Ultrastructural Study of Human Adrenal Cortical Hyperplasia in Cushing's Syndrome.

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INTRODUCTION

Correlations between the functional states and morphological changes in the animal adrenal cortex have been demonstrated in electron microscopic examination by some investigators[1,2,3,4,5]. However, as far as the authors know, only a few reports have appeared concerning the ultrastructure of the human adrenal gland in normal or pathologic conditions[6,7,8,9].

We had an oppotunity to examine the fine structure of the hyperplastic adrenal cortex from two patients with the classical clinical and laboratory features of Cushing's syndrome. Vacuoles were observed in the mitochondria of the cells in the zona fasciculata. Their nature and origin are briefly discussed.

MATERIALS AND METHODS

Case 1: The patient, a 36-year-old female, had suffered from hypertension, obesity and diabetes mellitus for six years. Physical examination showed typical manifestations of Cushing's syndrome such as moon face, red striae and buffalo hump. Daily urinary excretion of 17-OHCS was 16.8 mg and other endocrinological examinations were also diagnostic of Cushing's syndrome due to adrenocortical hyperplasia. A subtotal adrenalectomy was done. The left removed adrenal gland weighed 10.5 g and the right 12.5 g

Case 2: The patient, a 28-year-old female, was hospitalized because of obesity and amenorrhea. She had typical clinical manifestations of Cushing's syndrome such as moon face, buffalo hump, distended abdomen, purple striae and hypertention. A subtotal adrenalectomy revealed the left adrenal gland to be 6.3 g in weight and the right 7.4 g (Fig. 1).

Light microscopy: Sections of paraffin embedded tissue were stained with hematoxylin and eosin. Formalin fixed preparations were demonstrated with Sudan III, Sudan IV, Sudan black and Niel blue sulphate stains.

Electron microscopy: Small tissue blocks were fixed in 4.15% glutaraldehyde and 1% osmium tetroxide, dehydrated in graded alcohol and embedded in epoxy resin according to the method of Luft[10]. Sections cut with an Ivan Sorvall IIb ultramicrotome were stained with uranyl acetate and lead citrate for examination with a Hitachi HS-8 electron microscope.

OBSERVATION

Light microscopic examination: The cells in the zona fasciculata were markedly increased in size and number showing diffuse adrenal cortical hyperplasia in both cases (Fig. 2). In preparations with various fat stains, numerous lipid droplets were noted.

Electron microscopic examination: In both cases, dark cells and clear cells were increased in number in the zona fasciculata (Fig. 3). Various changes of the cytoplasmic inclusion and organelles were noted. Lipid droplets were numerous and varied in electron density, but most were electron lucent (Fig. 4). Mitochondria were increased in number and were enlarged. They were closely packed in numerous areas, and generally occupied a greater amount of the cytoplasm. The mitochondria varied in electron density. They were filled strikingly with vesiculated membranes. Intramitochondrial granules were also noted. Giant mitochondria were frequently seen. The central portion of such giant mitochondria were occasionally occupied by an amorphous material with resultant loss of central cristal elements. In some mitochondria the cristae appeared in a parallel stack or lamelliform arrangement in the peripheral portion. In giant mitochondria, vacuoles with a variety of sizes were noted. One had only a single vacuole (Fig. 5, 6) while another had several vacuoles (Fig. 7). Vacuoles were almost electron lucent. Some vacuoles abuted directly upon the outer membrane of the mitochondria (Fig. 6, 7). In addition, peculiar inclusions in the mitochondria which we have already reported were noted in case 1[11].

Smooth surfaced endoplasmic reticulum (sER) was increased in some cells (Fig. 8). The amount of sER appeared to vary inversely with the number of mitochondria and lipid droplets within the cytoplasm. Rough surfaced endoplasmic reticulum (rER) was increased in some cells (Fig. 8). The Golgi complex were well developed in some cells.

DISCUSSION

The clinical and laboratory findings of two patients were characteristic of Cushing's syndrome. The effectiveness of the subtotal adrenalectomies performed in both cases might be indicated by a decrease in excessive production of steroid hormone in the adrenal gland. Pathologic examination revealed diffuse adrenal cortical hyperplasia in both cases. Ultrastructural changes were characterized by an increase in lipid droplets, a large number of mitochondria, vacuoles in some mitochondria and the increase of sER in some cells.

Ashworth and Garvey[12] reported that the adrenal glands removed from 4 patients with Cushing's disease exhibited a zona of large, clear-staining cells in the outer portion of the zona fasciculata on light microscopic examination. Luse[5] described the ultrastructure of an adrenal adenoma in a patient with Cushing's syndrome. Reidbord and Fisher[8] reported the light and electron microscopic findings of the adrenal cortex from a 46-year-old man with classical laboratory features of Cushing's syndrome. They described characteristic ultrasructural changes such as increased mitochondria, mitochondrial swelling and increased lipid droplets. Light and electron microscopic examination of adrenal cortices from 4 cases with classical laboratory and clinical characteristics of Cushing's syndrome were also described by Mitschke et al[9], who revealed that the ultrastructural alterations were characterized by swelling of the very numerous mitochondria with a rather empty appearance, by prominent agranular endoplasmic reticulum with various degrees of dilatation and by reduced lipid vacuoles.

Although changes of mitochondria, lipid and sER were characteristic of Cushing's syndrome in our cases, the mitochondrial changes in our cases seemed to be more prominent than those reported by several authors[5,8,9]. The most prominent change was the manifestation of vacuoles in mitochondria which showed some variety in number. One had only a single vacuole, while another had several vacuoles which were located in the central portion and peripheral portion, and abuted on the inner and outer membrane of the mitochondria. Furthermore, some vacuoles were adjacent to the intracytoplasmic lipid.

Mitschke et al.[9] have suggested that there is a close structural relationship between lipid vacuoles and mitochondria with fusion or discontinuity of membranes on some occasions. Marek et al.[13] have reported that the ultrastructural findings of intramitochondrial lipid accumulation correlates well with the biochemical data concerning the intracellular

localization of corticosteroid biosynthesis and have supported the view that 5-pregnenolone synthesis takes place within the mitochondria and not on their surface.

To the contrary, Christensen[3] has suggested that the amount of sER in different species is directly proportional to the amount of precursor cholesterol synthesized in the cell. Furthermore, it has been suggested that the synthesized precursor cholesterol may be held in store within the structure of the sER membranes[4]. Long and Jones[7] also have suggested that the amount of sER might be related to the type of hormone synthesized and its rate of production.

In our cases, morphological changes of mitochondria were more prominent than sER. Our ultrastructural findings of the human intramitochondrial vacuoles support the view that synthesis of steroids takes place within the mitochondria. Furthermore, as was reported previously[11], peculiar structures were observed in the mitochondria and was suggested that they might be derived from the deposition of certain substances, probably lipids or their related compound, in the mitochondria due to the altered synthesis of steroids in Cushing's syndrome. Sabantini and Robertis[1] suggest that the vacuoles may be derived from the tubovesicular cristae.

In conculusion, the intramitochondrial vacuoles described in this paper might be associated with the deposition of steroids or their related compounds in the tubovesicular cristae.

SUMMARY

Light and electron microscopic changes of hyperplastic adrenal cortices from two patients with Cushing's syndrome were reported. The ultrastructural findings were characterized by the increase of lipid inclusions, a large number of mitochondria, vacuoles in mitochondria and also the increase of sER in some cells. Observations of the intramitochondrial vacuoles support the view that steriod synthesis may take place within the mitochondria, but their biological significance still remains to be elucidated.

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EXPLANATION OF FIGURES

Fig. 1. Slightly, though diffusely enlarged adrenal cortex. (Case 2)

Fig. 2. Increase of pale-staining cells in zona fasciculata. (Case 1) H. E. stain.

Fig. 3. Clear and dark cells in the hyperplastic adrenal cortex. (Case 2) × 18,000

Fig. 4. Numerous lipid inclusions are noted. (Case 2) × 7,000

Fig. 5. Single vacuole in the giant mitochondrion. (Case 1) × 13,000

Fig. 6. Single vacuole abuted directly upon the outer membrane of the mitochondrion. (Case1) × 16,000

Fig. 7. Many vacuoles with varied sized in a giant mitochondrion. (Case 1) × 16,000

Fig. 8. Well-developed sER and rER. (Case 1) × 29,000

Fig. 1.

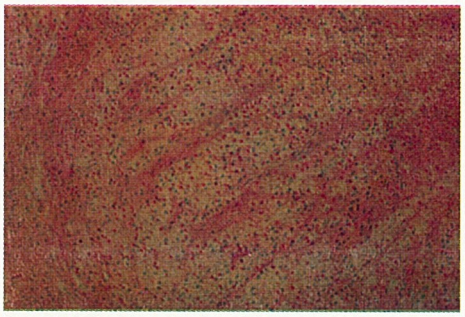

Fig. 2

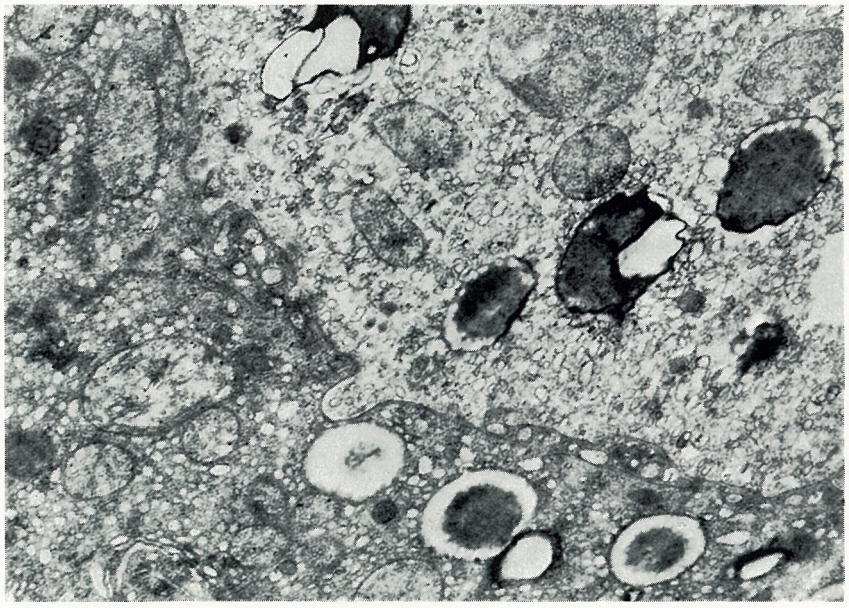

Fig. 3.

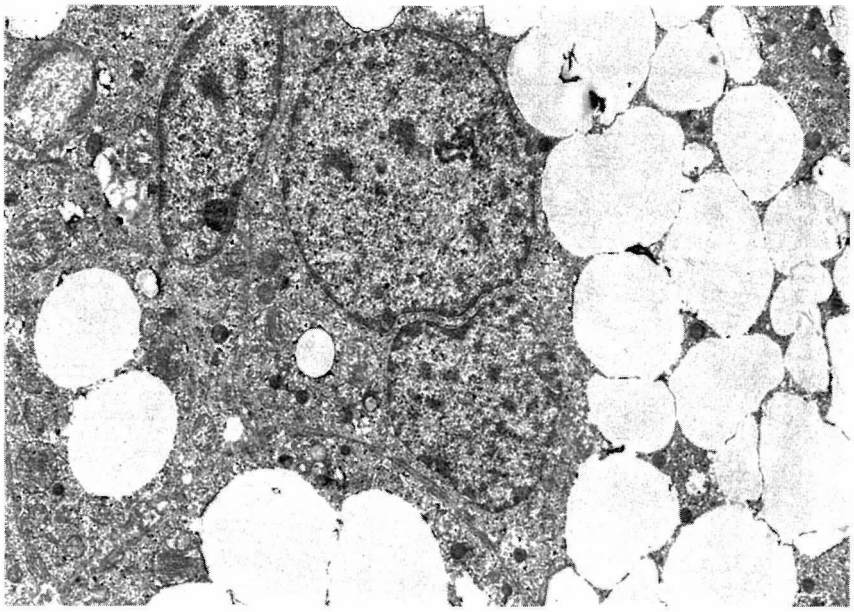

Fig. 4.

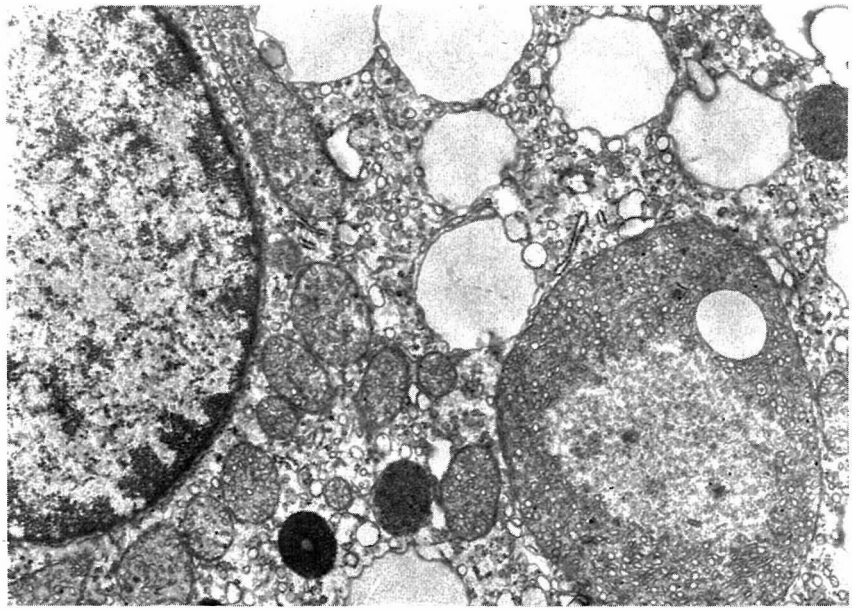

Fig. 5.

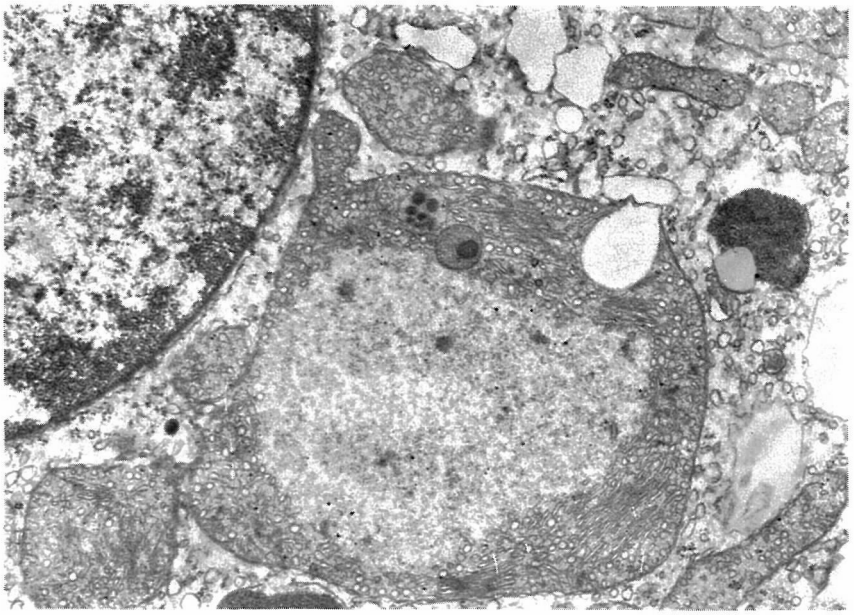

Fig. 6.

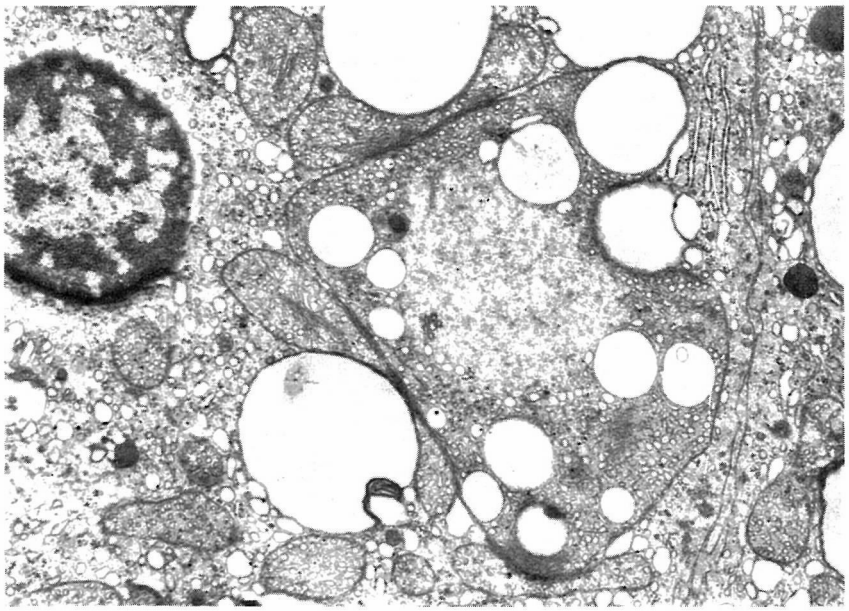

Fig. 7.

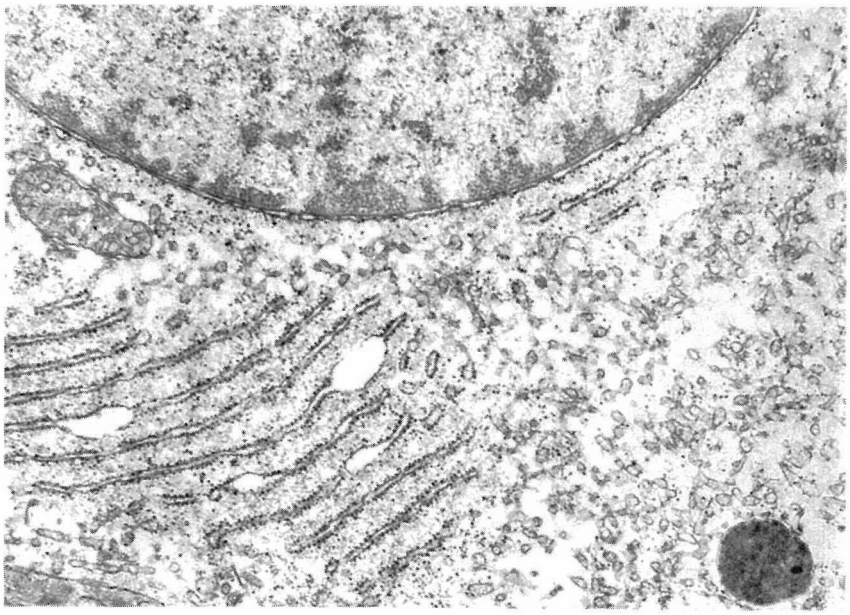

Fig. 8.