ULTRASTRUCTURAL ZONATION OF ADRENOCORTEX IN THE RAT

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ABSTRACT

The fine structure of the different zones in the adrenal cortex of the adult rat has been studied under the electron microscope. Four regions mainly differentiated by the mitochondrial morphology, the lipid droplets, and the structure of the ground cytoplasm were recognized. In the *glomerular zone* mitochondria are thin and elongated with an abundant matrix. The inner structure is characterized by the presence of tubules of 300 A that are straight or bend at an angle and which may be grouped in parallel array giving a pseudocrystalline pattern. The wall of each tubule is a finger-like projection of the inner membrane and its cavity corresponds to the outer chamber of the mitochondrion. In the *intermediary zone* mitochondria are larger and irregular. The matrix is filled with convoluted tubules and vesicular elements. The lipid droplets are larger and irregular in the glomerulosa and and small in the intermedia. The ground substance is dense and contains free ribosomes in the glomerulosa and starts to be vacuolated in the intermedia. In the *fasciculata* mitochondria are round or oval and are filled with vesicular elements with a mean size of 450 A. Larger vesicles and more clear elements (vacuoles) are seen near the edge as if their content was diluted. Some of these vacuoles protrude on the surface. In the *reticular zone* mitochondria are also vesicular but frequently show signs of alteration and disruption. Dense elements recognized as microbodies are observed in the fasciculata but they increase in number in the reticularis. These results are discussed on the light of the so called zonal theory of the adrenal cortex. Two stages in the differentiation of the mitochondria are postulated. The tubular structure of the glomerulosa undergoes a process of disorientation and dilatation of the tubules to form the tubulo-vesicular elements of the intermediary zone. In a second stage of differentiation, by fragmentation of the tubules, the vesicular structure of fasciculata is formed. These findings are discussed from the viewpoint of the relationship between mitochondria and synthesis of steroid hormones. A secretory process that starts within mitochondria by the formation of vesicles and proceeds into the ground cytoplasm, as extruded and more clear vacuoles, is postulated.

The use of classical cytological and cytochemical techniques in the normal and experimentally treated adrenal cortex has yielded a great deal of information regarding its physiological activity (see reference 1). Probably more than in other endocrine glands this histophysiological approach was very fruitful and lead to important concepts of adrenal physiology.

The more recent methods of steroid analysis, the experiments of perfusion or incubation with radioactive substrates and those of cell fractionation have resulted in the determination of different steps in corticosteroid synthesis and their relationship to subcellular particles (see reference 2). These advances made of considerable interest a comprehensive submicroscopic morphological analysis of the secretory function of the gland at a subcellular level of structure.

So far few papers have been devoted to this type of analysis (3–7). All of them have emphasized the great number of mitochondria present in adrenal cells, a fact that already was known from optical microscopy. This fact may be related to the oxidative metabolism (8) and particularly to the important function that mitochondria play in different steps of the synthesis of adrenal hormones (2).

The internal structure of mitochondria has also been found to differ from the more general model of Palade (9). A tubular inner structure was postulated not only for the adrenal cortex but also for other tissues secreting steroids such as the corpus luteum and the inner theca of the Graffian follicle (10). All these tissues are embryologically related belonging to a "steroid gland primordium" of mesoblastic cells and have functional and biochemical similarities (11).

This paper is the first of a series in which the submicroscopic morphology of the adrenal cell of the rat in different experimental conditions will be analyzed. It will be concerned mainly with the fine structure of mitochondria in the different regions of the adrenal cortex of the normal adult animal. The organization of the ground cytoplasm, the vacuolar system with the intracellular membranes, and the lipid droplets will be considered in lesser detail.

This analysis will show the existence of special patterns of submicroscopic organization in the different zones of the adrenal cortex. Furthermore- a secretory process starting within the mitochondria and continuing in the cytoplasmic vacuolar system will be postulated.

TECHNIQUES

Twenty young male rats (Stock and Wistar) weighing between 120 and 200 gm. were used. Under light ether anesthesia the adrenal gland was extirpated and immediately cut into small pieces within the fixative. The blocks were so oriented as to have a piece of capsule or adrenal medulla as point of reference.

The fixation for 2 hours was in a cold 1 per cent OsO_4 solution of periston (Bayer), a mixture containing polyvinilpyrrolidone and balanced ions. The pH was adjusted to 7.4. This type of fixation in which the osmotic and oncotic pressure and ion composition of the blood are maintained is particularly fitted to avoid artifacts of fixation in this tissue which otherwise is particularly labile. After a stepwise dehydration in ethanol the material was finally embedded in a prepolymerized butyl-methylmethacrylate mixture 9:1.

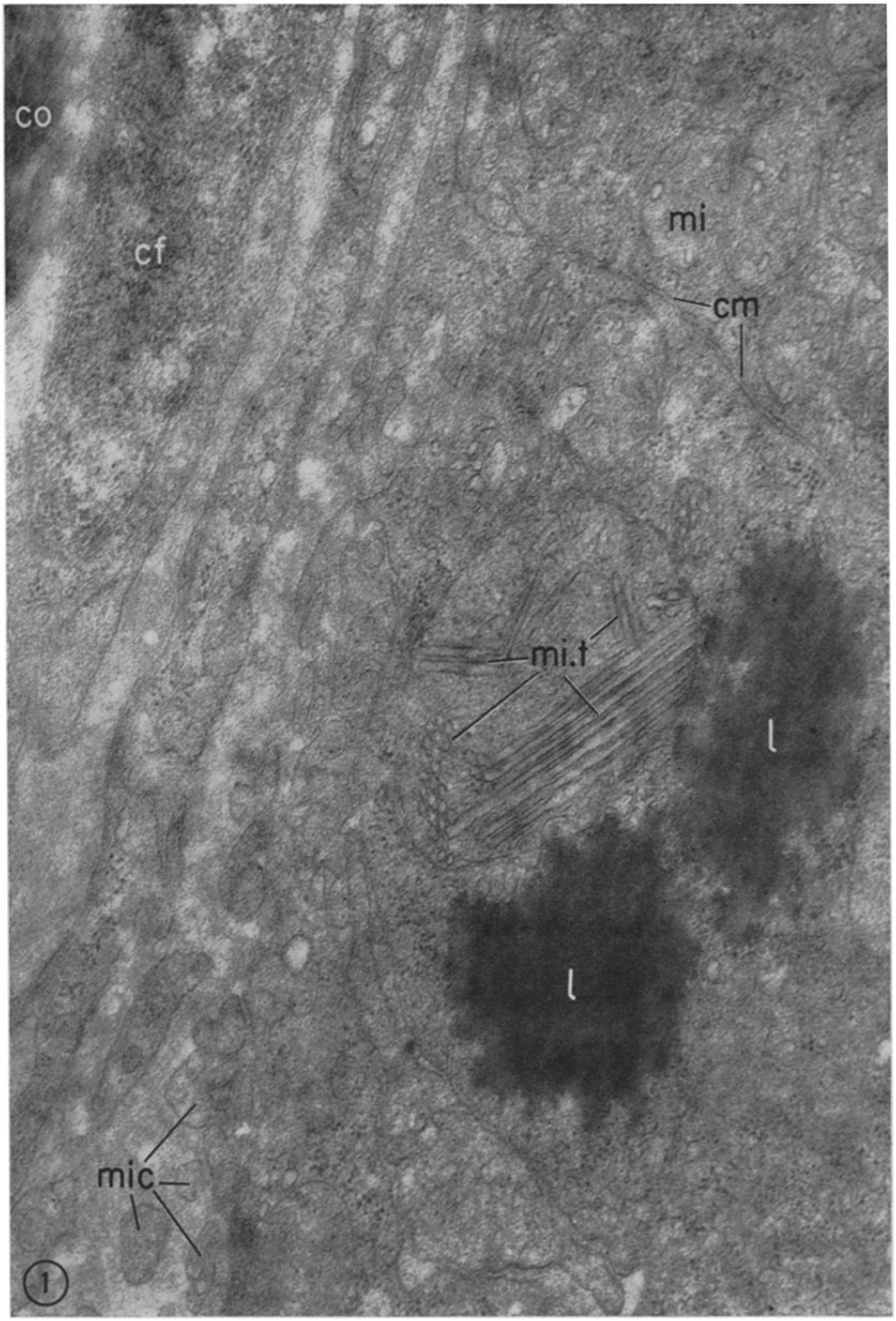
For orientation 1 μ thick sections were observed under the phase microscope, trying to recognize the different regions of the gland by their structure or the presence of the connective capsule or the cathecol containing cells. The thin sections, with silver or gray colors, were observed with an RCA EMU 2E electron microscope with a compensated objective lens provided with a Canalco stigmator.

Explanation of Figures

ami	altered mitochondria
cf	capsular fibroblast
cl	capillary lumen
cm	cell membrane
co	collagen
ct	convoluted tubule
en	endothelium
er	erythrocyte
eva	extruding vacuole
l	lipid
li	lipochrome
ma	matrix of the mitochondrion
mb	microbody
mi	mitochondrion
mic	microvilli
min.	mitochondrial membrane
mit	mitochondrial tubules
mv	mitochondrial vesicles
N	nucleus
p	pore of the endothelium
r	ribosomes
va	vacuoles

FIGURE 1

Electronmicrograph of the outermost cell layer of the glomerulosa. Showing fibroblast-like cells (*cf*) in the capsule separated by a fibrillar matrix and collagen fibers (*co*). Typical irregularly shaped lipid droplets and a mitochondrion containing tubules in parallel array are seen. × 48,000.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OBSERVATIONS

Four regions corresponding approximately to the glomerulosa, intermedia, fasciculata, and reticularis can be differentiated by some cytological characteristics. These refer mainly to mitochondria, lipids, amount and structure of ground cytoplasm, and so forth.

A. *Zona Glomerulosa*

The cells situated immediately below the capsule are characterized by the high density and abundance of the cytoplasmic matrix, the presence of large, irregularly shaped lipid droplets, and the elongated mitochondria varying in width between 0.2 and 1 μ. The mitochondrial structure is most typical and permits *per se* the identification of the cell type (Figs. 1 to 3). The mitochondrial matrix is abundant, amorphous, and of high density and the two mitochondrial membranes are clearly observed. A varying portion of the mitochondrial matrix is occupied by tubules of about 300 A in diameter that are frequently grouped in parallel with an ordered array. Longitudinal and cross-sections of these groups of tubules cut at different incidences are found (Figs. 1 and 2). Because of this regular patterns some parts of the mitochondrion may have a pseudocrystalline aspect (Figs. 1 and 3).

The mitochondrial tubules have straight portions of 1 micron or more in length but frequently they bend at an angle (Fig. 1). In some cases there are dilatations of 400 A in diameter disposed at the same level in the several tubules. In favorable sections it can be observed that the tubules are finger-like invaginations of the inner mitochondrial membrane. The less dense content of the tubule thus corresponds to the interspace or outer chamber in between the membranes. In addition to this special type of mitochondria there are smaller ones with abundant dense matrix in which the tubules are not straight or in regular array but with more dilated portions. Between the two types of mitochondria there are all transitional forms.

The *ground cytoplasm* is of high density and contains groups of free ribosomes. The vacuolar system (endoplasmic reticulum) is poorly developed in these cells. In the deeper regions of the cell a Golgi complex with typical flattened sacs and some clear vesicles can be observed.

At the edge of the cell in contact with the capsule or the blood capillaries there are irregular microvillous projections interspaced in the tissue spaces and having a dense cytoplasmic matrix (Fig. 1). In between the microvilli deep invaginations of the cell membrane penetrating into the peripheral cytoplasm may be found.

B. *Zona Intermedia*

While the outer limit of the glomerulosa layer is clearly marked by the capsule with their fibroblast-like elements (Fig. 1) in deeper regions, there is a gradual change in fine structure that leads to the so called *intermediary* (12) or *transitional zone* (13) that in the male adrenal corresponds approximately to the sudanophobic region. In these cells mitochondria are larger, more numerous, and have irregular contours. The mitochondrial matrix is less abundant than in the glomerulosa, being occupied by profiles of convoluted and dilated tubulo-vesicular elements. The cavity of these is much larger and more irregular than in the glomerulosa. The size of the tubules increases near the edge of the mitochondria (Fig. 4).

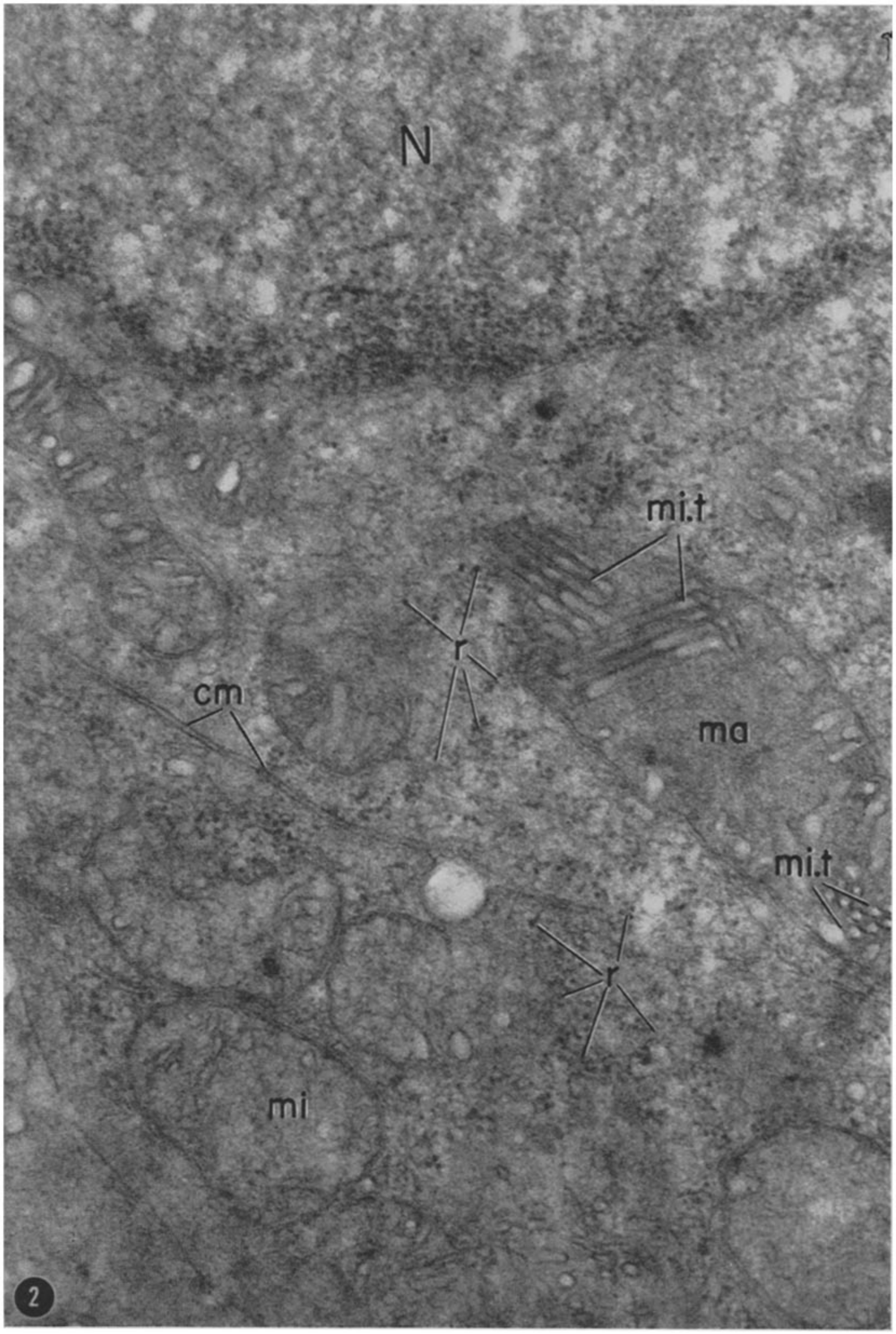
The *ground cytoplasm* is less abundant than in the glomerulosa and contains numerous vacuoles with a content of low density many of which are adjacent to mitochondria. Sometimes the contact is so intimate that the outer membrane of the mitochondria is lacking (Fig. 4). Small irregular *lipid* droplets are observed intermingled with the mitochondria and cytoplasmic vacuoles.

C. *Zona Fasciculata*

This region of the gland is also characterized by the fine structure of mitochondria. These are of varying size and of oval or round shape. Within the double membrane most of the space is occupied

FIGURE 2

Portion of a cell of the glomerulosa showing the nucleus, the dense ground cytoplasm with free ribosomes, and mitochondria with tubular structure. Note the large amount of mitochondrial matrix. $\times$ 62,000.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by tightly packed vesicular elements of about 450 A with little matrix in between. The entire mitochondria can be compared to a "bag" filled with vesicles (Figs. 5 to 7).

The closer observation of the edge of certain mitochondria may show at certain points the lack of outer membrane and the projection of larger vesicles (vacuoles) on the surface (Figs. 6 and 7). The content of these vesicles is less dense than that of the inner ones, as if the material was being progressively diluted. They resemble the numerous clear vacuoles found in the ground cytoplasm surrounding the mitochondria and there are transitions in size, aspect, and position between both types.

The *lipid droplets* are more osmiophilic roundly shaped and with wavy edges. They are not grouped as in the glomerulosa but dispersed in the cytoplasm. Frequently they bear a close relationship with mitochondria and the cytoplasmic vacuoles (Fig. 5).

In addition to mitochondria and lipid droplets, there are smaller bodies which have been called *microbodies* (5) or *globules* (7). Usually round in shape and having a single membrane, they are found in all regions of the cortex but are more numerous in the deeper ones. The content of the microbody is generally dense and finely granulous; sometimes small vesicles and curved membranes or a dense deposit may be present (Fig. 7). While the *nucleus* may be elongated and parallel to the surface in the outer glomerulosa, it is round in the deeper cells of this zone and also in the intermedia and fasciculata. In these the nucleolus is prominent and chromatin clumps may be found near the nuclear membrane. In this region and throughout the entire cortex there is a subendothelial pericapillary space similar to that found in other endocrines (see reference 14). The edge of the cell projects into this space by means of long and wavy microvilli, and there are also deep invaginations of the cell membrane (Figs. 5 and 6). Some of the vacuolar elements of the cytoplasm may be found in closed relationship with these invaginations.

D. *Zona Reticularis*

In this deepest region of the cortex, *mitochondria* are also filled with vesicles but frequently have irregular edges, are vacuolized or disrupted, or have dense deposits (Fig. 8). The ground cytoplasm is of low density with few ribosomes and lacking a special organization. There are microbodies with dense granular deposits and large dense granules of irregular shape and variable size which probably correspond to the lipochromes or pigment granules of optical microscopy. Some of these granules show similarities with microbodies (Fig. 8).

All these and other characteristics that may be indicative of an involution of the cell starting in the inner fasciculata but becoming more prominent in the reticular region proper.

DISCUSSION

Submicroscopic Morphology and the Zonal Theory of the Adrenal Cortex

As Chester Jones (15) has graphically mentioned, so far "the zonation of the eutherian adrenal cortex is based, for better or worse, on histological criteria, that is the appearance, shape and grouping of cells by routine methods." This morphological approach gave rise to the now old theory of cellular migration (16–21) and to the more recent zonal theory of cortical function.

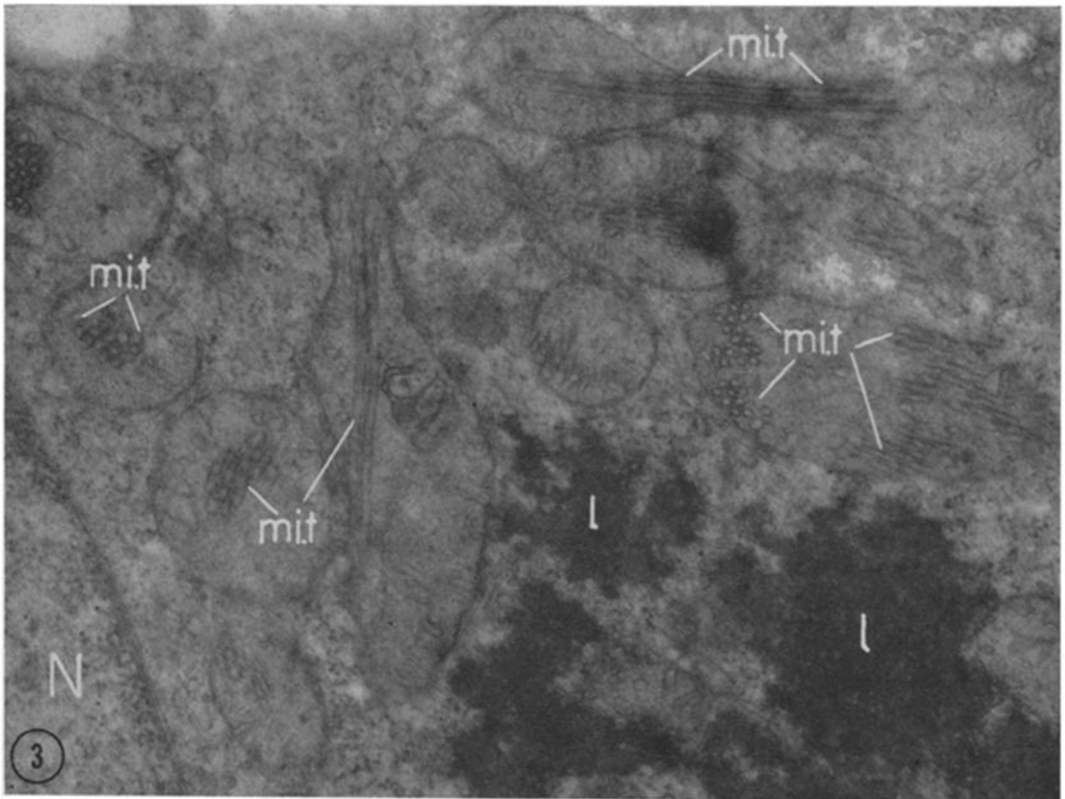
The concept of cellular migration implying a process of cytomorphosis starting at the periphery of the gland and ending near the adrenal medulla with a single secretory cycle cannot be maintained at present without qualifications. The finding of

FIGURE 3

Part of a cell of the glomerulosa with typical lipid droplets and mitochondria with array of tubules sectioned in several orientations. × 40,000.

FIGURE 4

Part of a cell of the transitional zone showing large and irregularly shaped mitochondria filled with tubulo-vesicular elements. See that the edge of the mitochondria is poorly defined and that clear vacuoles (*va*) are present in the surrounding cytoplasm. Lipid droplets are very small. × 52,000.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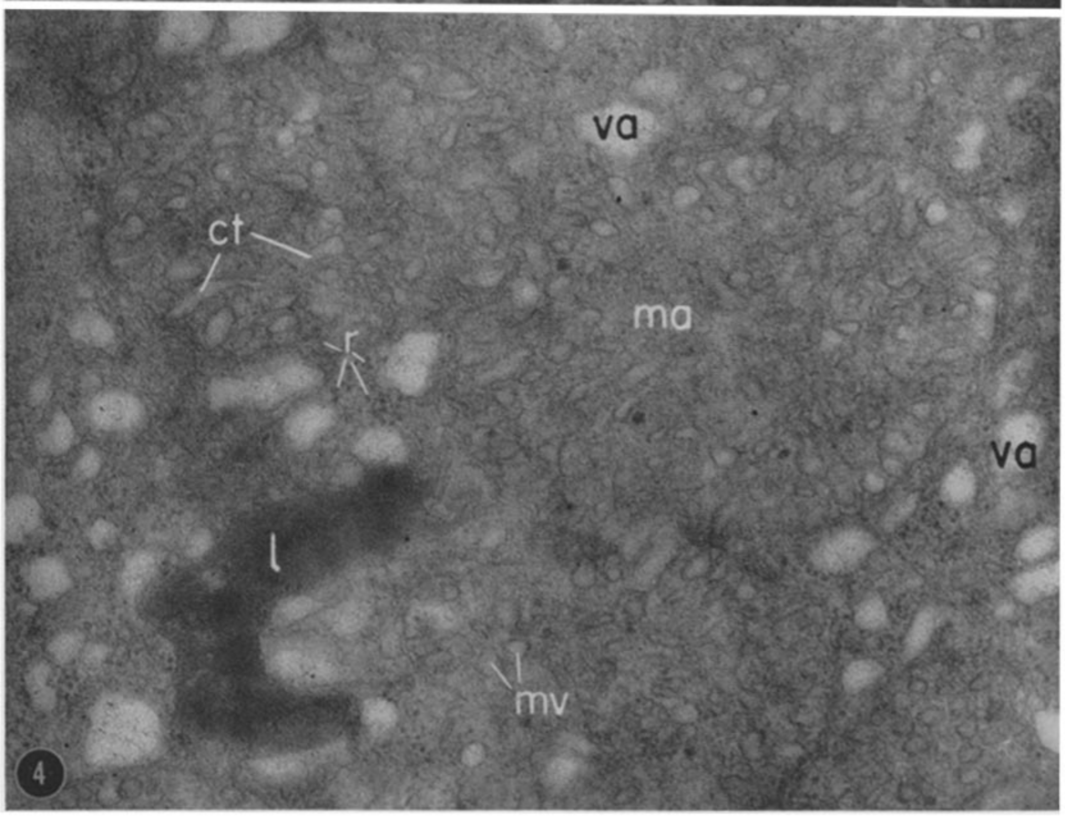
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several bands of mitosis (22, 23) within the cortex was a heavy blow to this theory, but the migration phenomenon can still be explained by assuming the existence of several cell generations undergoing cellular differentiation. Among the possible factors regulating this process ACTH has been considered important in inducing the transformation of the glomerular into fascicular cells (24, 26). This interpretation is reinforced by the finding that the glomerular zone tends to disappear after chronic administration of ACTH (24–26, 15).

The now widely accepted zonal theory that postulates a functional autonomy for the different zones of the cortex may not imply a complete anatomical independence as far as the origin and destiny of the cells is concerned. As we shall see later, the hypothesis of a special cellular differentiation may probably be combined with that of functional autonomy.

Starting from histophysiological and histochemical evidence the zonal theory (27–30), in recent years, has acquired a solid biochemical background. By incubating different zones of the cortex it was shown that aldosterone—the chief mineralocorticoid—is produced in the glomerulosa while the glucocorticoids are mainly manufactured by the fasciculata and reticularis (31).

Stanchenko and Giroud (32) have recently brought forth important evidence for an *enzymatic zonation* of the adrenal cortex of the ox that would explain the different regional metabolic pathways. They find, for example, that the 3β-dehydrogenase and the 11- and 21-hydroxylase activities are present throughout the gland while the 18-oxygenation needed for aldosterone is localized in the glomerulosa and 17-hydroxylase activity in the fasciculata-reticularis. An enzymatic zonation has also been found in the rat (33).

The submicroscopic analysis of the normal rat adrenal reported here has revealed interesting data backing the zonal theory and the possibility of a special sequence in cell differentiation. Two essentially different submicroscopic patterns were found in the glomerular and the fascicular zones. These patterns are, however, not sharply delimited and between them there is a transitional or intermediary zone suggesting a possible cellular transformation. The reticular zone seems to be a continuation of the fasciculata in which an involutional process takes place.

These topographic characteristics and the sequence in cell differentiation are mainly seen in the mitochondrial structure but can be observed as well in the intervening ground cytoplasm. As described above, mitochondria of the outermost cell layers are characterized by the small size, the large amount of mitochondrial matrix, and the presence of oriented tubules. The sequence of changes probably consists in the disorientation of the tubules and the increase in number of tubulo-vesicular projections with the consequent reduction in mitochondrial matrix. The outcome of this process is probably represented by the intermediary zone with the larger and irregular mitochondria filled with tubulo-vesicular elements.

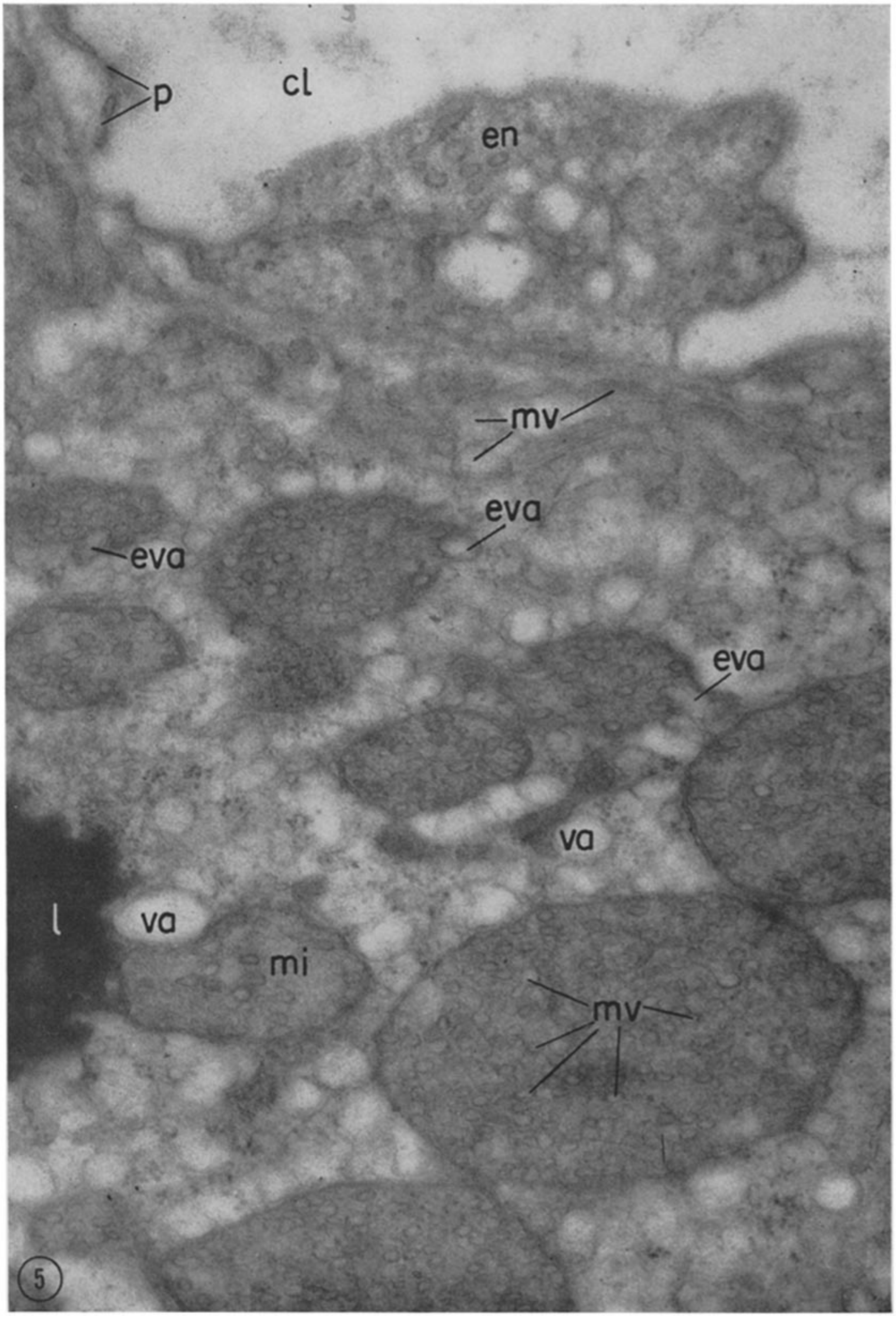
Continuing with this interpretation, the mitochondria of the fasciculata would correspond to a second stage of differentiation in which by the fragmentation of the tubules an essentially vesicular structure is formed. Finally in the reticularis, mitochondria still maintain a similar fine structure but the involutive changes are added.

It is interesting to mention here that in the hamster, although the tubular structure is maintained throughout the fasciculata, in the deeper regions there are striking mitochondrial changes that also suggest a process of involution (34).

In addition to these zonal patterns of mitochondrial structure there are also topographic differences in the rest of the cytoplasm. Thus, the lipid droplets that are large, irregularly shaped, and polarized in the glomerulosa become smaller

FIGURE 5

Part of a cell of the fasciculata showing a capillary with an endothelium provided with pores (*p*). The edge of the cell shows microvilli that occupy the subendothelial space. Mitochondria have a vesicular structure (*mv*) with some larger and clearer vesicles (vacuoles) near the edge. Some of these intramitochondrial vacuoles protrude on the surface of the mitochondrion. The ground cytoplasm is more abundant, contains few ribosomes, and is filled with clear vacuoles. × 52,000.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and less numerous in the intermediary zone and again larger, denser, and with smoother edges in the fasciculata. The Golgi complex that is compact in glomerular cells, becomes fragmented in the fasciculata and the microbodies increase in size and number in the deeper regions. Other interesting zonal changes are related to the ground cytoplasm proper of hyaloplasm. Thus in the glomerulosa it is denser and contains free ribosomes, and few elements of the vacuolar system. In the intermedia and fasciculata the number of clear vacuoles increases while in the reticularis the ground substance appears disorganized.

Mitochondria and the Secretory Function of the Adrenal Cortex

Lehninger (35) has emphasized the fact that mitochondria, in addition to functioning in cellular oxidations, phosphorylation, and energy transfers, generally have a secretory activity. This is particularly evident in the adrenal cortex where it has been demonstrated that most of the steps in the biosynthesis of the corticosteroids occur in mitochondria or are closely related to the mitochondrial fraction (2).

In bovine adrenals, mitochondria contain the enzymatic system that degrades the lateral chain of cholesterol to produce pregnenolone. Since this system is apparently not involved in the biosynthesis of aldosterone it would be confined exclusively to mitochondria of fascicular cells. The other mitochondrial system producing 11-hydroxylation is instead present throughout the entire cortex.

The finding of a special tubular and vesicular structure in mitochondria of the adrenal cortex suggests that this might be related to the special secretory activity they perform. This interpretation is supported by the observation, which will be published elsewhere, of a striking modification in the fine structure of mitochondria in experimental conditions known to produce changes in secretory activity (36).

From all observations made so far, it seems to us that the mitochondrial structure can be best interpreted in terms of a secretory activity occurring within the organoid and continuing in the surrounding cytoplasm. The presence of vesicles in mitochondria of the fasciculata may represent a more dynamic type of structure than the plate-like crests found in most tissues (9). The presence of vesicular elements and the corresponding reduction in matrix results in a considerable increase in membrane surface where enzymes may be bound. Furthermore, vesicles are free structures that probably wander within the mitochondria and may even be extruded from it. Indeed, several observations show that largest and less dense vesicles are frequently found near the periphery of mitochondria and that they even protrude on the surface.

In the description it was emphasized that the cavity of the vesicle probably originates as a dependence of the outer chamber of the mitochondria, which may increase and become isolated into special units. We may postulate that the process of corticosteroid biosynthesis results in the accumulation of secretory products within the vesicle. A further stage would be the increase in volume of of the vesicle with reduction in density of the content (vacuole) and its passage into the surrounding cytoplasmic vacuolar system. There this vacuole, which may be still attached to mitochondria, increases in size and has a ligher content. This sequence of secretory events may also be correlated with the fact that during biosynthesis the water polarity of the corticosteroid molecule increases.

This dynamic interpretation of the mitochondrial fine structure in the different zones of the cortex is, for the moment, only tentative and may serve as a working hypothesis and basis for discussing the experimental results that will be reported elsewhere.

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FIGURE 6

Similar description as that of Fig. 5. The edge of the cell shows one deep indentation of the subendothelial space. Two microbodies, one of them with a dense deposit, are seen. × 52,000.

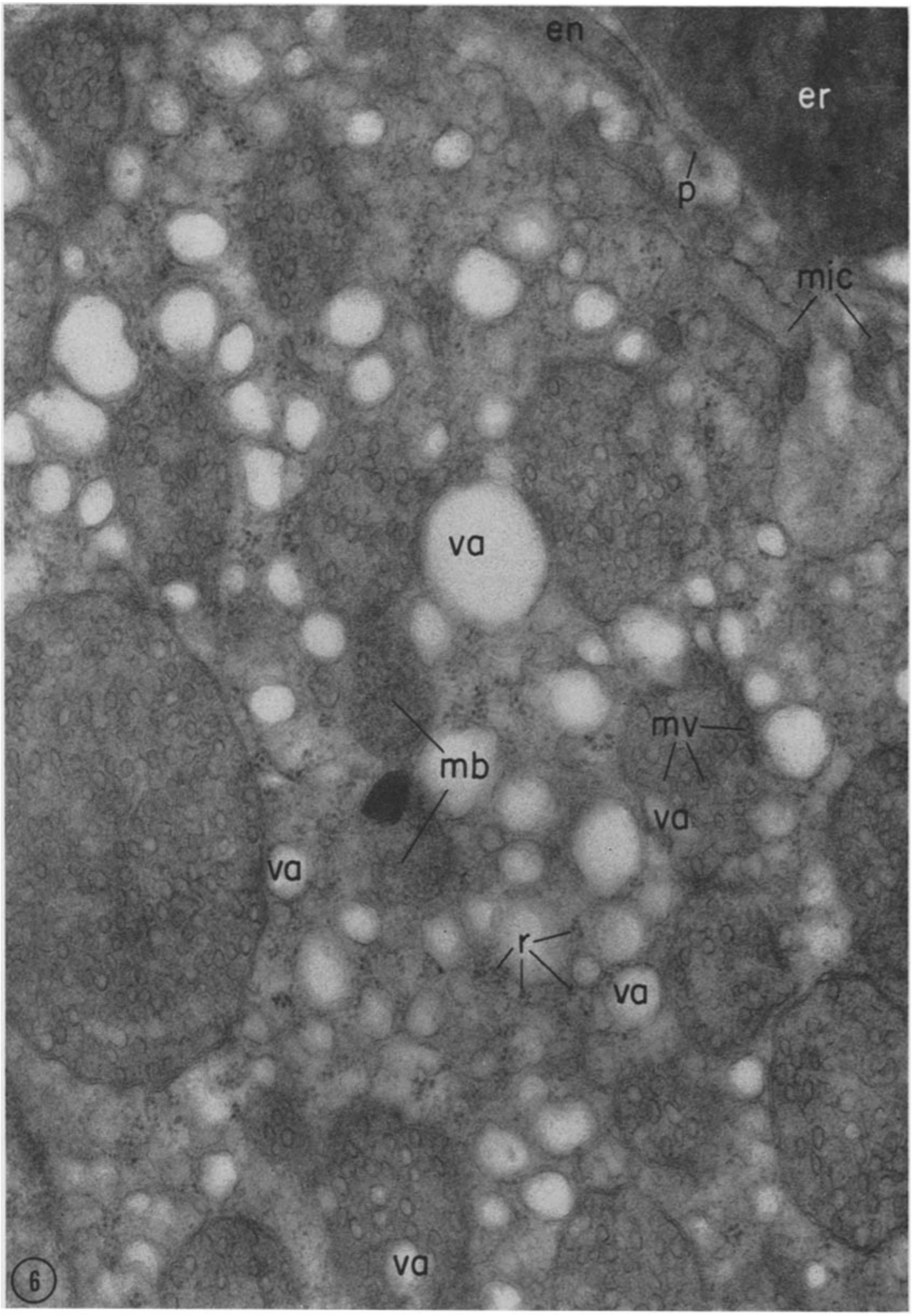
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FIGURE 7

Similar description as that of Fig. 6 at higher magnification. See the fine structure of a mitochondrion with the two outer membranes and the vesicles. With arrows is indicated one vesicle that communicates with the cytoplasm. Microbodies show only a single membrane. × 93,000.

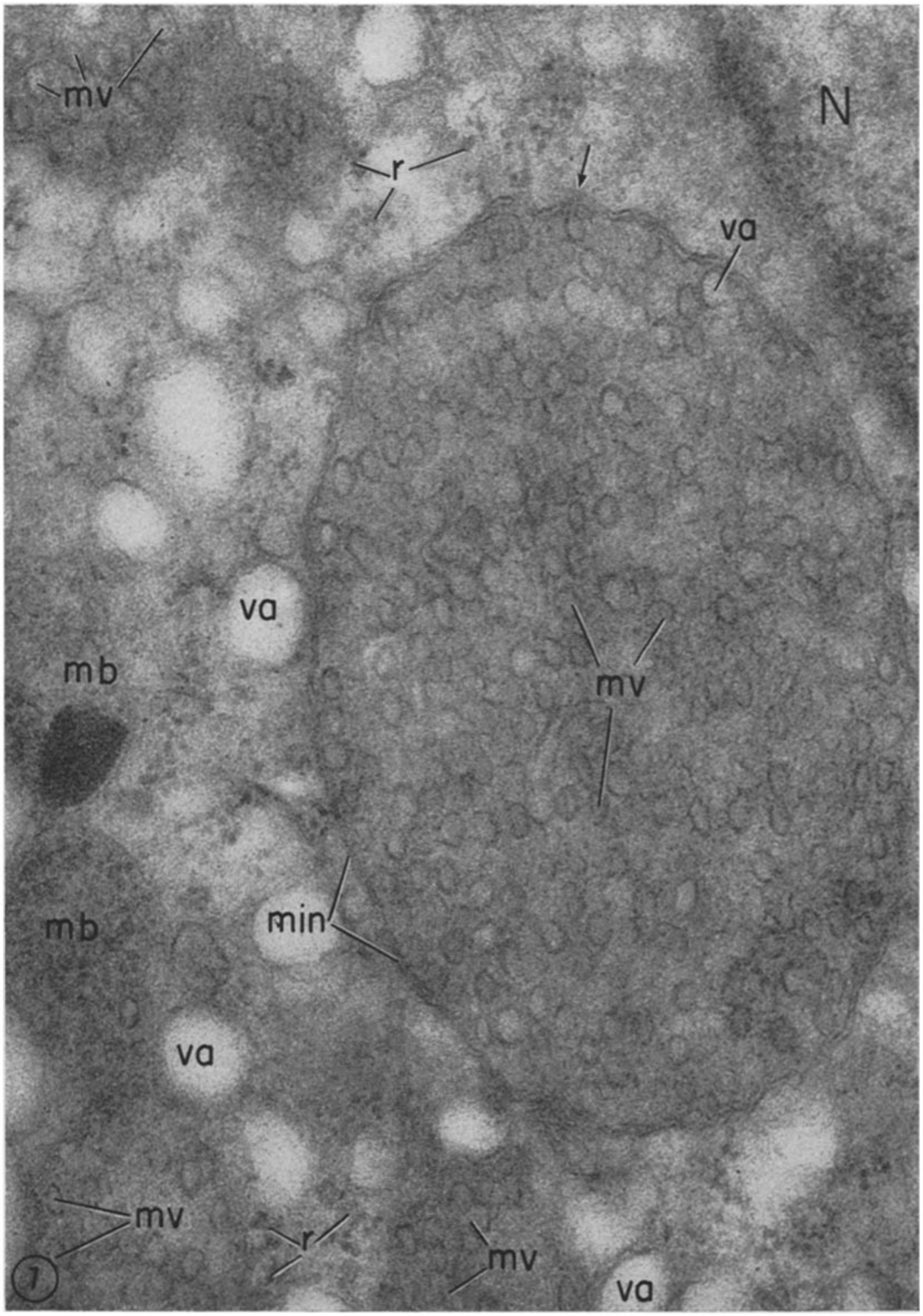
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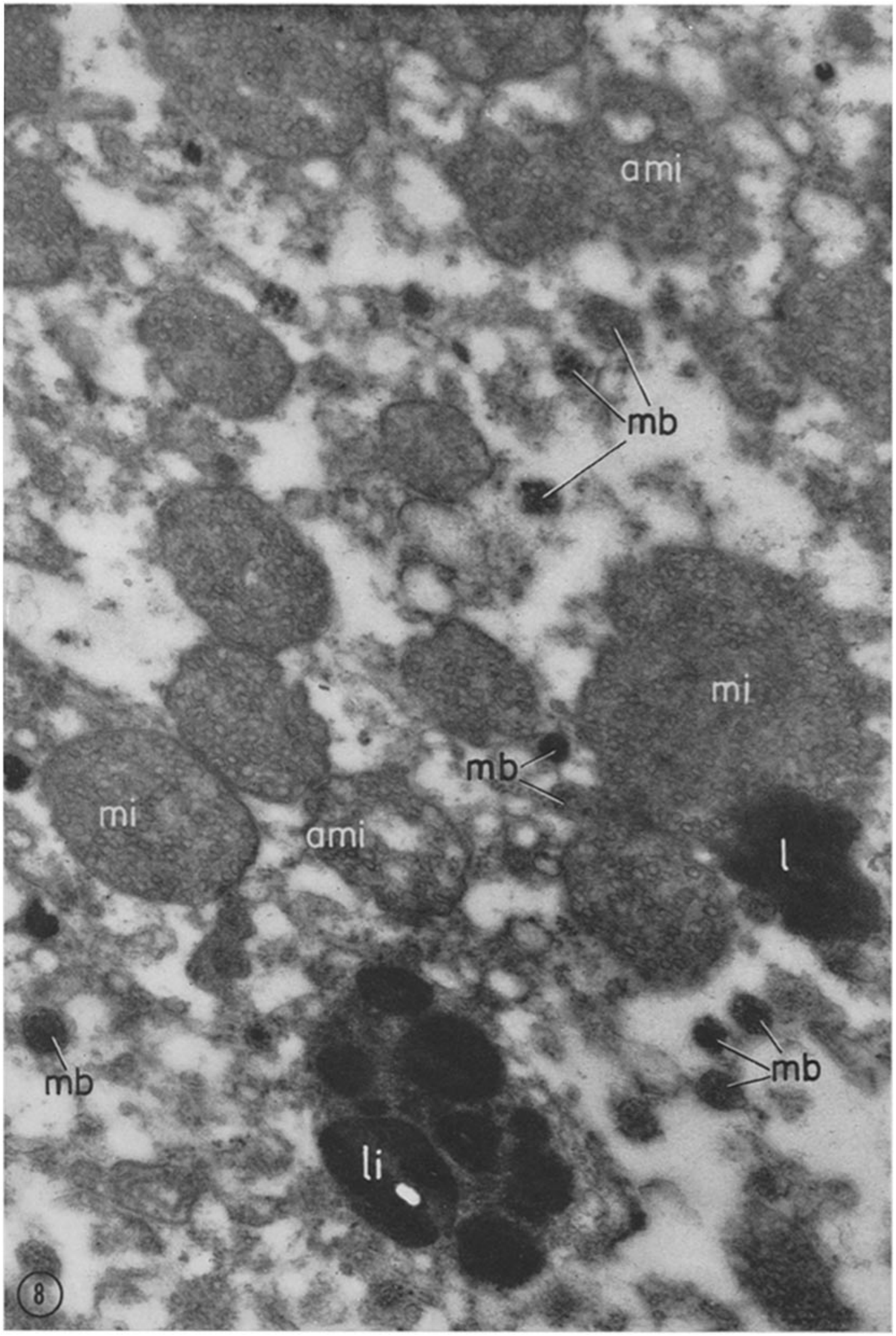
FIGURE 8

Part of cell from the reticularis showing altered mitochondria, lipochrome inclusions (*li*), and numerous dense microbodies (*mb*). The ground cytoplasm is also disorganized. × 44,000.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