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Fine Structure of Adrenal Adenomata Causing Cushing's Syndrome

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Summary. Adenomata taken from nine patients with Cushing's syndrome were observed by electron microscopy. Agranular endoplasmic reticulum was prominently developed in all cases, occasionally fine dotty granules were observed in the tubules of agranular endoplasmic reticulum.

Mitochondria showed a wide variety of changes in size, shape and internal structure, and somtimes contained electron dense droplets in their matrix. Mitochondria resembling those in the normal zona fasciculata intermingled with pathologically altered ones, suggesting that the adenomata which caused Cushing's syndrome were derived from the zona fasciculata.

Fibrous structures were seen in the cytoplasm in one case. It is proposed that all of the changes in cellular organelles of the adenoma cells are correlated with increased secretory activity.

Key words: Cushing's syndrome – Adrenal adenomata.

Introduction

Cushing's syndrome due to adrenal adenoma is no longer an uncommon disease. Its clinical, biochemical and pathological features have been well documented but only several electron microscopic studies have been made. To the best of the authors' knowledge studies dealing with more than five cases of the adenoma have been reported only by Mackay (1969) and Tannenbaum (1973). It is known that ultrastructural features of adenoma cells differ markedly from those in the normal zona fasciculata and zona reticularis, that agranular endoplasmic reticulum may be unusually developed, and that mitochondria undergo striking changes in size, shape and internal structure. Such changes in the organelles in tumor cells are not readily seen with the light microscope, electron microscopy is more useful, although it is not always easy to obtain well-fixed tissues of the adrenal adenomata.

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In the present study the ultrastructural changes of adrenal adenomata which caused Cushing's syndrome are observed and discussed with special reference to organelles (agranular endoplasmic reticulum and mitochondria) involved in steroid biosynthesis.

Materials and Methods

Adrenal tissues for electron microscopy were taken from nine patients during surgery for Cushing's syndrome due to adrenal adenoma. Adenoma was verified histologically in all of them. Data for the patients and tumors are given in Table 1.

The specimens were fixed immediately either in 2% osmium tetroxide buffered with cacodylate (pH 7.4) or in 2.5–3.5% glutaraldehyde buffered with cacodylate or phosphate (pH 7.4), the latter being followed by 2% osmium tetroxide. After dehydration with ethanol the specimens were embedded in Epon epoxy resin (Luft, 1961). Thin sections for electron microscopy were made with glass knives on a Porter-Blum MT-I microtome, and stained with uranyl acetate and lead (Millonig, 1961). Electron micrographs were taken with a Hitachi HS-7s or Hitachi HU-125DS microscope.

Table 1. Data of nine patients with adenoma

Case	No., Age, Sex	Date of operation	Size of tumor (cm)	Weight of tumor (g)	Urinary 17-OHCS mg/day	Plasma cortisol μg/dl
1	M.S., 9 mo, ♀	Feb. 17, 1965	2.0 × 1.4 × 1.3	3.0	4.5	not determined
2	S.M., 12 yr, ♀	Oct. 23, 1967	2.3 × 2.0 × 1.8	5.3	15.4	29.9
3	H.T., 43 yr, ♀	Mar. 27, 1970	4.4 × 3.5 × 2.4	21.0	7.3	8.7
4	S.K., 27 yr, ♀	Sep. 3, 1971	3.7 × 2.7 × 1.6	11.0	12.7	12.6
5	R.H., 46 yr, ♀	Dec. 17, 1971	4.5 × 3.8 × 3.2	26.0	29.1	16.1
6	S.O., 34 yr, ♀	Mar. 31, 1972	4.0 × 3.3 × 3.0	26.0	20.3	18.4
7	M.K., 35 yr, ♀	May 17, 1972	5.0 × 4.8 × 2.0[a]	9.5[a]	20.2	11.8
8	M.O., 29 yr, ♀	Jan. 19, 1973	3.8 × 3.0 × 2.0	10.0	114.0	31.0
9	R.K., 24 yr, ♀	June 6, 1973	4.0 × 3.0 × 3.0	7.0	14.3	13.2

[a] Together with adrenal tissue

Observations

Endoplasmic Reticulum

Agranular endoplasmic reticulum was prominently developed in all cases, showing a mostly tubular pattern. In Cases 1 and 7, however, it showed mainly vesicular profiles. Occasionally one or a few dotty granules, 30–100 Å in size, were observed in the tubules of agranular endoplasmic reticulum (Fig. 1).

Development of granular endoplasmic reticulum varied in amount from case to case and it generally consisted of a parallel unit (Fig. 1, and also Fig. 4). Continuity between the granular and agranular endoplasmic reticulum was found (Fig. 1).

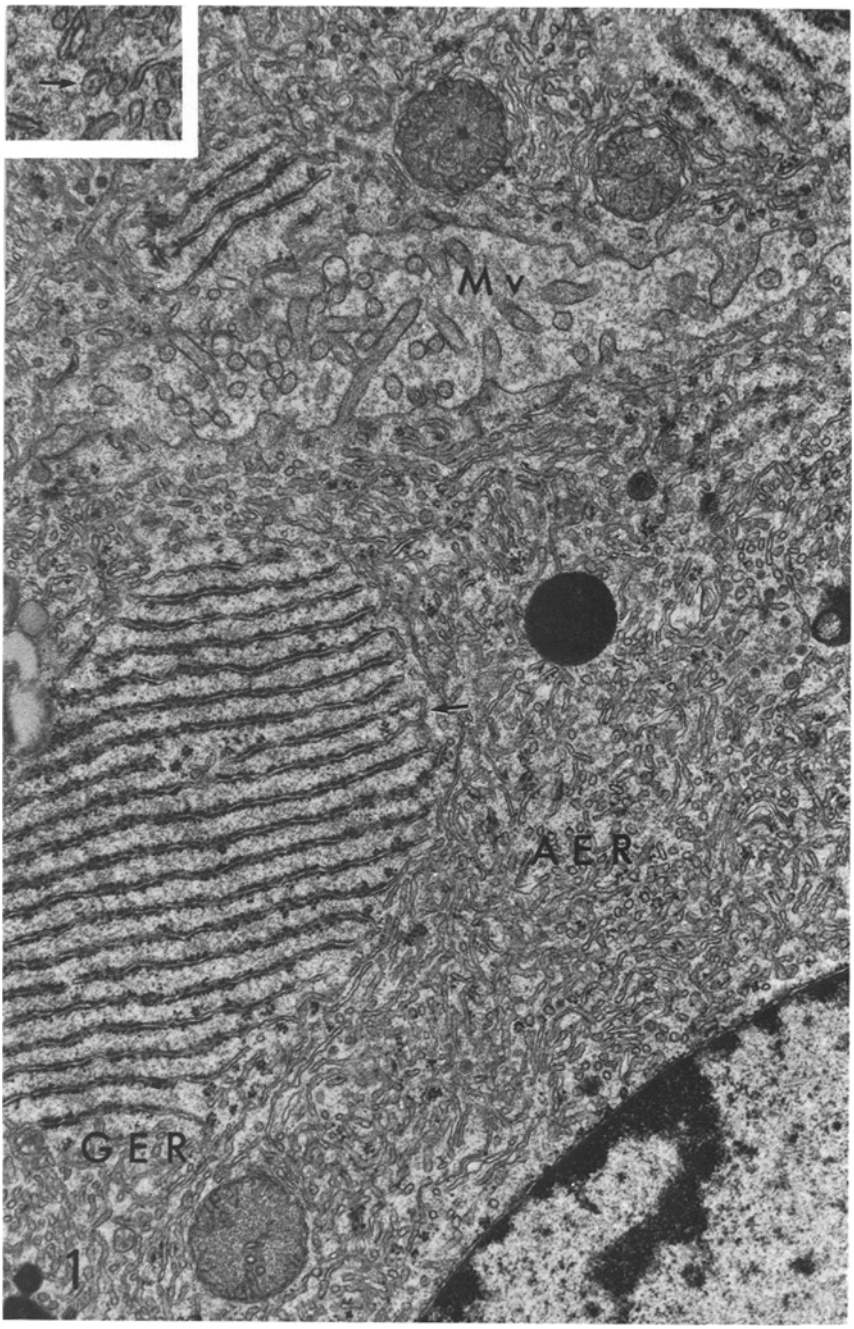

Fig. 1. Parts of two adenoma cells. Granular endoplasmic reticulum (*GER*) is markedly developed showing a stack of more than twenty parallel cisterns. The cytoplasm is filled with tubules of agranular endoplasmic reticulum (*AER*). Continuity between the granular and agranular endoplasmic reticulum is indicated (arrow). The apical cell surface is provided with microvilli (*Mv*). Case 2, ×18,000. Inset: Dotty granules within a tubule of agranular endoplasmic reticulum (arrow)

Mitochondria

The most characteristic feature of the fine structure of adenoma cells was the appearance of the mitochondria. They showed a conspicuous variation in size and shape as well as in arrangement of cristae from cell to cell or even in the same cell.

Large mitochondria, with only a few cristae in the peripheral portion were occasionally encountered. Spherical or oval mitochondria with many lamellar cristae were also observed, this latter type closely resembled the mitochondria of normal zona glomerulosa cells. These two types of mitochondria occurred in the same tumor (Fig. 2).

Mitochondria of elongated rod-like shape, which could not be found in the normal adrenal, were observed in the vicinity of the nucleus, usually making a cluster (Fig. 3). The cristae ran parallel to the longitudinal axis of the mitochondrion in a wavy pattern. Electron dense droplets were occasionally contained in the mitochondrial matrix. Extremely large spherical mitochondria possessing tubular cristae were observed in a few cells, and were characterized by a heterogeneous matrix, consisting of a fine granular area with many tubular cristae and a coarse area without cristae, the former being more electron dense than the latter (Fig. 4). One of the largest mitochondria measured 8μ in diameter. Many electron dense droplets were found in the matrix of large mitochondria with few cristae (Fig. 5).

In Cases 2, 6 and 8 some of the cells were packed with a number of round or oval mitochondria with lamellar cristae. Moreover, in Case 8 mitochondria of this type were intermingled with much larger round ones with few cristae. The matrix of larger mitochondria were speckled with dark inclusions of irregular shapes (Fig. 6). In Case 6 most cells contained large mitochondria with well-developed tubular cristae arranged in parallel and/or concentrically. Some of them had an electron dense droplet of round or irregular shapes measuring about 0.7 μ in diameter in their matrix (Fig. 7).

In all of the cases examined in this study, mitochondria resembling those of the normal zona fasciculata cells were found among pathologically changed mitochondria of various types.

Other Cellular Organelles

Generally the Golgi complex was more developed in adenoma cells than in normal zona fasciculata and zona reticularis cells. In Cases 5 and 6, however, few Golgi complexes were encountered. Lipid droplets were seen in all cases, though they varied in number from cell to cell and from case to case.

Fibrous structures of unknown nature, measuring 1.5–4.5 μ in length and 0.3–1.5 μ in width, were observed in the cytoplasm of Case 6. They were not found in any other cases. These structures ran in parallel with or rarely perpendicular to the cell membrane, and were partially attached to it (Fig. 8). In the vicinity of the fibrous structures the intercellular space was dilated and was filled with an amorphous substance of moderate electron opacity which did not occur elsewhere (Fig. 8).

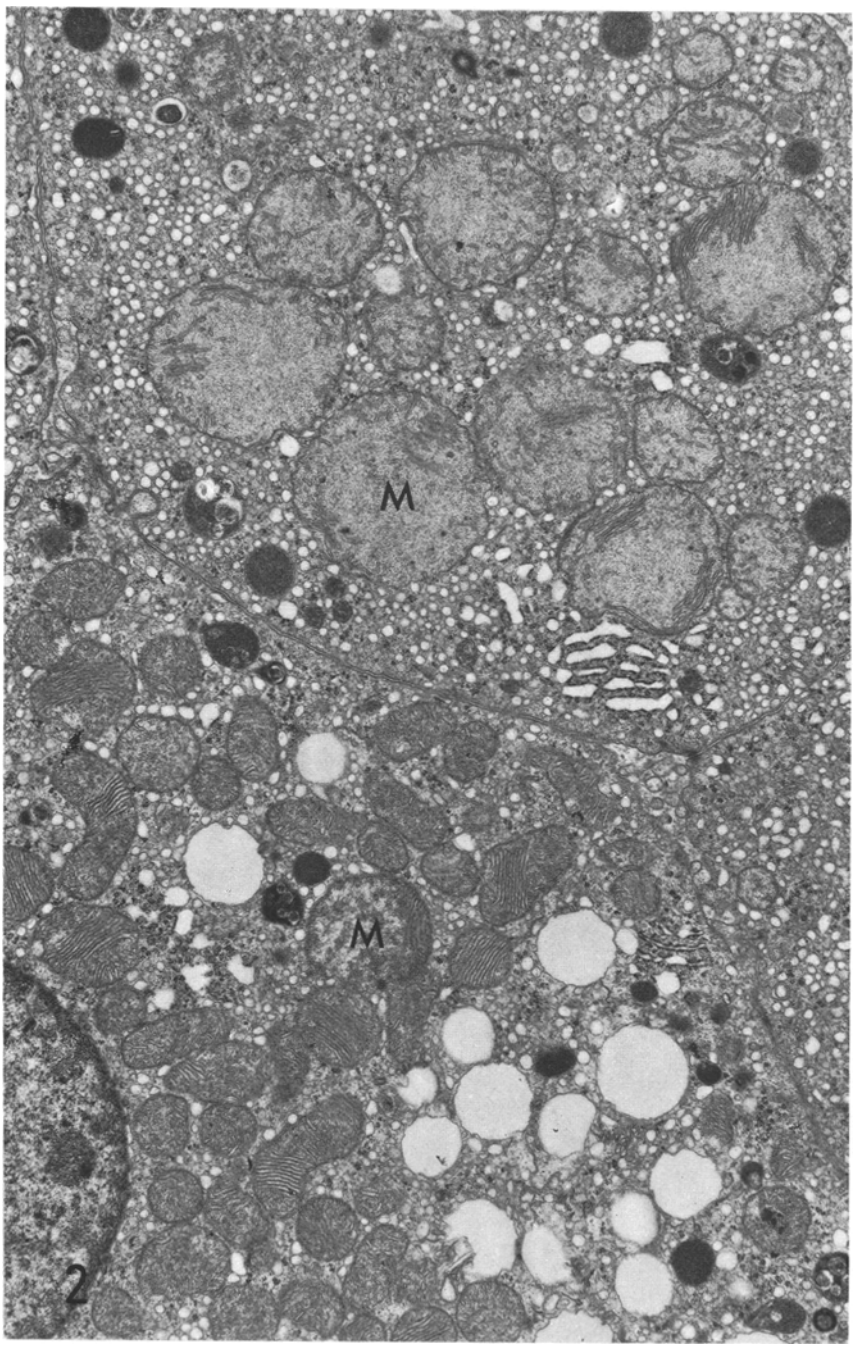

Fig. 2. Four adenoma cells. Large, round mitochondria (*M*) with a few cristae in their peripheral portion are seen in the upper cell, whereas spherical or oval mitochondria with lamellar cristae are observed in the lower cell. Case 1, ×8,400

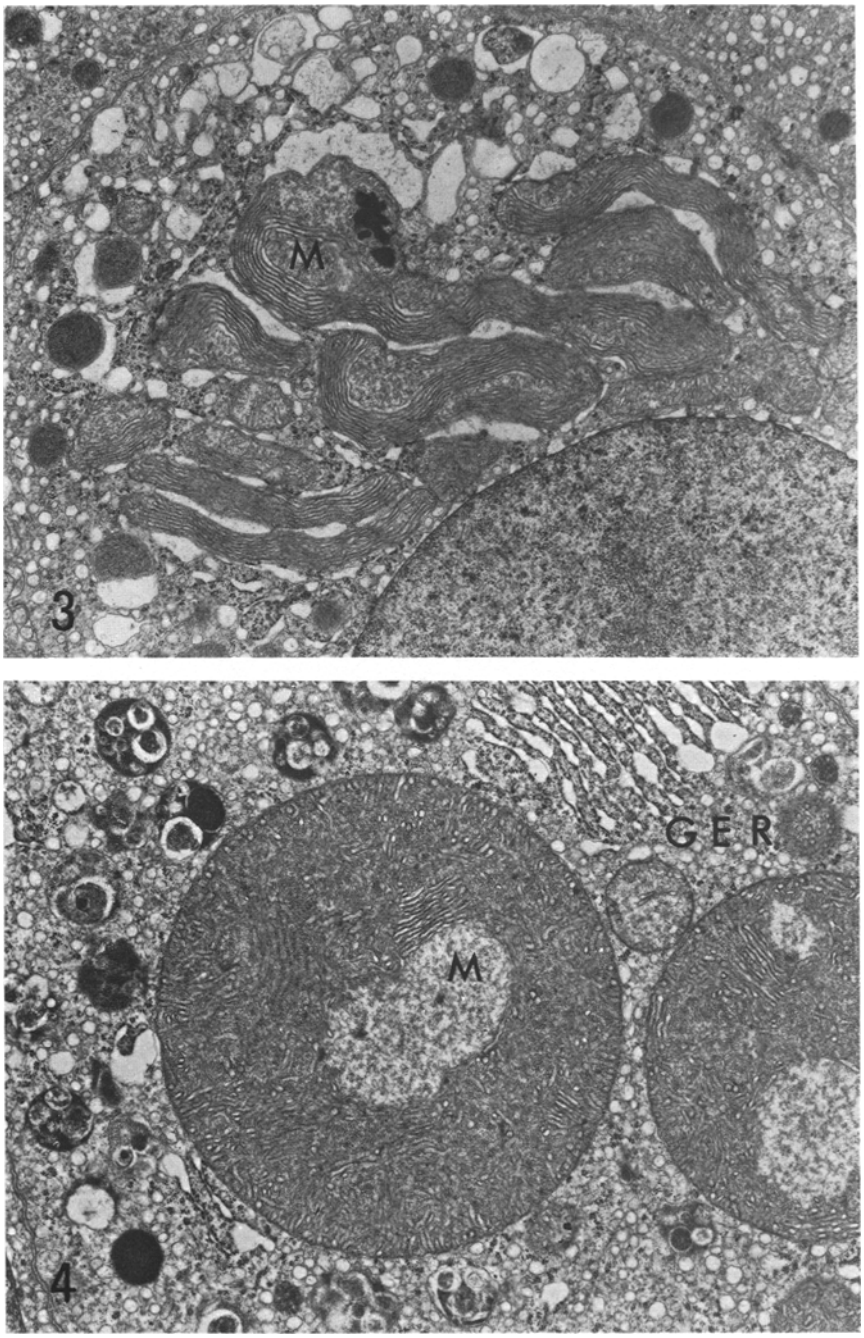

Fig. 3. A stack of mitochondria of rod-like shape (*M*) near the nucleus. The cristae run wavy and parallel to the longitudinal axis of mitochondria. Electron dense droplets are seen in the matrix of mitochondria (*M*). Case 1, ×9,500

Fig. 4. Large mitochondria (*M*) with tubular cristae. The peripheral part of the matrix with many tubular cristae is denser in electron opacity as compared with the central part. Case 1, ×11,000

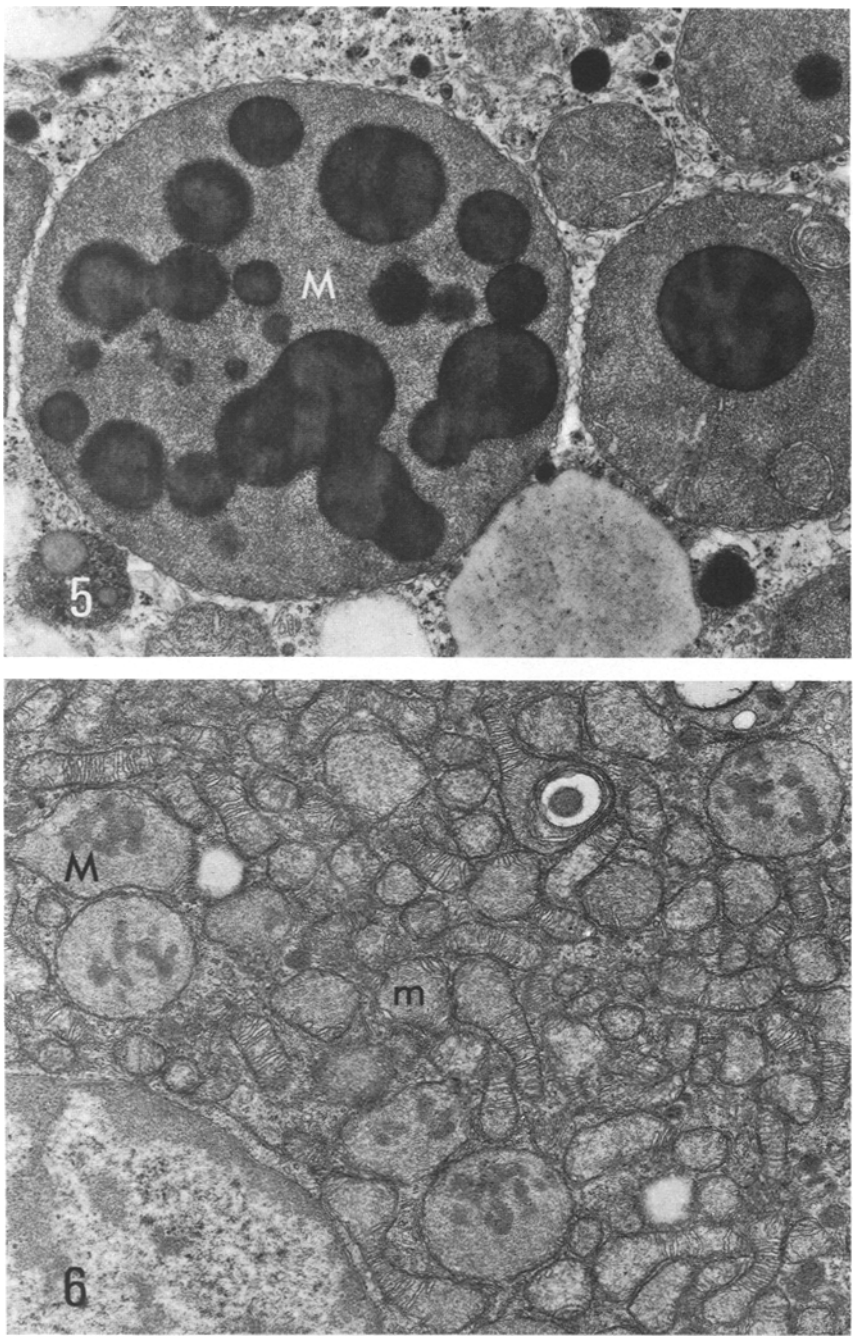

Fig. 5. Large spherical mitochondria with few cristae containing electron dense droplets. Case 3, ×23,000

Fig. 6. Part of an adenoma cell where the cytoplasm is packed with mitochondria. The larger ones with few cristae (*M*) are speckled with inclusions. Case 8, ×23,000

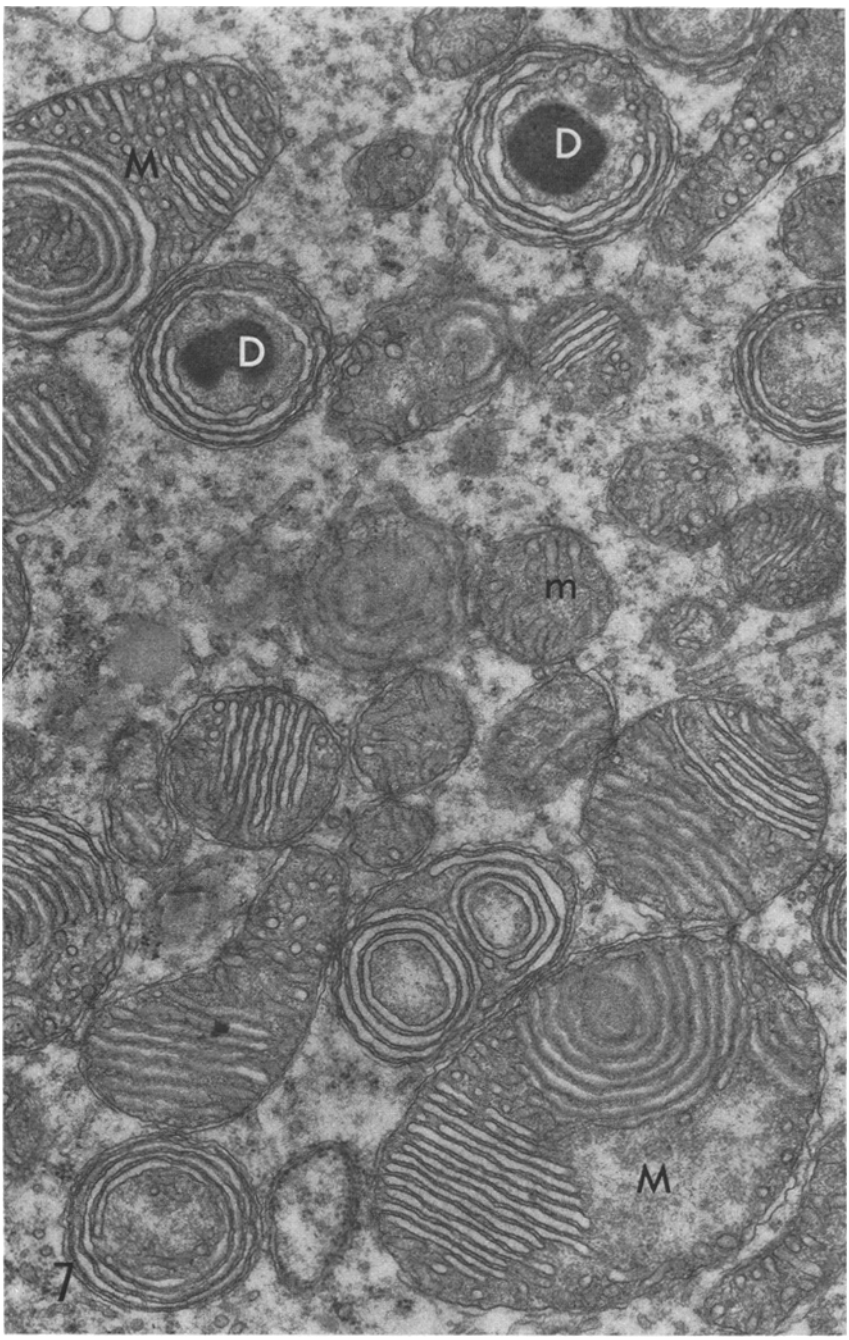

Fig. 7. Large mitochondria (*M*) with parallel and/or concentrically arranged cristae of tubular type. Some of them have an electron dense droplet (*D*) in the matrix. Mitochondria of normal size (*m*) as found in the normal zona fasciculata are intermingled with large ones. Case 6, ×18,000

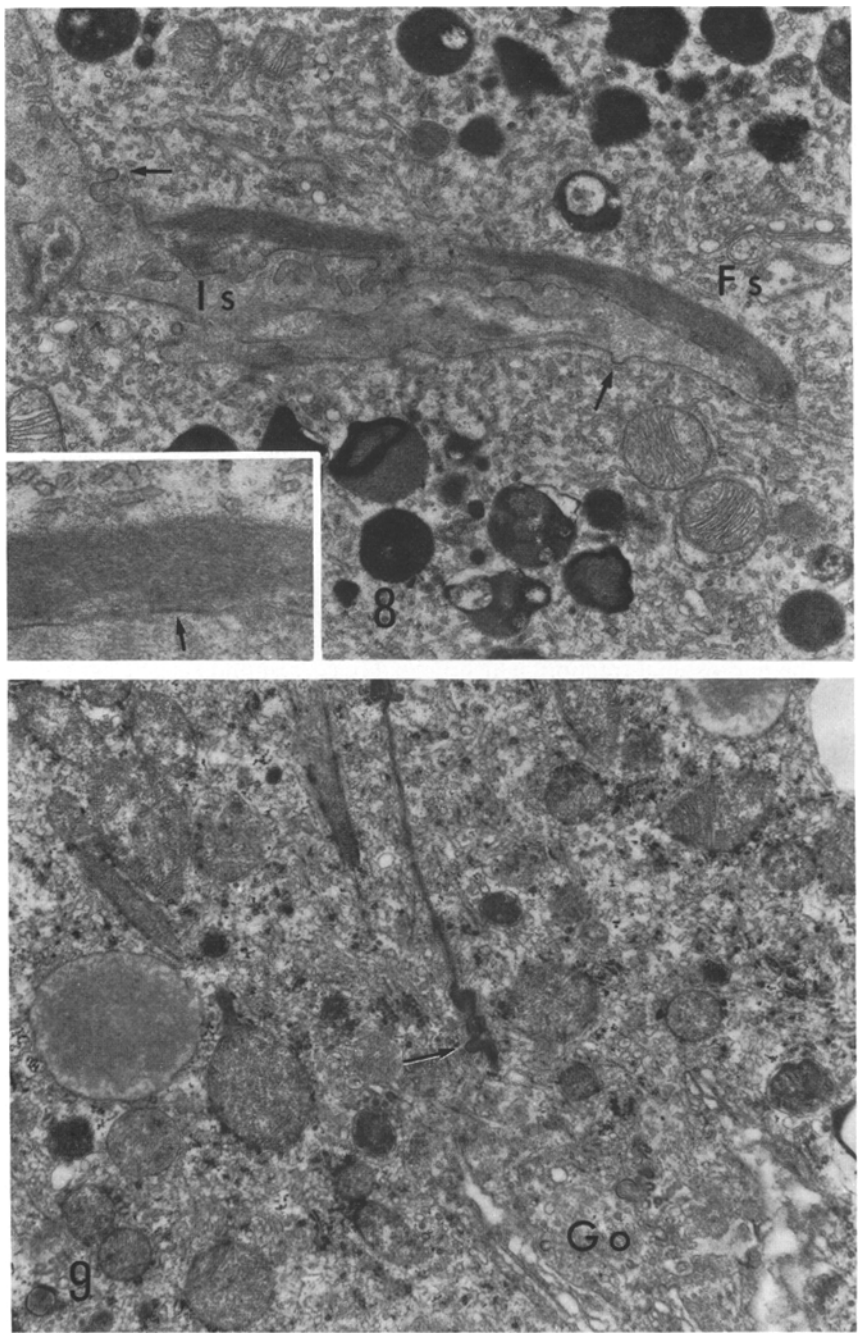

Fig. 8. Fibrous structures (*Fs*) running in parallel with the cell membrane. In the vicinity of these structures the intercellular space (*Is*) is dilated and filled with an amorphous substance. Note also numerous lysosomes and coated vesicles (arrow). Cases 6, × 14,000. Inset: The fibrous structure partially attached to the cell membrane (arrow)

Fig. 9. Infolding of the cell membrane and Golgi complex (*Go*). Numerous coated vesicles are seen in the Golgi complex. The end of infolding is attached by a coated vesicle (arrow). Case 4, × 11,000

In Case 4, a deep invagination of the cell membrane occasionally reached to near the Golgi complex (Fig. 9), but its possible continuity with the Golgi complex was not assessed. One of these infoldings of the cell membrane was attached terminally by a coated vesicle. These vesicles occurred frequently near the Golgi complex and are thought to be involved in the GERL system (Novikoff et al., 1971).

Discussion

It is widely accepted that well-developed agranular endoplasmic reticulum characterizes steroid-secreting cells such as interstitial cells of the testicle (Christensen et al., 1961; Christensen, 1965), corpus luteum cells of the ovary (Enders, 1962; Yamada et al., 1960) and cortical cells of the adrenal (Brenner, 1966; Long et al., 1967). Christensen and Fawcett (1961) paid attention to the amount of agranular endoplasmic reticulum in testicular interstitial cells of the opossum and subsequently Christensen (1965) suggested that membranes of this organelle are the site of enzymes involved in androgen biosynthesis. It is known that several enzymes in the synthetic pathway from cholesterol to cortisol are contained in the microsomal fraction, which probably corresponds to the agranular endoplasmic reticulum. A large accumulation of agranular endoplasmic reticulum has been observed in hyperfunctional states of the adrenal cortex such as Cushing's syndrome, a finding confirmed in this study.

Fine dotty granules in the tubules of the agranular endoplasmic reticulum were observed in some well-fixed specimens in the present study. Long and Jones (1967) recognized dense particles (500 Å in diameter) in the zona fasciculata cells of the opossum, without knowing their chemical nature. These particles may correspond to the fine dotty granules in this study, though they differ in size. Their situation suggests a possible correlation with the biosynthesis of steroids.

The morphological changes in mitochondria in the cortical cells of the adrenal are related to the secretory activity of steroid hormones (Luse, 1967; Nishikawa et al., 1963; Yates, 1965; Mitschke et al., 1973). A variety of changes in shape and internal mitochondrial structure has been recorded in cases of Cushing's syndrome due to both adenoma and carcinoma (Mackay, 1969; Mitschke et al., 1973; Tannenbaum, 1973; Thiele, 1974; Bahu et al., 1974). In the present study the size, shape and internal structure varied from case to case, but mitochondria of rod-like shape (Fig. 3), large mitochondria with tubular cristae (Fig. 4) and giant mitochondria with electron dense droplets (Fig. 5) have not previously been described.

Elongated, rod-like mitochondria (Case 1) resembled the long and thin mitochondria with one to three rows of vesicles reported by Sharawy and Peney (1973), who found them in zona fasciculata cells of hypophysectomized rats. Electron dense droplets in mitochondria were frequently observed in the adenoma cells. They had a tendency to appear in larger or giant mitochondria with few cristae. Several articles have dealt with similar intramitochondrial droplets and some investigators have regarded them as a lipid accumulation

(Lever, 1955). Giacomelli et al. (1965) suggested that intramitochondrial electron dense droplets in the zona glomerulosa cells of the rat might represent aldosterone and its intermediates. We speculate that the intramitochondrial droplets may present a stage in the production of steroid hormones, since mitochondria contain enzymes such as pregnenolone synthetase and 11β-hydroxylase.

Spherical mitochondria with tubular cristae found in the normal zona fasciculata, were also found in variable numbers in adenomata intermingling with pathologically altered mitochondria (Figs. 3, 4 and 6). It therefore seems likely that the adenomata which caused Cushing's syndrome are derived from the zona fasciculata.

With regard to cytoplasmic filaments, Nickerson et al. (1970) reported their occurrence in the zona fasciculata and zona reticularis cells of androgen treated rats. According to their report the filaments either completely filled the cytoplasm, or several bundles of the filaments were seen to extend through it. Macadam (1970) described the cytoplasmic inclusions which consisted of the bundle of the microtubules within an enclosing unit membrane in the cells of the adrenal adenoma causing Cushing's syndrome. The fibrous structures which were observed in Case 6 of this study ran in parallel with the cell membrane as a rule without showing single clear-cut fibers of filaments. They differed from those of Nickerson et al. and Macadam and always lay close to the cell membrane and the neighboring intercellular space was filled with moderately electron dense substance. The structure corresponding to the GERL (Golgi-associated ER from which lysosomes are thought to arise) of Novikoff was found in Case 4. It was always observed in close vicinity to the Golgi complex, and was differentiated from the latter by the presence of acid phosphatase activity (Novikoff et al., 1971). This structure in the adenoma cells is supposed to relate to secretory activities.

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