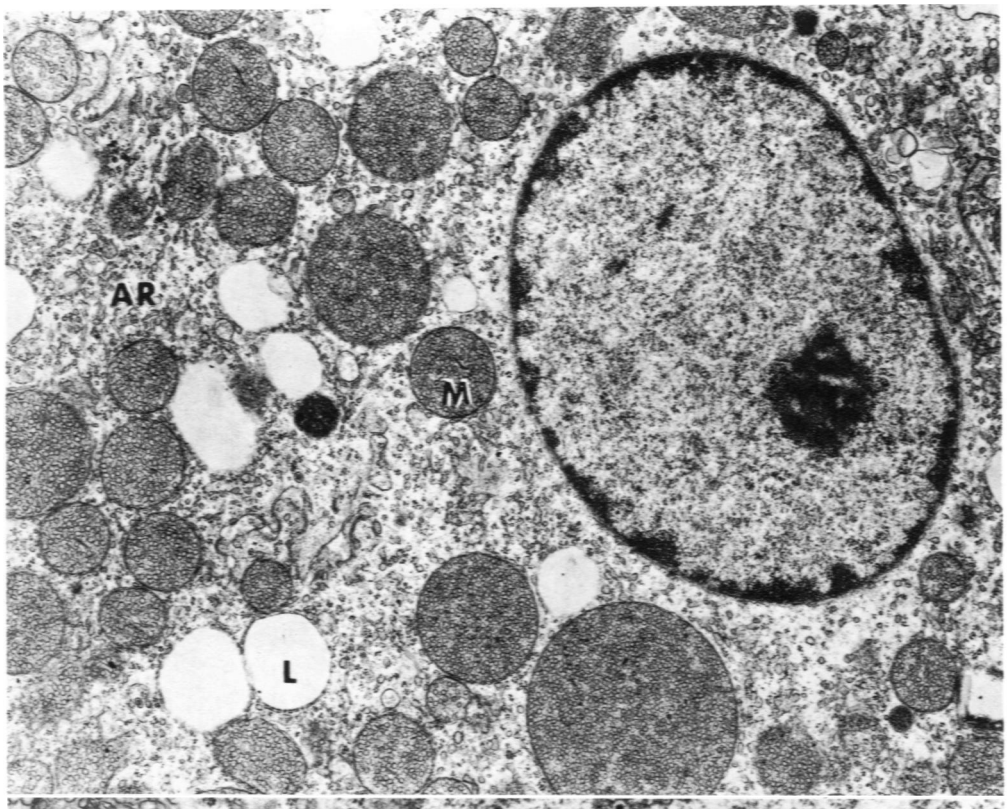
[*Illustrations follow*]

Legends for Figures

All ultrathin sections for electron microscopy were stained with methanolic uranyl acetate and lead citrate; the 1-μ light microscopic section in Fig 8 was stained with toluidine blue.

Fig 1. Zona fasciculata cell from adrenal of control animal 2 days after uninephroadrenalectomy. Density of cytoplasm appears electron lucid. These typical zona fasciculata cell mitochondria *(M)* are round and contain closely packed cristae, usually appearing as vesicles. Lipid droplets *(L)* and tubular segments of smooth endoplasmic reticulum *(AR)* are dispersed throughout the cytoplasm. × 9000.

Fig 2. Zona fasciculata cell from adrenal of control rat 56 days after uninephroadrenalectomy. Mitochondria contain vesicular cristae *(VC)* although these cristae are sometimes interconnected into system of tubules *(arrow)*. Lipid droplets *(L)* without discernible limiting membrane are seen throughout cytoplasm. Vesicular material *(V)* is sometimes incorporated into matrix of dense bodies presumed to be lysosomes *(LY)*. × 18,000.



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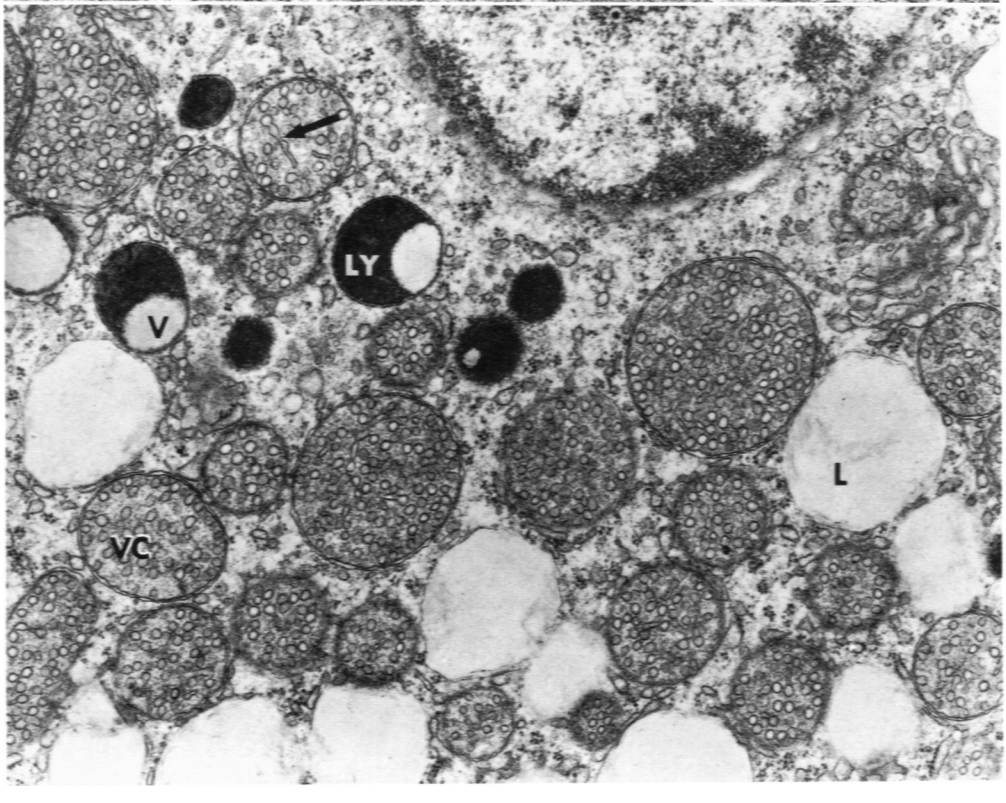

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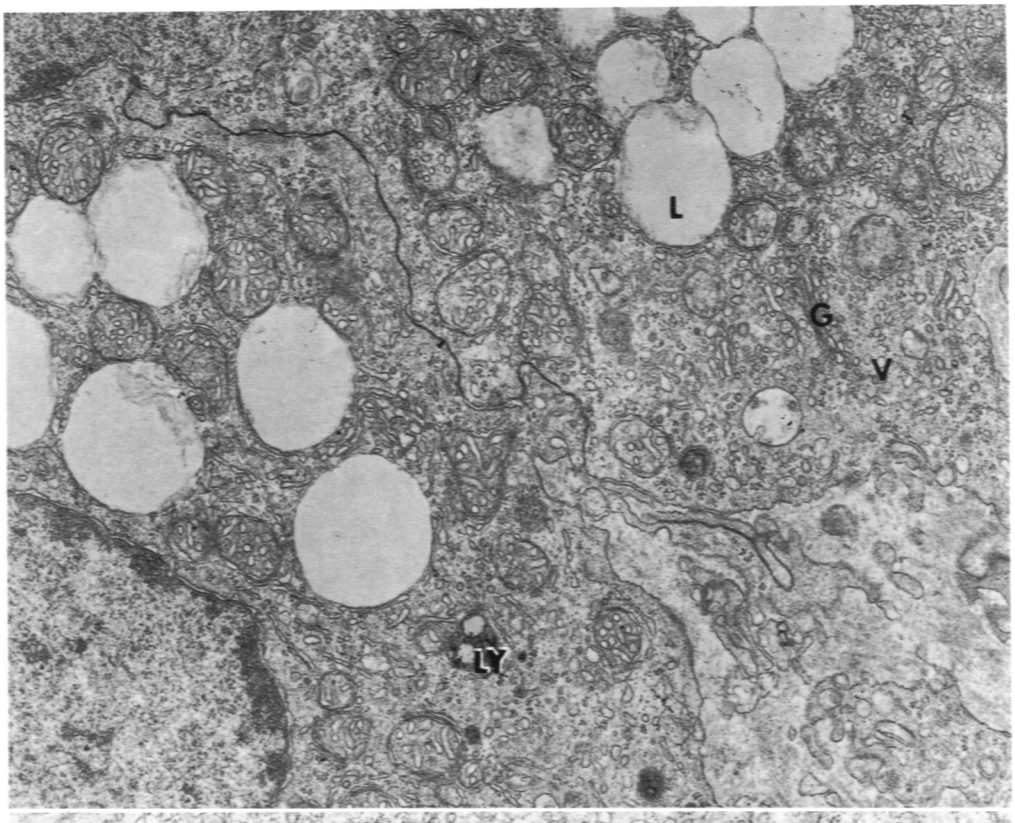
Fig 3. Portions of two zona glomerulosa cells from adrenal of control rat 70 days after uninephroadrenalectomy. Mitochondria are round and contain tubular cristae which frequently show connections with inner mitochondrial membrane. Several lipid droplets (*L*) are seen in cytoplasm. Golgi apparatus (*G*) in upper cell is well developed and vesicles (*V*) can be seen at lateral edge of cisternae. *LY*, lysosome. × 9000.

Fig 4. Portions of cell in zona glomerulosa region of regenerate 2 days after adrenal enucleation. Some cristae appear as vesicles connected to inner mitochondrial membrane (*arrow*), whereas others form tubular segments (*Double arrow*). Smooth endoplasmic reticulum (*AR*) is virtually normal. Numerous polysomes (*R*) are dispersed throughout cytoplasm. × 18,000.



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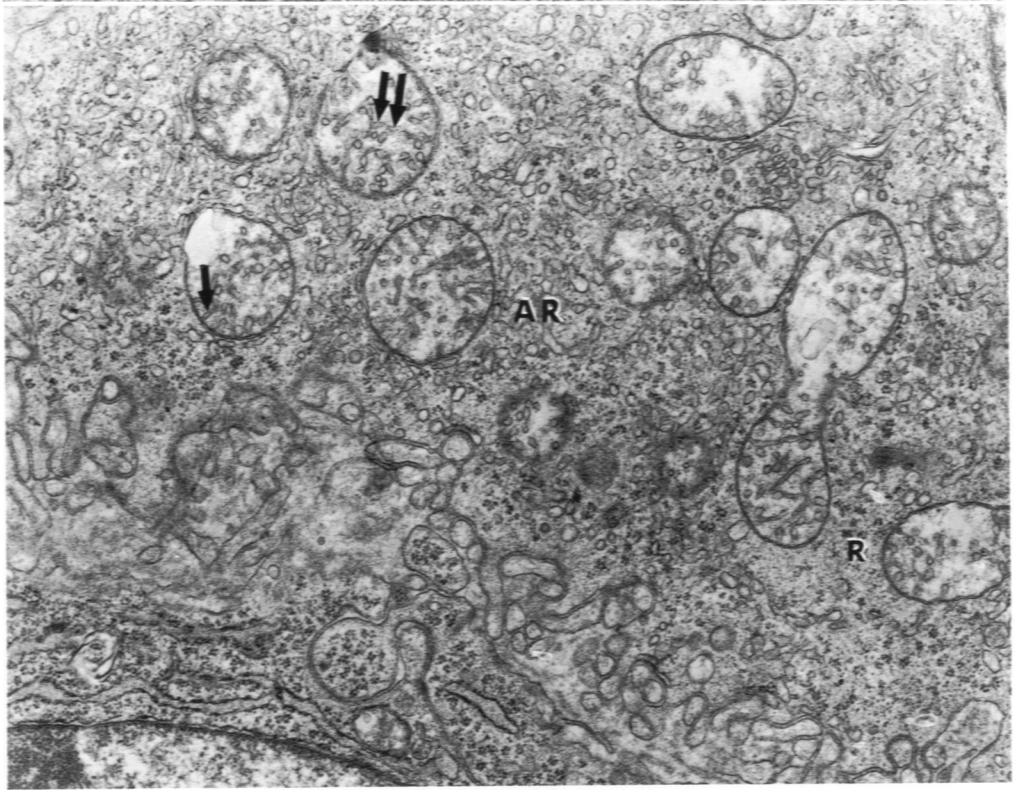

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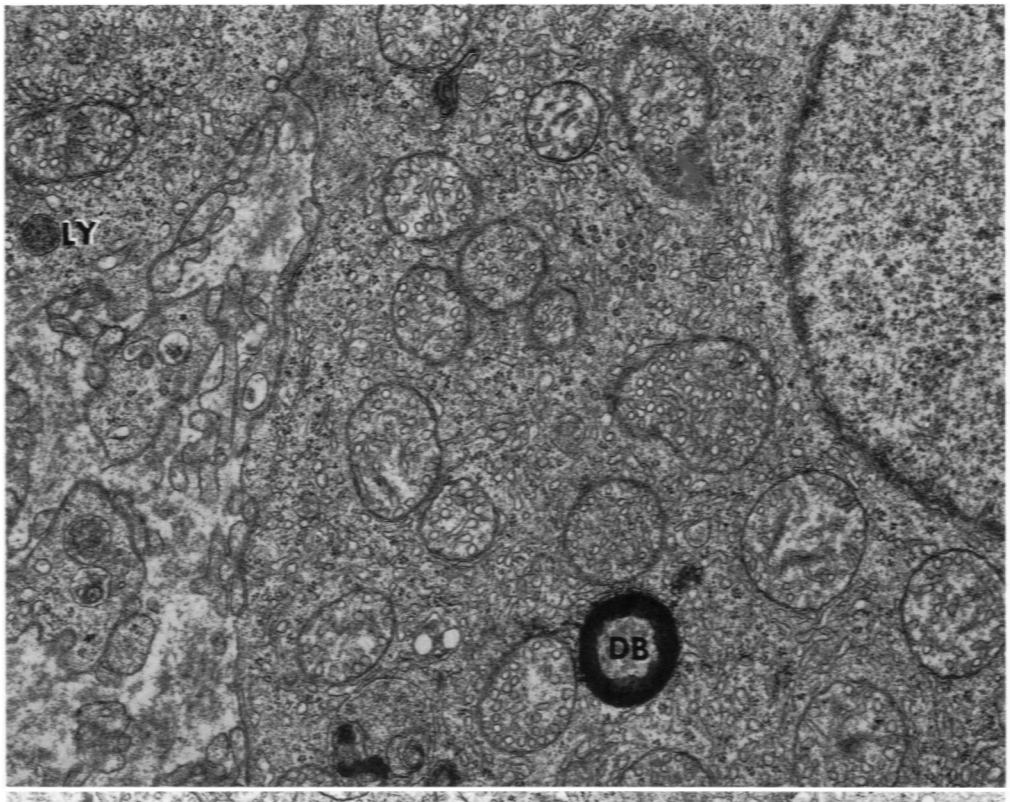
Fig 5. Cortical cell immediately adjacent to capsule at 4 days after adrenal enucleation. A non-membrane-limited osmiophilic body with dark peripheral ring (*DB*) and less dense core is seen in cytoplasm; this structure is distinct from lysosome (*LY*). Mitochondrial cristae appear largely as vesicles. × 19,000.

Fig 6. Portion of several cells in inner region of adrenal cortical regenerate 7 days after enucleation. Numerous vesicles of smooth endoplasmic reticulum (*AR*) are seen throughout cytoplasm. Little rough endoplasmic reticulum is present, although cells contain abundant polysomes (*R*). Cristae of mitochodria are predominantly vesicular although number of these cristae is reduced as compared to those in mitochondria of control fasciculata adrenal cortical cells at same time interval. *LY*, lysosome; *DB*, dense body. × 9000.



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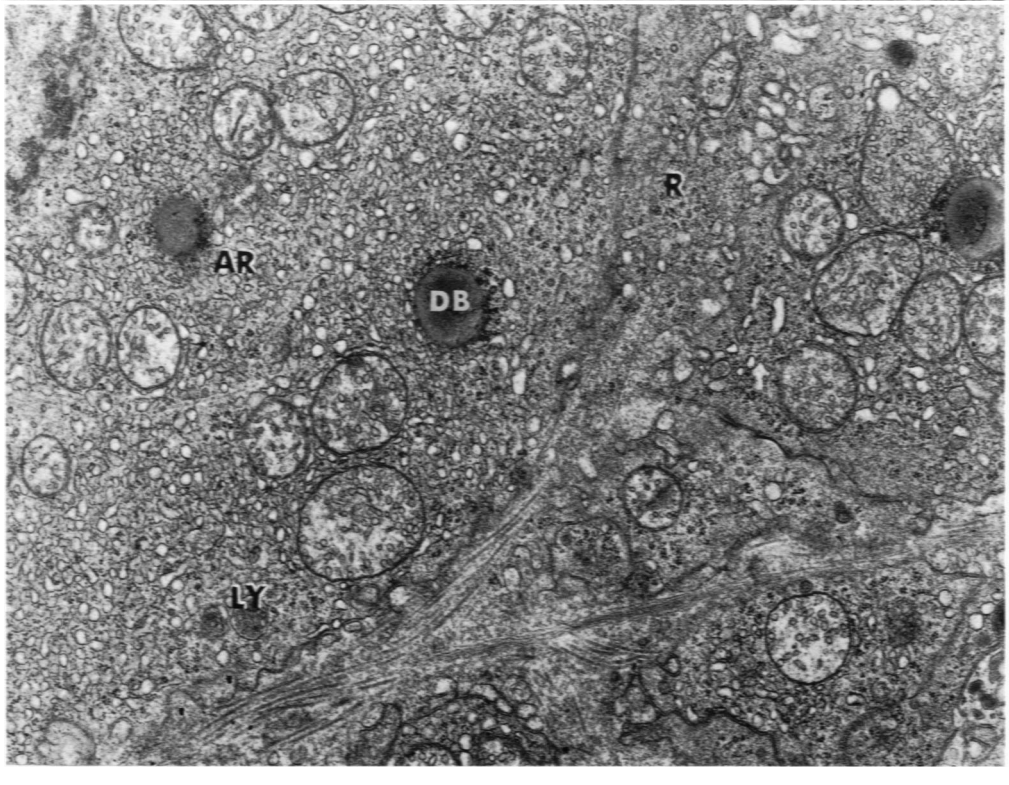

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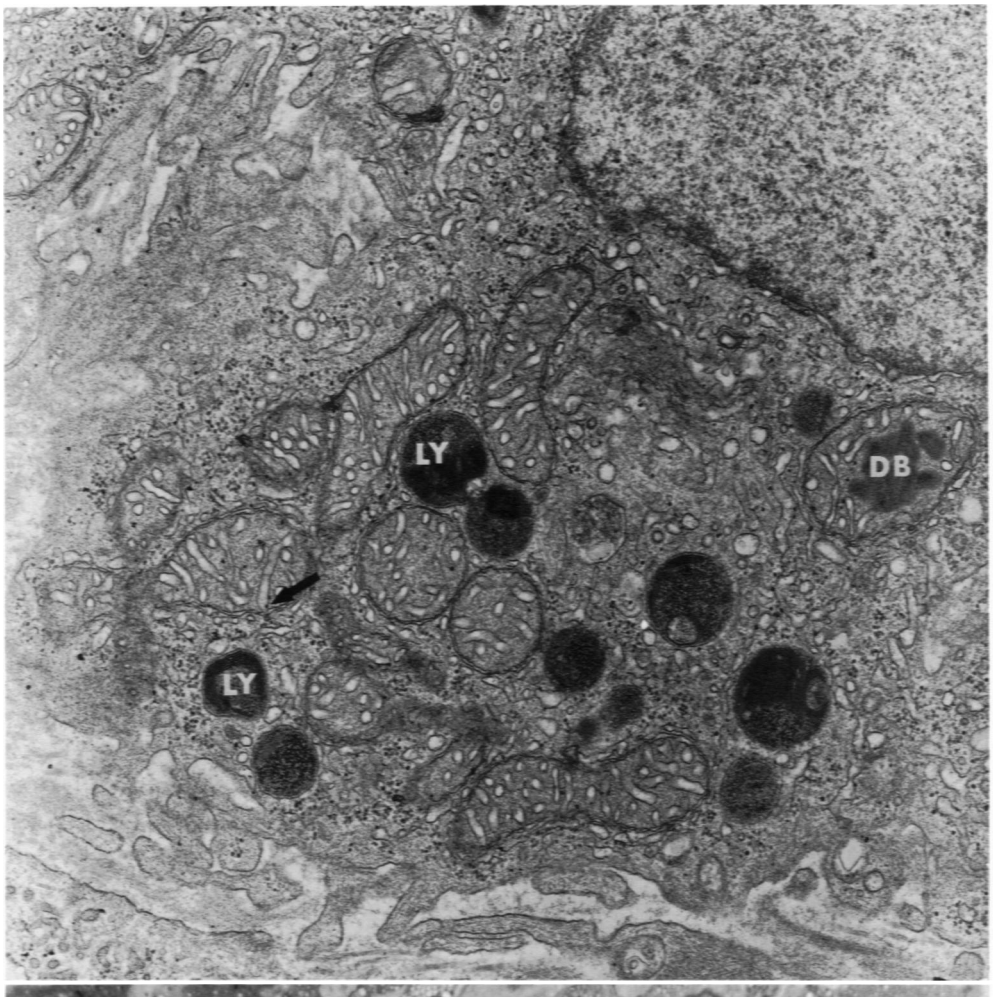
Fig 7. Typical zona glomerulosa cell now seen after 7 days in outer zone of adrenal regenerate. Mitochondria are elongated and contain tubular cristae frequently connected to inner mitochondrial membrane (*arrow*). One mitochondrion contains amorphous, electron dense structure (*DB*) whose significance is unknown. Numerous lysosomes (*LY*) containing a crystalloid material are seen in cytoplasm. × 24,000.

Fig 8. Light micrograph of 1-micron thick toluidine blue stained section of 10-day adrenal regenerate. Cells with light cytoplasm (*LC*) containing densely packed vacuolar structures are seen interspersed among darkly staining cells (*DC*). Nuclei (*N*) in light cells stain consistently darker than those in adjacent dark cells. × 1050.



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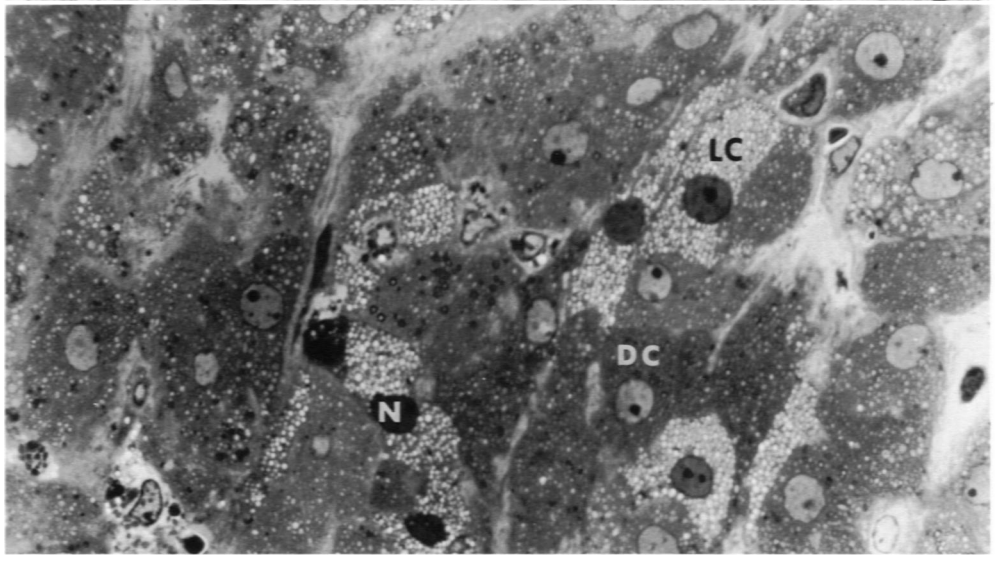

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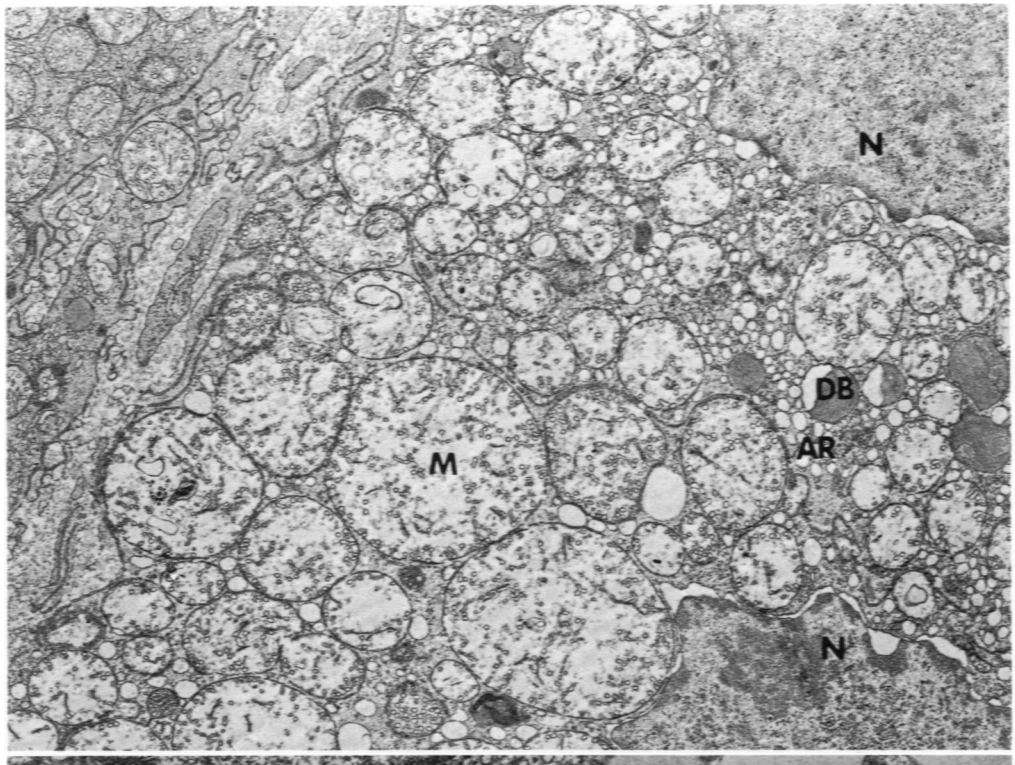
Fig 9. Electron micrograph of zona fasciculata cells corresponding to light cells seen in area similar to that shown in previous toluidine blue stained section. In these cells enlarged mitochondria (*M*) with few cristae and electron lucid matrix correspond to vacuoles seen by light microscopy. Other characteristics include dilated, vesicular endoplasmic reticulum (*AR*), irregularly shaped nucleus (*N*), and electron dense cytoplasmic bodies (*DB*) scattered throughout the cytoplasm. × 12,150.

Fig 10. Electron micrograph of zona fasciculata cell corresponding to dark cells seen in toluidine blue stained section. Mitochondria in this cell are less closely packed than those seen in light cells. This cell contains numerous tubules and vesicles of smooth endoplasmic reticulum (*AR*). Moderate number of lipid droplets (*L*) are present in cytoplasm. Number of vesicular cristae in mitochondria appears greater than at previous times. Many of these cristae can be seen forming interconnecting networks of tubules (*arrow*). × 18,000.



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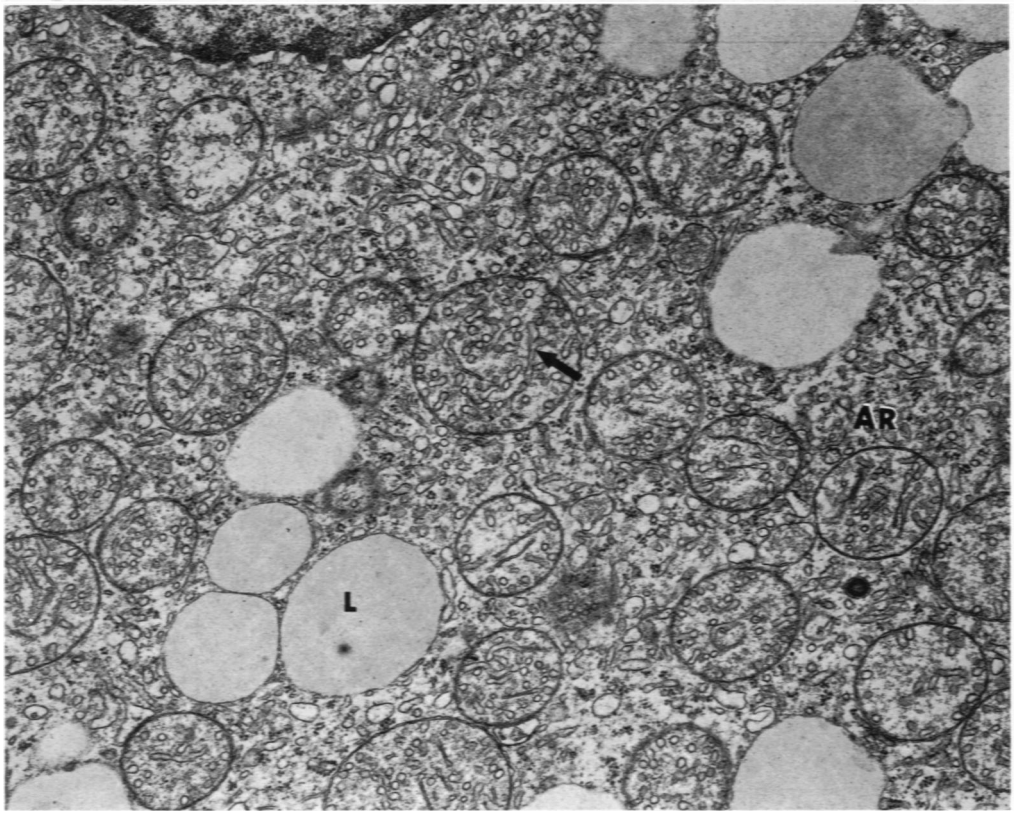

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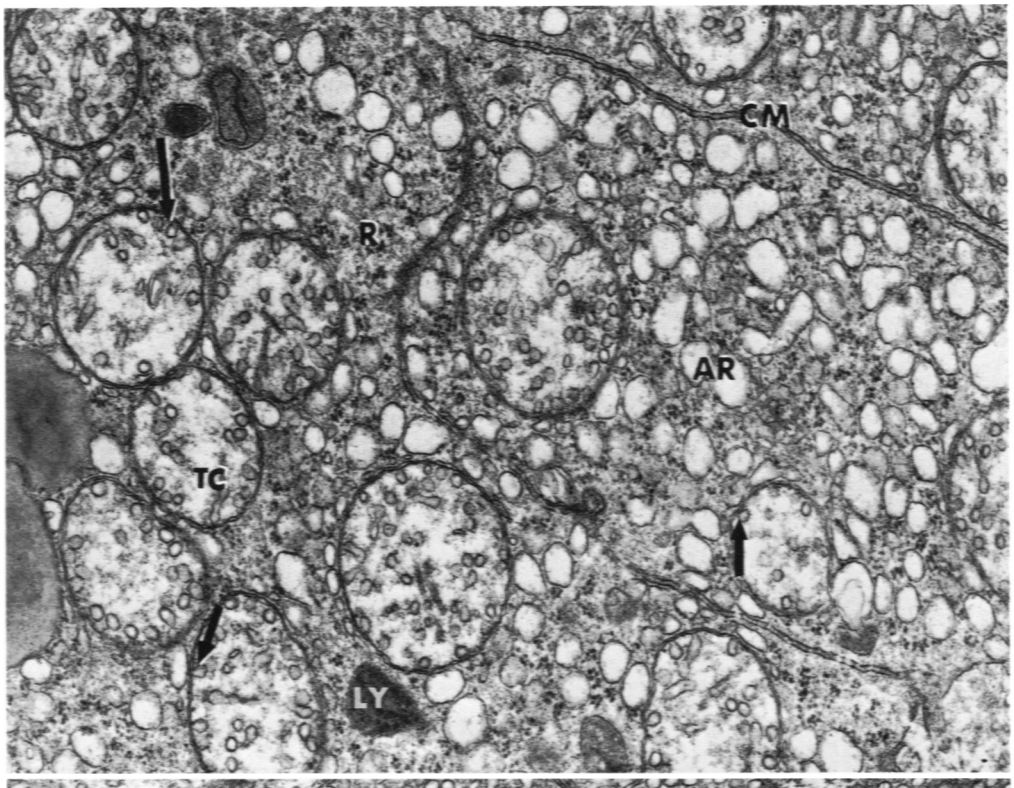
Fig 11. Dark zona fasciculata cells from adrenal which has regenerated for 10 days. Many mitochondrial cristae in these cells are located peripherally and appear to be continuous with inner mitochondrial membrane (*arrow*). Endoplasmic reticulum (*AR*) is vesicular and dilated. *R*, polyribosomes; *CM*, cell membranes; *TC*, tubular cristae; *LY*, lysosome. × 22,000.

Fig 12. Dark zona fasciculata cell in 35-day adrenal regenerate. Most mitochondria contain nearly normal number of vesicular cristae, although some mitochondria are small and have few cristae (*arrow*). Endoplasmic reticulum is predominantly smooth surfaced (*AR*). Lysosomes can be seen in cytoplasm (*LY*). × 18,000.



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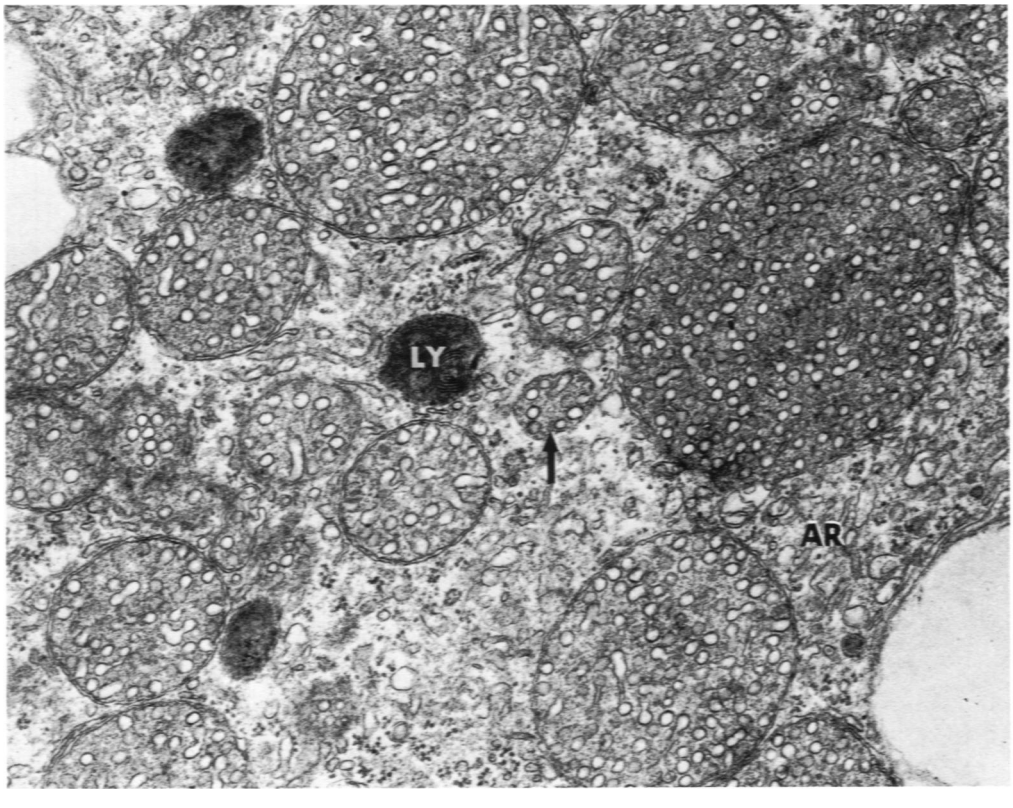

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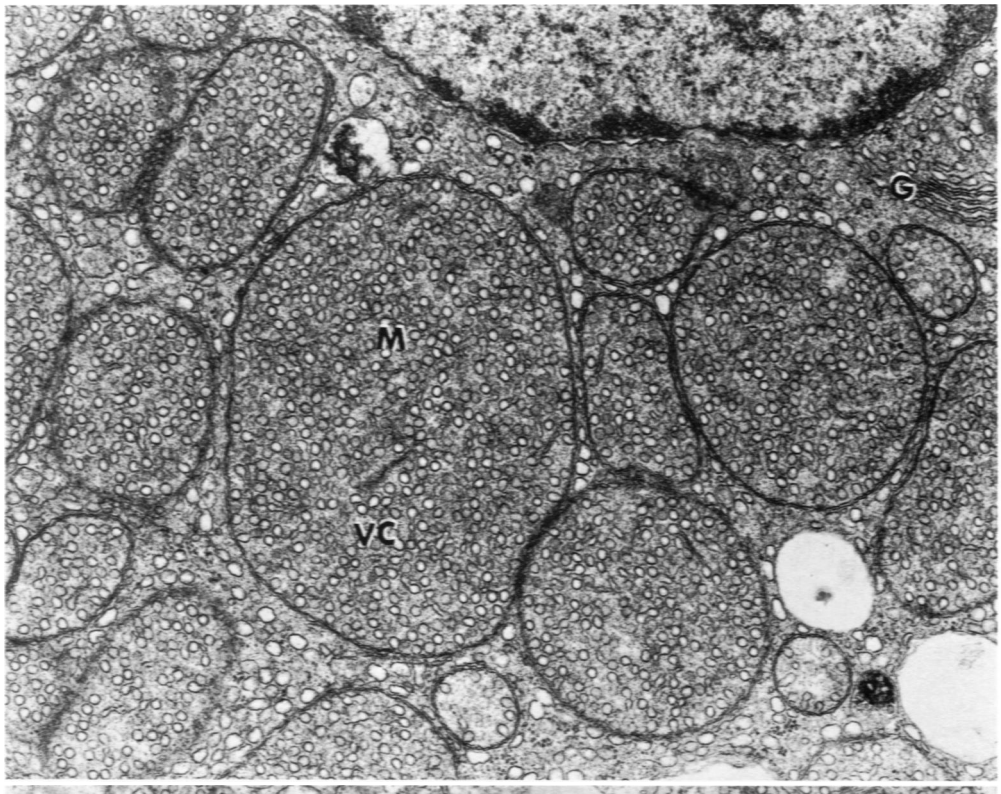
Fig 13. Zona fasciculata cells from adrenal which has regenerated for 56 days. Giant spherical mitochondrion (*M*) is seen at center of micrograph. Cristae are almost all vesicular in type (*VC*). (*G*), Golgi apparatus. × 18,000.

Fig 14. Zona fasciculata cell after 70 days of adrenal cortical regeneration. Numerous tubules of smooth endoplasmic reticulum (*AR*) are seen near cell membrane. × 18,000.



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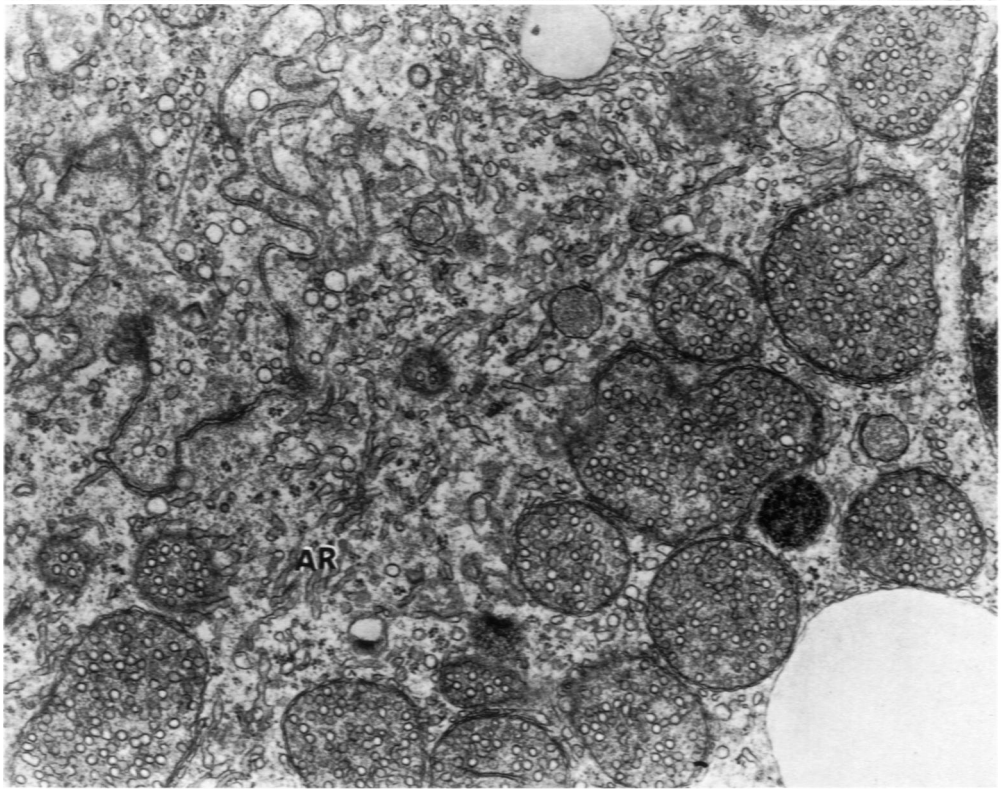

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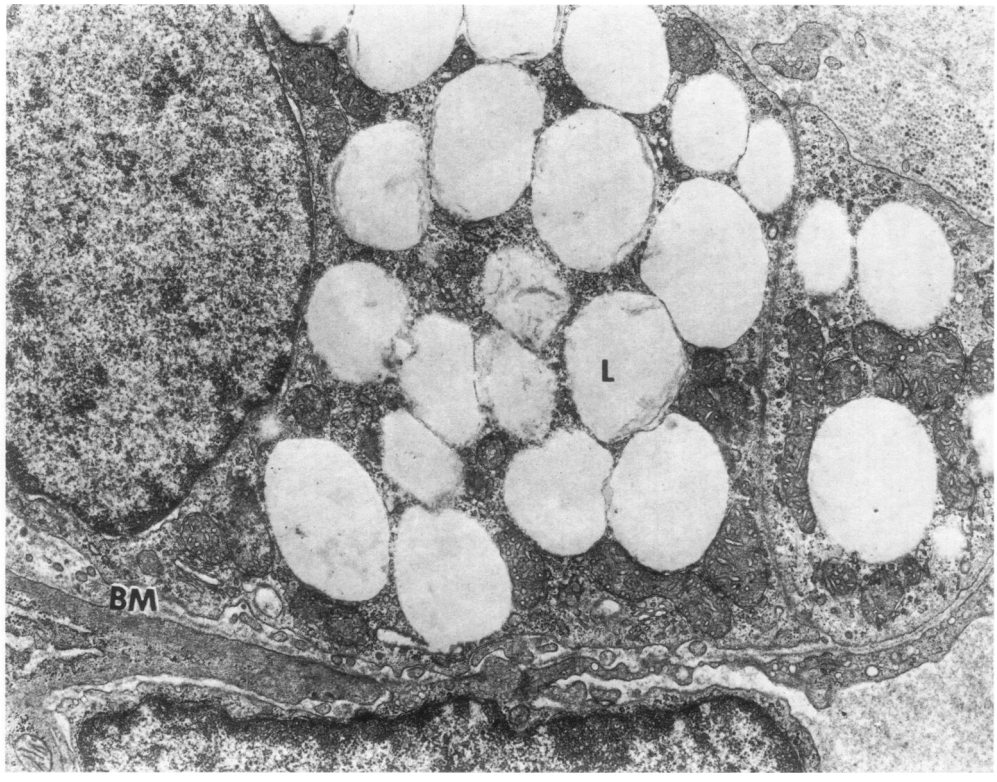

Fig 15. Zona glomerulosa cell from 56 day adrenal regenerate. Numerous lipid droplets (*L*) almost fill entire cell cytoplasm. Mitochondrial cristae are similar in appearance to those seen in zona glomerulosa cells of control animals. (*BM*), basement membrane. × 9000.