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Androgen Producing Adrenocortical Carcinoma

A Histological and Ultrastructural Study of Two Cases

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Summary. Two cases of androgen secreting adrenocortical carcinoma have been described by light and electron microscopy. The histological and ultrastructural features of the tumour cells were similar to those of compact cells of zona reticularis and to those described in virilizing adenomas. They possess numerous mitochondria with lamellar and tubular cristae, abundant smooth endoplasmic reticulum, lipofuscin bodies and scanty lipid. Irregularly shaped, crenated mitochondria, with outpouchings of the outer limiting membrane have also been observed. The clusters of neoplastic cells were surrounded by basement membrane which demonstrated a focal discontinuity, probably reflecting malignancy of the tumours. Hyperplasia of smooth endoplasmic reticulum and the presence of outpouchings of the mitochondrial outer limiting membrane might be the morphological manifestation of endocrine activity of the tumours.

Key words: Adrenal cortex hormones – Endocrinology – Microscopy, Electron – Neoplasms – Virilism.

Introduction

Adrenocortical carcinomas are relatively rare neoplasms (Hutter and Kayhoe, 1966) which are of particular interest. Although malignant, they may exhibit endocrine activity, thus producing specific clinical features. Huvos et al. (1970) reported 34 cases of adrenocortical carcinoma, among which 18 were endocrinologically active. A similar incidence was observed by Hajaar et al. (1975) who, in a study based on 32 malignant cases, described Cushing's syndrome in 7 patients, Cushing's syndrome and virilization in 4, and virilization alone in other 4.

Although the ultrastructural features of carcinomas producing Cushing's syndrome have been described (Mitschke et al., 1973; Tannenbaum, 1973; Thiele, 1974), no ultramicroscopic investigation of androgen secreting malignant tumours has been published. However the ultrastructure of two cases of andro-

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gen secreting adenoma has been reported (Fisher and Danowski, 1973; Akhtar et al., 1974).

We report here clinical, histological and ultrastructural data from two patients with adrenocortical carcinomata causing virilization.

Case Reports

Case 1

A 2.5 year old boy was admitted to the University Hospital with the somatic picture of the adrenogenital syndrome. The testes were normal but the penis was precociously developed and pubic hair was present. Muscular hypertrophy was also observed, and the voice was deep and coarse. Routine laboratory studies gave normal results, but urinary 17-Ketosteroid values were 15 mg per day (normal values up to 2 mg); 17-OH corticosteroid values were normal; dexamethasone administration did not produce any change. A retroperitoneal pneumogram showed an enlarged right adrenal gland covering the renal shadow in part. The diagnosis of adrenocortical tumour with androgen secretion was made. The child underwent surgical exploration and right adrenalectomy was performed. The post-operative course was uneventful and 17-Ketosteroids fell to normal values. Five months after the operation the patient was readmitted to the hospital because of progressive liver enlargement. Surgical exploration disclosed multiple hepatic metastases and a biopsy was taken. The patient died one month later; autopsy could not be carried out.

Case 2

A 13 year old prepubertal girl was admitted to the University Hospital with signs of virilization. She had facial acne and growth of facial, axillary and pubic hair with a male distribution. The voice was deep. The breasts were not developed and the external genitalia were hypoplastic. Routine laboratory findings were in the normal range. X rays of the hands showed ossification of the epiphyseal plates. The 17-Ketosteroid value was 24.8 mg per day (normal values up to 12 mg) and there was no change after dexamethasone administration; 17-OHcorticosteroid values were normal. Gas chromatography revealed a pronounced increase in dehydroxyepiandrosterone. A retroperitoneal pneumogram showed a large mass above the right kidney shadow. The diagnosis of androgen secreting adrenocortical tumour was made and the girl underwent surgery. Right adrenalectomy was performed. The patient had a normal post-operative course and was discharged with normal 17-Ketosteroid values. Unfortunately we were not able to follow up this case.

Material and Methods

Portions of surgically removed adrenal tumours of both patients and a biopsy of a hepatic metastasis in Case I, were fixed in formalin and embedded in paraffin. 5–7 micron thick sections were stained with hematoxylin and eosin and PAS for histological examination.

For the ultrastructural study, fragments of both tumours were immediately fixed in 4% phosphate-buffered glutaraldehyde (pH 7.2), post-fixed in osmium tetroxide and embedded in Epon. Semithin sections (0.5–1 micron) were stained with toluidine blue and observed at the light microscopy. Ultrathin sections (600–700 Å) of selected areas were stained with uranyl acetate and lead citrate for electron microscopic observation.

Results

Gross Findings

The two adrenal neoplasms had similar macroscopic features, appearing as brownish encapsulated irregular masses, about 4–5 cm in diameter. Small yellow

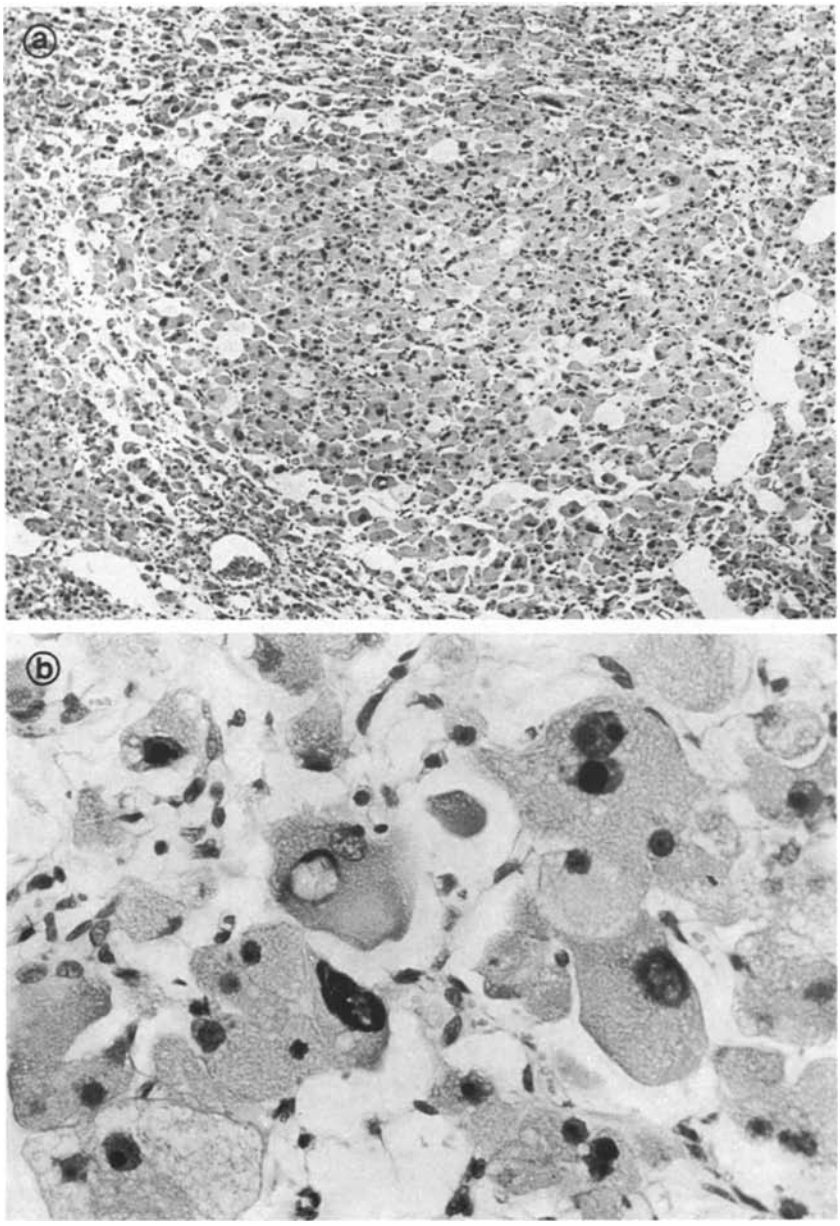

Fig. 1 a and b. Case 1. Histological appearance of adrenocortical tumour, mainly consisting of cords of compact cells (**a**), with finely granular eosinophilic cytoplasm and nuclear pleomorphism. (**b**) H.E. Stain, ×56 (**a**), ×350 (**b**)

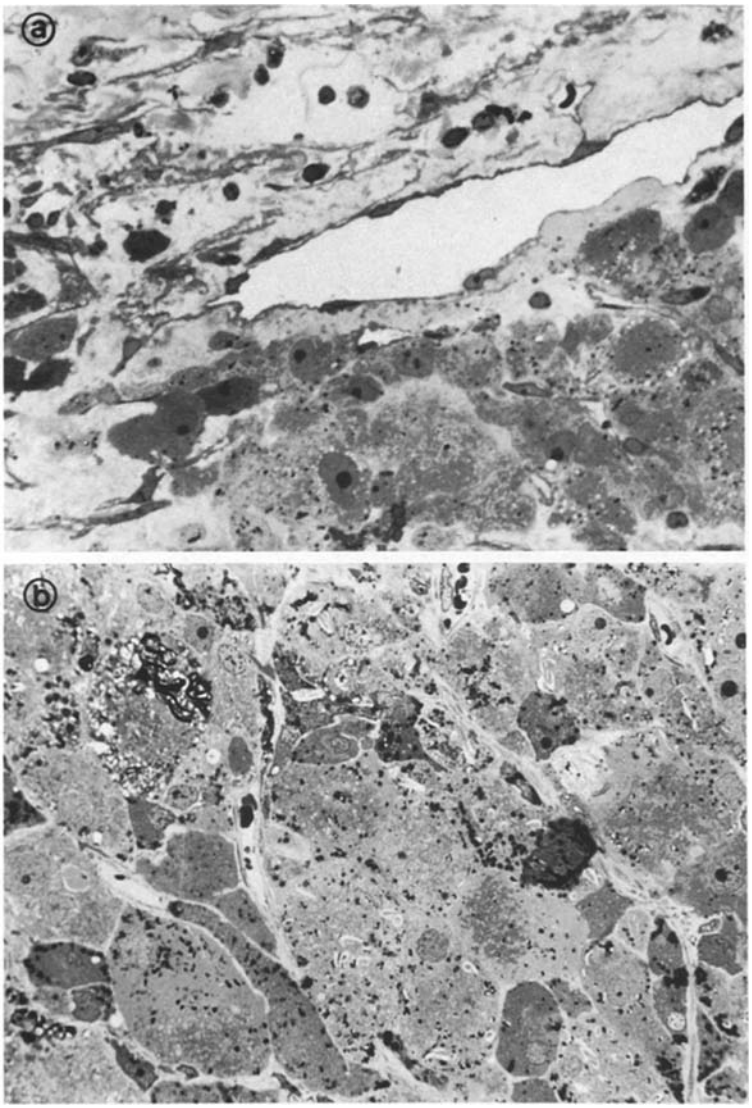

Fig. 2 a and b. Case 1. Semithin sections: **a** The neoplastic cells infiltrate the capsular connective tissue; **b** The cells are arranged in clusters surrounded by basement membrane. Note the presence of mitosis and osmiophilic inclusions. Toluidine blue, **a** ×480, **b** ×600

zones and hemorrhagic areas were seen on the cut surfaces. Residual adrenal parenchyma was compressed and capsular infiltration was suspected.

Histological Findings

Paraffin Sections (Fig. 1). Both tumours consisted of cords of epithelial cells, at times tightly packed, with intervening sinusoidal spaces. There was focal

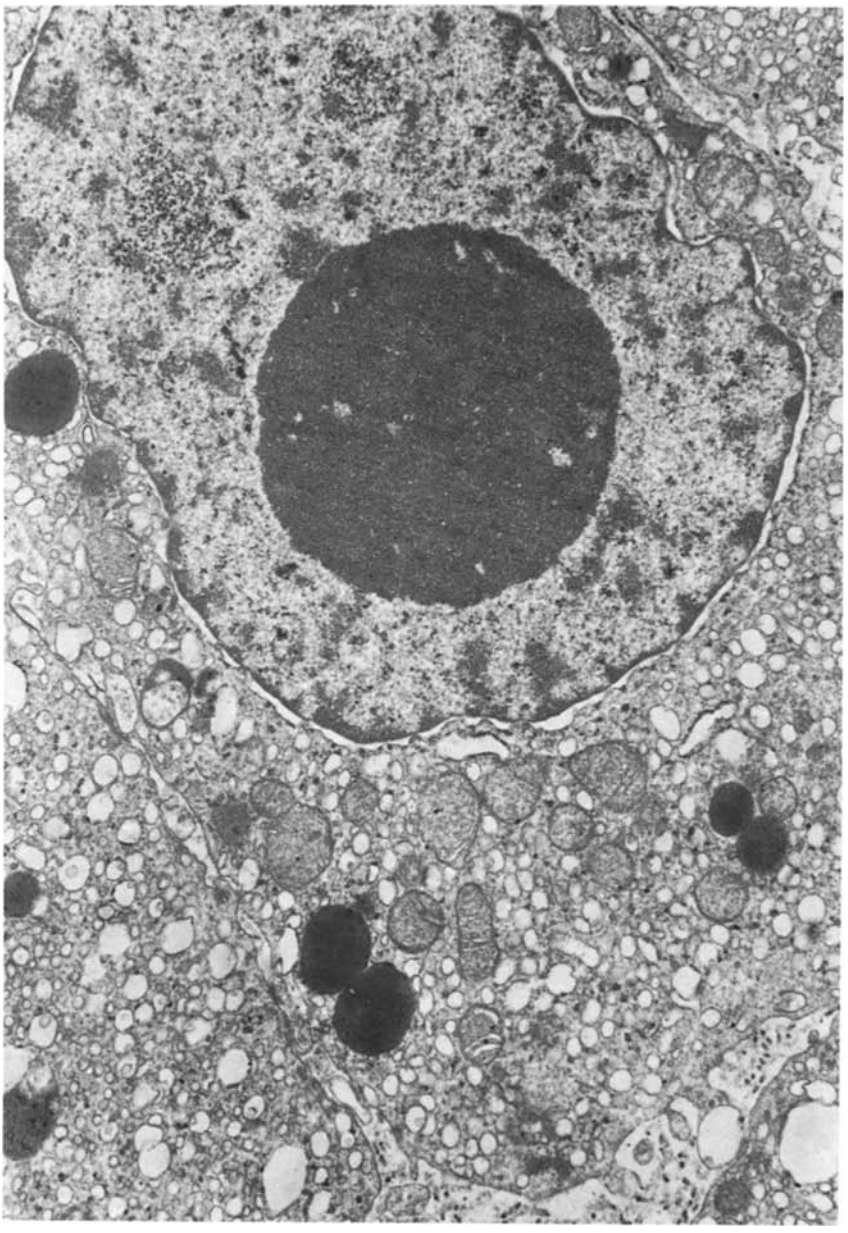

Fig. 3 Case 1. Tumour cell presenting with large nucleolus and chromatin aggregations along nuclear membrane, and short microvilli on the cellular surface. ×14,000

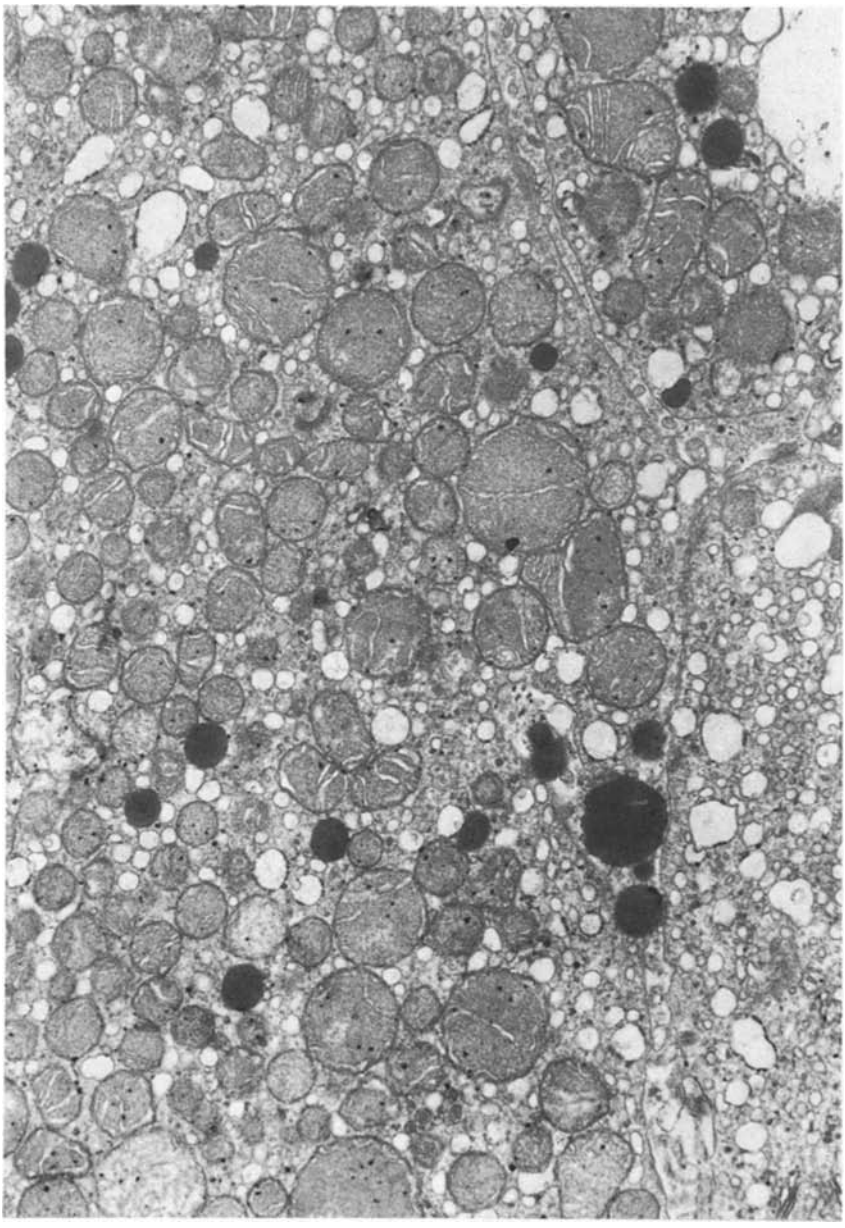

Fig. 4. Case 1. Very numerous and irregularly sized mitochondria, with dense, finely granular matrix and few plate-like and tubular cristae. ×14,000

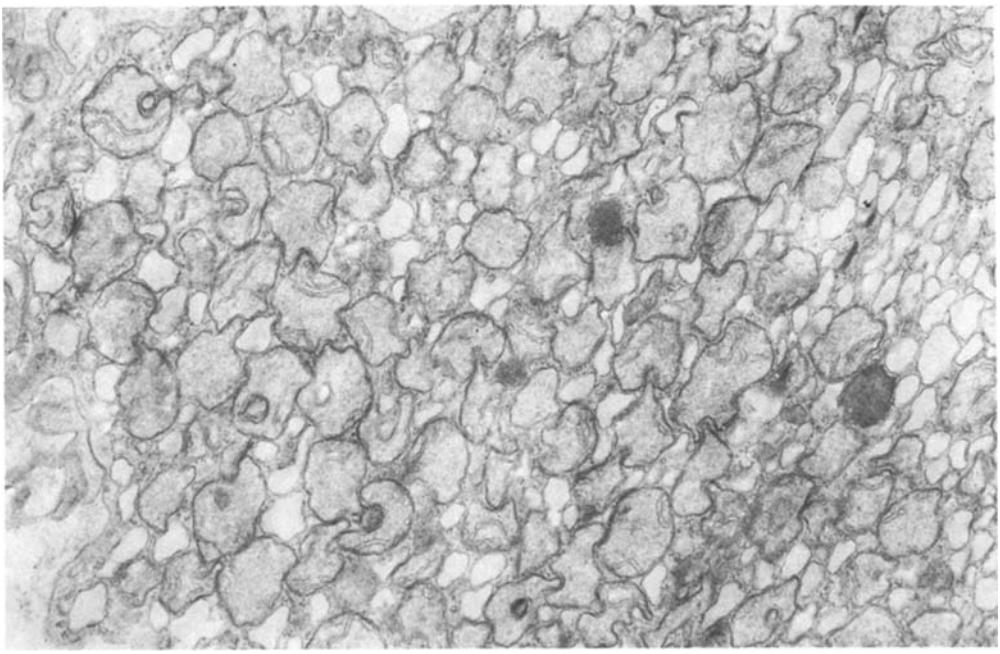

Fig. 5. Case 2. Mitochondria with indented borders and outpouching of the outer limiting membrane. Note the close relationship between mitochondria and smooth endoplasmic reticulum. ×24,000

capsular infiltration. Most of the cells had a large eosinophilic, finely granular cytoplasm; the nuclei were mostly round, vescicular and nucleolated; nuclear polymorphism and hyperchromia and giant multinucleated cells were occasionally seen. Some foamy or vacuolated cells were scattered among the acidophilic cells. The hepatic metastasis showed more severe cellular atypia and nuclear pleomorphism.

Semithin Sections (Fig. 2). Neoplastic cells varied in size and were grouped in clusters surrounded by basement membrane. A few mitoses were observed.

Ultrastructural Findings

The electron-microscopic features of both tumours were also similar. The neoplastic cells had abundant cytoplasm, short and isolated microvilli, and rounded nuclei with large nucleoli and some chromatin aggregations along the nuclear membrane (Fig. 3). Mitochondria were numerous and variable in size and shape; sometimes they were round, with lamellar and tubular cristae (Fig. 4). Some cells also showed "crenated mitochondria" with outpouchings and discontinuity of the outer limiting membrane (Fig. 5); mitochondria were not infrequently associated with smooth endoplasmic reticulum and microbodies. There was

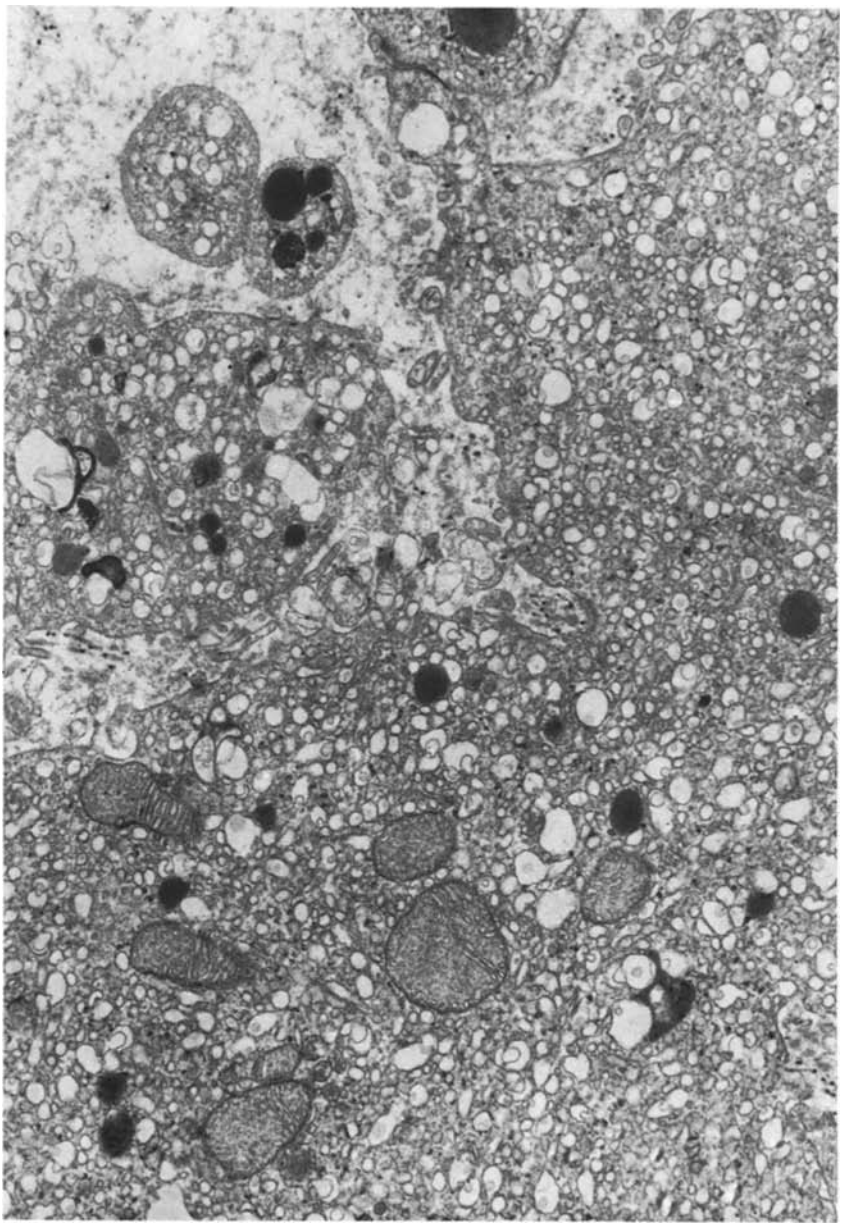

Fig. 6. Case 1. The very abundant smooth endoplasmic reticulum gives the cytoplasm of the neoplastic cell a honeycomb appearance. ×14,000

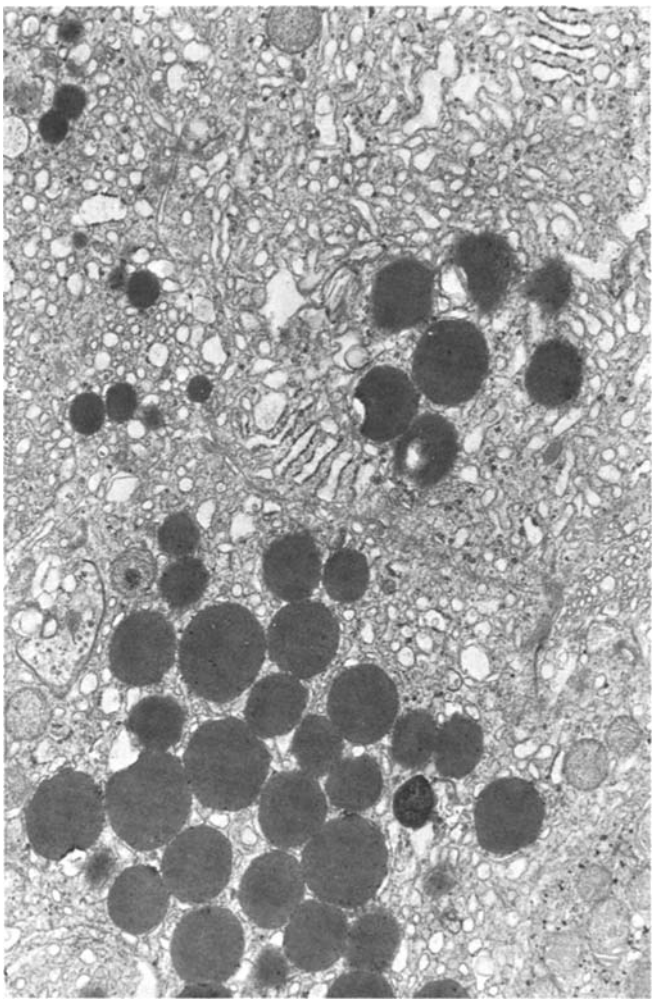

Fig. 7. Case 1. Scattered rough-surfaced endoplasmic reticulum. ×14,000

an extensive, often swollen smooth endoplasmic reticulum, the cytoplasm sometimes resembling a honeycomb (Fig. 6), while rough endoplasmic reticulum was scarce and scattered (Fig. 7). The Golgi complex was inconspicuous. Lysosomes and lipofuscin bodies were present in variable amount (Fig. 8). There were only a few lipid droplets. The basement membrane which surrounded the clusters of neoplastic cells showed focal interruptions. The sinusoidal vessels were lined by fenestrated endothelium resting on a basement membrane. Most of the perisinusoidal space between the two opposing basement membranes was potential rather than realised: it appeared to be widened and occupied by cellular debris where the epithelial basement membrane was missing (Fig. 9).

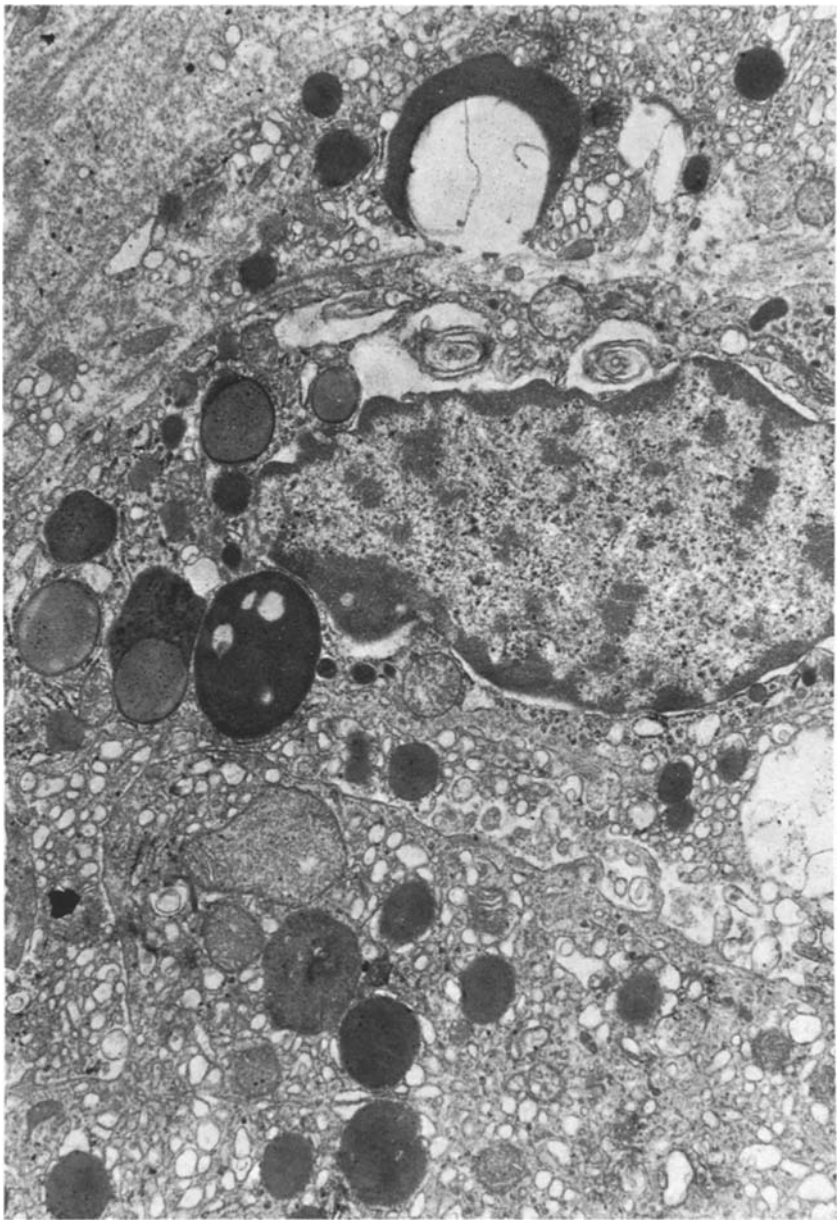

Fig. 8. Case 1. Many lysosomes and lipofuscin bodies. ×14,000

Discussion

The androgen secreting adrenocortical tumours described here had caused virilism in both cases, with features of sexual precocity in the former and masculinization in the latter. Androgen levels dropped to normal and signs of virilism were partially lost after adrenal surgery.

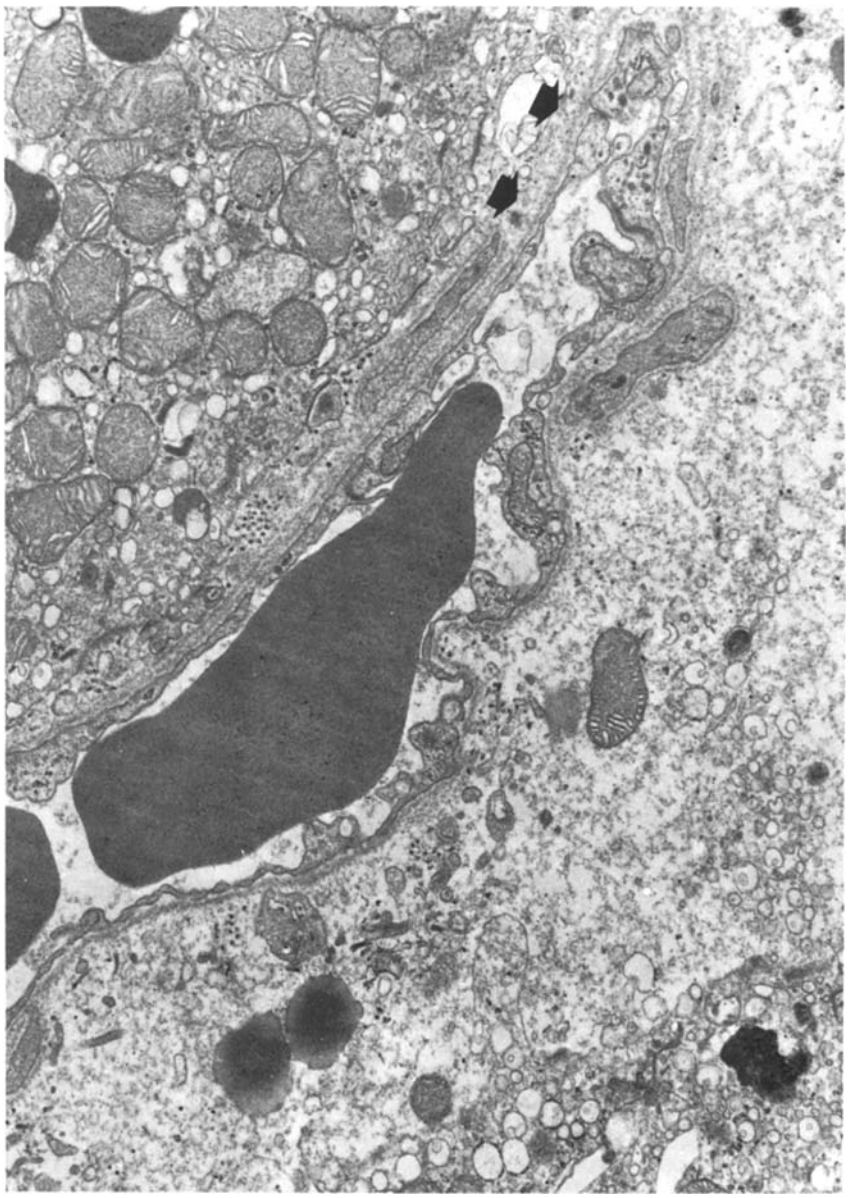

Fig. 9. Case 2. Capillary with intraluminal red cell, endothelial lining and basement membrane. On the upper left side of the capillary the neoplastic cell with packed mitochondria exhibits partial disappearance of the epithelial basement membrane (arrows). On the right side of the capillary the epithelial basement membrane is absent diffusely, the perivascular space being widened and occupied by cellular debris. ×14,000

The tumours consisted of irregularly surfaced brownish masses, suggesting cellular proliferation of zona reticularis. In addition there were hemorrhagic areas and few small scattered yellow zones. In agreement with this macroscopic appearance, polygonal cells with eosinophilic finely granular cytoplasm formed the bulk of the tumor, few clear cells were present. Malignant behaviour, established by capsular infiltration, was confirmed in the first case by the liver metastases detected some months after adrenalectomy.

The ultrastructural study showed that the tumour cells were quite similar to the compact cells of the human zona reticularis, as described by Carr (1961). The tumour cells presented with abundant mitochondria and smooth endoplasmic reticulum, microvilli, lipofuscin bodies and scanty lipid. Mitochondria had few lamellar or tubular cristae. These cytological ultrastructural findings parallel those of two cases of androgen secreting adenoma reported by Fisher and Danowski (1973) and by Akhtar et al. (1974). In addition we observed focal disruption or absence of basement membrane surrounding the clusters of neoplastic cells, which has not been reported in the ultrastructural descriptions of androgen secreting adenoma. MacKay (1969) and Tannenbaum (1973), describing these discontinuities of basement membrane in carcinoma producing Cushing's syndrome, postulated that it should be regarded as distinctive ultrastructural finding in differentiating malignant from benign proliferative processes leading to this syndrome. Even though we have no personal experience of electron microscopy of androgen secreting adenoma, we believe that this distinction may also apply to functional carcinomas. Interruption of the basement membrane of neoplastic cells may reflect their metastatic potential. In addition in our cases some cells presented with irregularly shaped, indented mitochondria, with outpouchings and sometimes discontinuity in the outer limiting membrane. These mitochondrial evaginations and defects have already been described in the human fetal cortex (Ross et al., 1958; Luse, 1967), in the cortical cells of ACTH-treated dogs (Bloodworth, 1966), in adrenocortical hyperplasia associated with Cushing's syndrome (MacKay, 1969; Hashida et al., 1970), in the normal cells of the human inner zona fasciculata (Tannenbaum, 1973) and in virilizing adrenocortical adenoma (Akhtar et al., 1974). MacKay (1969) interpreted these features as artefacts; Hashida et al. (1970) observing outpouchings both in specimens fixed in osmium tetroxide alone and prefixed in 2% glutaraldehyde, and mitochondrial outer limiting membrane discontinuities only in tissues fixed in osmium tetroxide, suggested that only these latter were artefacts.

More recently Merry (1975) postulated that mitochondrial outer membrane protrusions might have a functional significance, playing a role in the transfer of cholesterol to the inner mitochondrial membrane desmolase complex, thus facilitating side chain cleavage of cholesterol to pregnenolone. According to this hypothesis, mitochondrial outpouchings may represent, together with hyperplasia and dilatation of smooth endoplasmic reticulum, the morphological basis of endocrinological activity of adrenocortical tumours.

In conclusion, our ultrastructural findings in androgen secreting adrenocortical carcinomas revealed that neoplastic cells are similar to the compact cells of zona reticularis. Mitochondrial features and the large amount of smooth endoplasmic reticulum we observed suggest functional activity. Partial disappea-

rance of the basement membrane applied to clusters of neoplastic cells probably reflects malignancy of the tumours. It is important to note that the cells proliferating in neoplasms associated with Cushing's syndrome also present the features of compact cells. Tannenbaum (1973) suggested that the difference between cortisol producing cells in Cushing's syndrome and androgen producing cells in adrenogenital syndrome might be the arrangement of the smooth endoplasmic reticulum, which assumed parallel arrays only in the former. In accordance with these findings, stacked parallel arrays of the smooth endoplasmic reticulum were not observed in our cases.

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References

Akhtar M., Gosalbez, T., Young, I.: Ultrastructural study of androgen-producing adrenocortical adenoma. Cancer **34**, 322–327 (1974)

Bloodworth, J.M.B.: The adrenal. In: Sommers S.C. (ed.) Pathology annual. New York: Appleton 1966

Carr, I.A.: The ultrastructure of the human adrenal cortex before and after stimulation with ACTH. J. Path. Bact. **81**, 101–106 (1961)

Fisher, E.R., Danowski, T.S.: Ultrastructural study of virilizing adrenocortical adenoma. Am. J. Clin. Pathol. **59**, 480–489 (1973)

Hajjar, R.A., Hickeey, R.C., Samaan, N.A.: Adrenal cortical carcinoma: A study of 32 patients. Cancer **35**, 549–554 (1975)

Hashida, Y., Kenny, F.M., Yunis, E.J.: Ultrastructure of the adrenal cortex in Cushing's disease in children. Hum. Pathol. **1**, 595–614 (1970)

Hutter, A.M., Kayhoe, D.E.: Adrenal cortical carcinoma: Clinical features of 138 patients. Am. J. Med. **41**, 572–580 (1966)

Huvos, A.G., Haidu, S.I., Brasfield, R.R., Foote, F.W. Jr.: Adrenal cortical carcinoma. Clinicopathologic study of 34 cases. Cancer **25**, 354–361 (1970)

Luse, S.A.: Fine structure of adrenal cortex. In: Eisenstein A.B. (ed.) The adrenal cortex. Boston: Little, Brown 1967

MacKay, A.: Atlas of human adrenal cortex ultrastructure. In: Symington T. (ed.) Functinal pathology of the human adrenal gland. Baltimore: Williams & Wilkins 1969

Merry, B.J.: Mitochondrial structure in the rat adrenal cortex. J. Anat. **119**, 611–618 (1975)

Mitschke, H., Saeger, W., Breusted, H.G.: Zur Ultrastruktur der Nebennierenrindentumoren beim Cushing Syndrom. Virchow Arch. Abt. A Path. Anat. **360**, 253–264 (1973)

Ross, M.H.: Electron microscopy of the human foetal adrenal cortex. In: Currie A., R., Symington T. and Grant J.K. (eds.). The human adrenal cortex. Baltimore: Williams & Wilkins 1962

Tannenbaum, M.: Ultrastructural pathology of the adrenal cortex. In: Sommers S.C. (ed.) Pathology annual. New York: Appleton 1973

Thiele, J.: Feinstrukturelle Untersuchungen an einem endokrin aktiven Carcinom der Nebennierenrinde. Virchows Arch. B Cell. Path. **17**, 51–62 (1974)

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