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Feminizing Adrenocortical Tumor

Histological and Ultrastructural Study

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Summary. A case of a feminizing adrenocortical tumor associated with Cushing's syndrome in a 29 year old male is presented. The ultrastructural features are compared with adrenal tumors secreting aldosterone, glucocorticoids or androgens. As in adrenal carcinomas, this tumor demonstrates nuclear pleomorphism with enlarged nucleoli and nuclear pseudoinclusions. The cytoplasmic organelles show some parallels between feminizing and androgen-secreting adrenal tumors. Different types of mitochondria occur with varying amounts of smooth endoplasmic reticulum. Numerous microbodies are present.

Histological and ultrastructural signs indicating probable malignancy are discussed and it is noted that most of the feminizing adrenal tumors are carcinomata. Neither local recurrence nor distant metastases have yet been detected in this case, two years after excision of the tumor.

Key words: Adrenal glands – Adrenal adenoma – Adrenal ultrastructure – Feminizing syndrome.

Introduction

Adrenal feminization caused by an oestrogen producing adrenocortical tumor is a very rare form of the adrenogenital syndrome, in its broad sense. It usually affects men between the ages of 25 to 50 years, but has also been observed in children (Dhom, 1965 Lit.) and menopausal women (Mathur et al., 1973). In 70 children with hormonally active adrenal tumors, Wilkins (1948) found 8 cases with oestrogen secretion. In a survey Gabrilove et al. (1970) reported 61 cases with this syndrome. Clinical symptoms are mainly gynecomastia, diminished libido and sexual potency, feminizing hair change and atrophy of the testes with azoospermia. In 50% of these cases the adrenal tumor was clinically

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palpable, and their size varied between 10 and 2000 g (Symington, 1969). Occasionally hypertension, obesity, acne and striae are present. The clinical syndrome can be complicated by simultaneous hypercortisolism and virilisation (Dohm, 1965). The oestrogen/androgen relation is obviously more important than the absolute levels of each hormone in determining the clinical picture (Gabrilove et al., 1970).

During the last 10 years we have been able to examine one oestrogen producing adrenocortical tumor among 82 surgical specimens of adrenal glands. While increasing attention has been given to the clinical and biochemical aspects of this tumor, morphological examinations have been rather limited and confined to scanty light microscopic observations. The differentiation between adenoma and carcinoma presents a problem in at least some of these cases. With these aspects in mind, a comparative ultrastructural examination with androgen or cortisol producing adrenal tumors seemed to be indicated. We have attempted to demonstrate a specific morphological basis for the altered steroid hormone secretion, and we have made an ultrastructural comparison with adrenocortical adenomas and carcinomas.

Report of a Case and Methods

Our patient was a 29 year old male who developed increasing gynecomastia for three years before the operation. Sex chromatin was negative. Hormone analysis indicated a tumor producing massive amounts of oestrogen. Oestrogen secretion could be further stimulated by gonadotropins. Additionally, hypercortisolism was present. The high oestrogen levels induced a marked testosterone reduction due to decreased LH-secretion. The right-sided tumor, which had been demonstrated by scintigraphy, was removed. In addition to routine light microscopic examination, tissue from various tumor areas and from the remaining atrophic cortex were fixed in cacodylate-buffered glutaraldehyde, postfixed in osmiumtetroxyde and embedded in epon. A selection of ultrathin sections (Reichert ultramicrotome) was made after examining toluidine blue-stained semi-thin sections. Staining of the ultra-thin sections was done with uranyl acetate and lead citrate. For electron microscopy the Zeiss EM 9 and the Phillips EM 300 were used.

Results

The clinical and hormonal results will be reported elsewhere (Breustedt et al., in preparation). The tumor weighed 120 g and was well defined with a capsule, and a light brown cut surface, the attached cortex was atrophic. Histologically (J-Nr. 24919/75) the tumor was made up of predominantly uniform cells separated by a very scant stroma, consisting of reticulin fibres and capillaries. Variable cell differentiation was conspicuous at the light microscopic level. A preponderance of cells contained vesicular nuclei with partly distinct nucleoli (Fig. 1 a). The cytoplasm was moderately abundant and weakly eosinophilic. Cells with abundant lipid with a spongiocytic appearance occured in isolated areas only. Alveolar arrangement of cells was seen; these cells were characterized by a considerably wider cytoplasm and larger pleomorphic nuclei containing prominent nucleoli (Fig. 1 b). Their cytoplasm was intensively eosinophilic and finely granular, almost oncocytic. The nuclei were sometimes arranged spherically

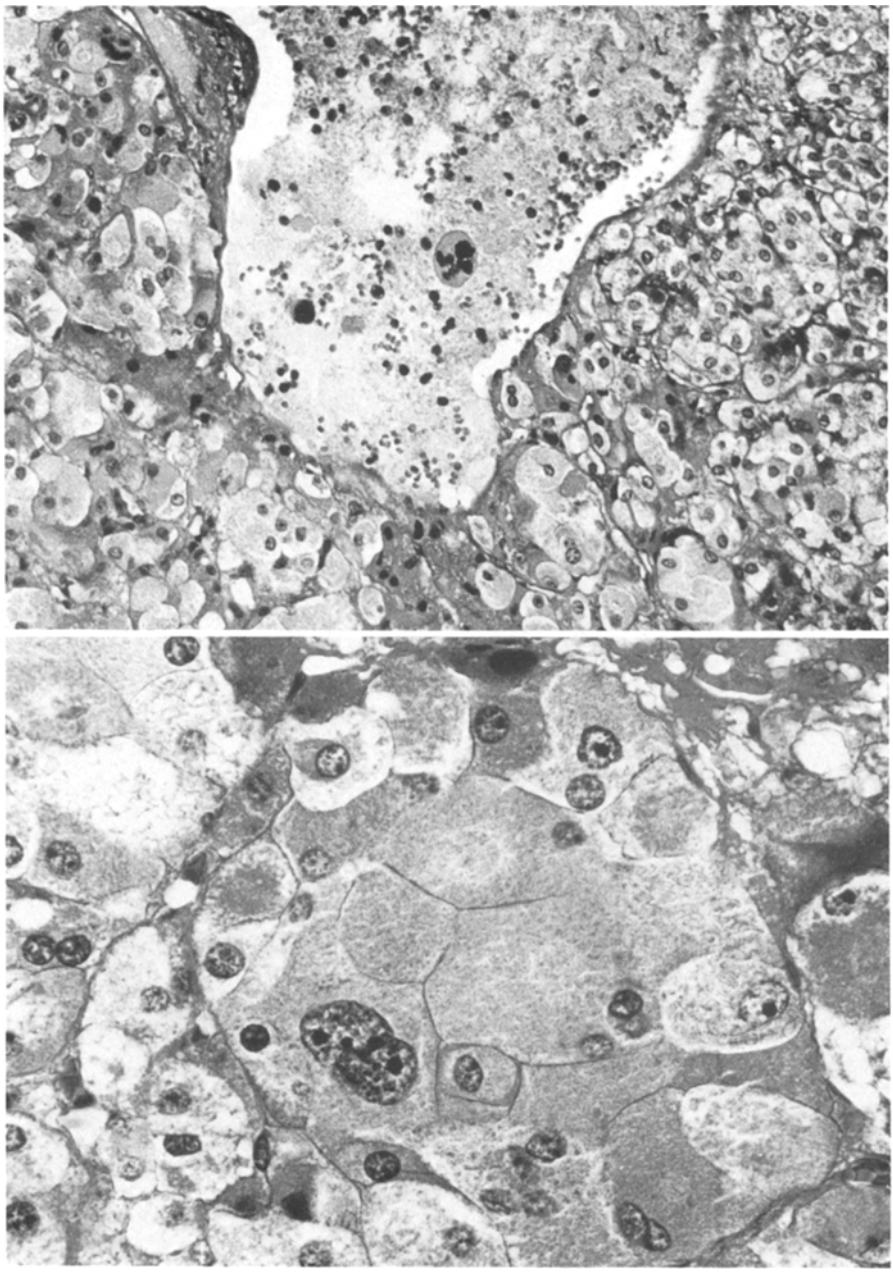

Fig. 1. a Feminizing adrenocortical tumor with a sinusoidal blood vessel containing scattered tumor cells with giant hyperchromatic nuclei. H. & E., ×250. **b** Alveolar arrangement of tumor cells with finely granular cytoplasm and pleomorphic nuclei including prominent nucleoli. H. & E., ×630

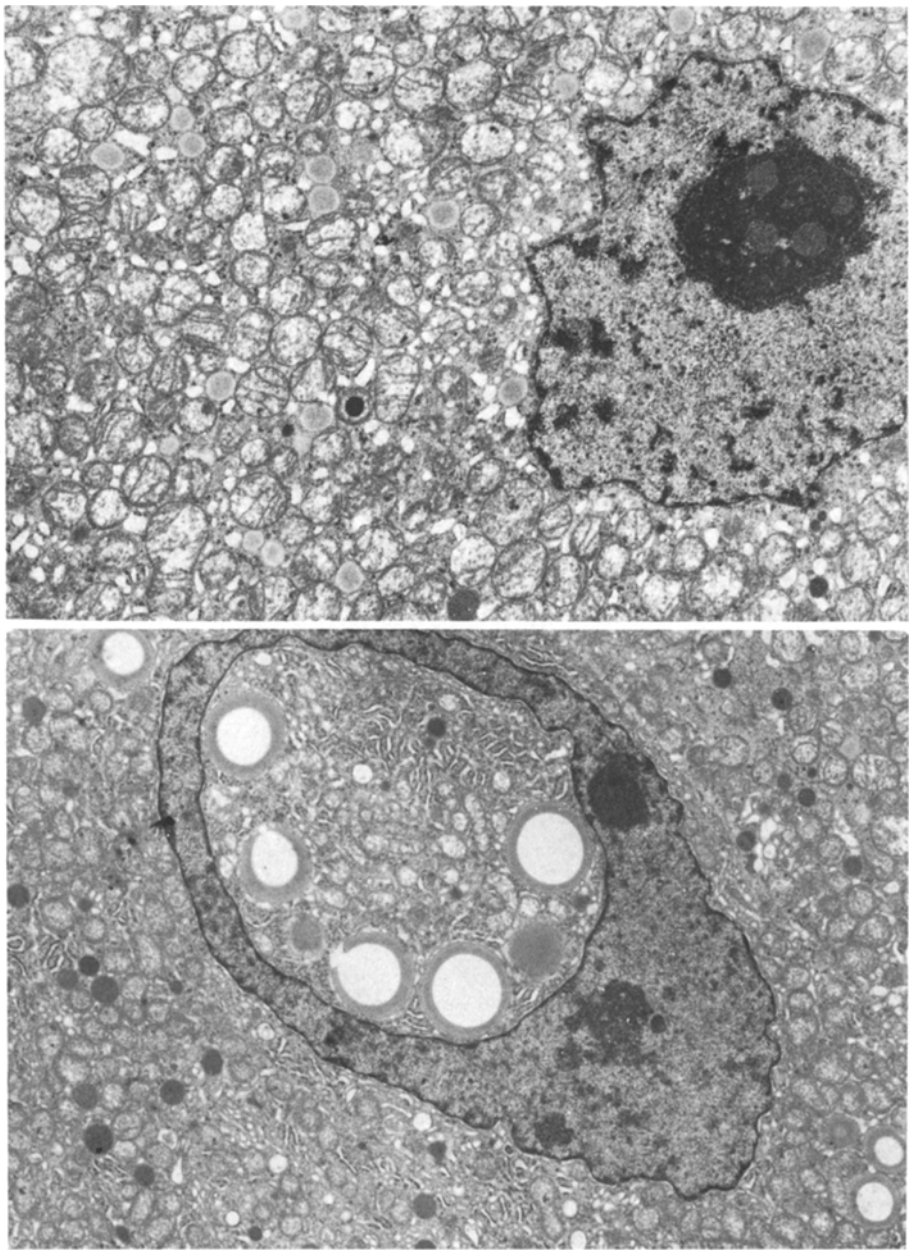

Fig. 2. a Compact tumor cell with numerous mitochondria and vesicular profiles of smooth endoplasmic reticulum. Euchromatin-rich nucleus with enlarged nucleolus. ×8600. **b** Nuclear pseudoinclusion containing granular and smooth endoplasmic reticulum, very few mitochondria and well preserved liposomes. ×7760

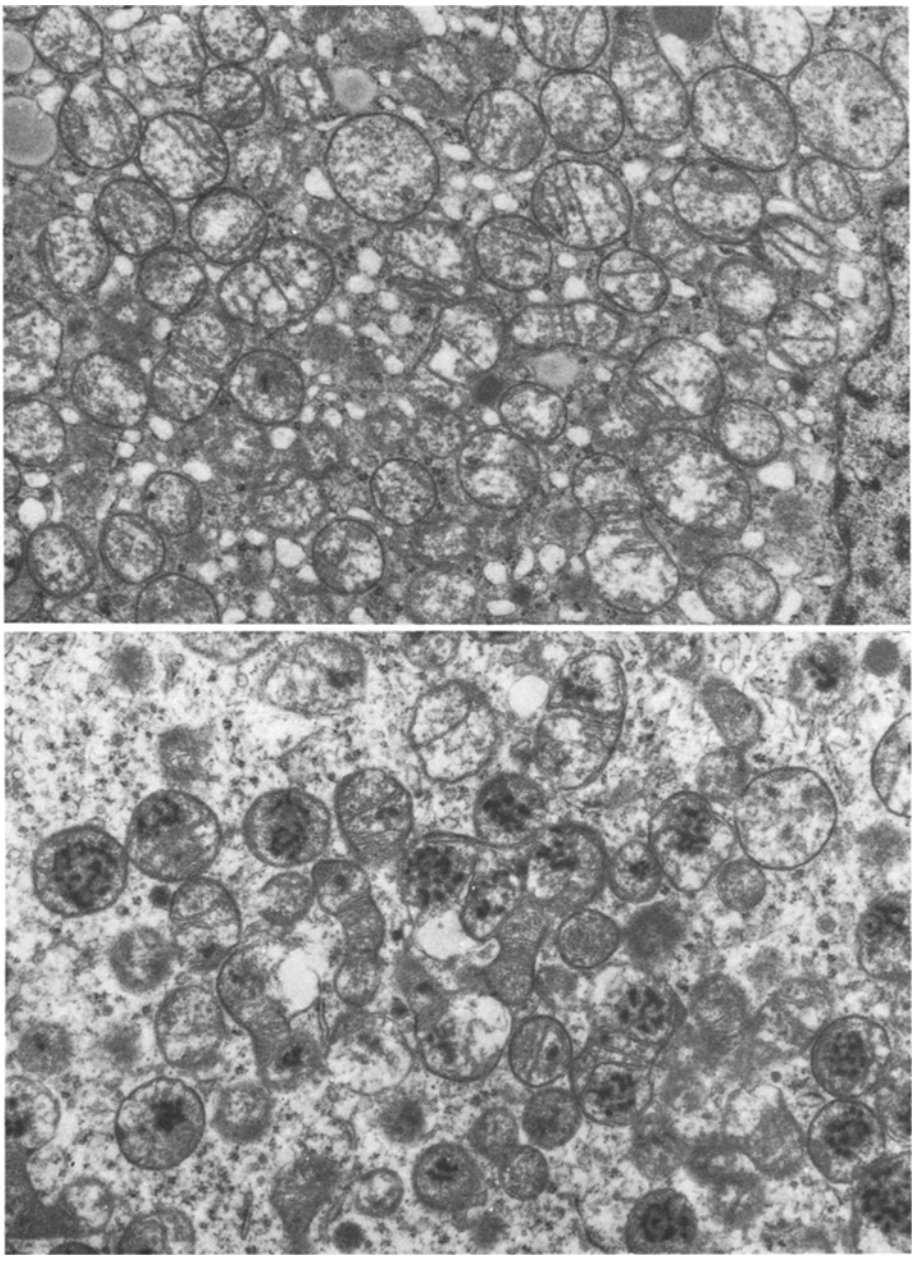

Fig. 3. a Predominant type of mitochondrion with lamellar cristae and a rather pale matrix. Besides the mitochondria, vesicular smooth endoplasmic reticulum and scattered ribosomes can be observed. ×16,450. **b** Irregular shaped mitochondria with electron-dense intra-mitochondrial granules, lamellar cristae partially preserved. The endoplasmic reticulum is very sparse or even absent in these cells. ×16,920

around PAS-positive cytoplasmic areas, corresponding to earlier reports of so-called giant spheres (Dohm, 1965). Additionally, pseudoinclusions in the nuclei and multinuclear giant tumor cells could be found. Mitoses were not demonstrable on numerous sections. The capillaries usually showed a narrow lumen, although sinusoidal vessels with a wider lumen appeared focally. Here cell protrusions projecting into the vascular lumen could be demonstrated and although the perivascular basement membrane was well defined in most areas, on some sections isolated tumor cells could be observed intravascularly (Fig. 1a). At the periphery the tumor was enclosed by collagenous tissue and adjacent adrenocortical tissue which was atrophic. Intracapsular tumor invasion was not present, and no central regressive changes in the form of hemorrhage or necrosis were seen.

Ultrastructurally the variability of the *nuclei* was conspicuous. Besides euchromatin-rich, round nuclei (Fig. 2a), very bizarre forms with deep invaginations were present. Pseudoinclusions were also found which contained in part well preserved cell organelles (Fig. 2b). The nucleoli were considerably enlarged (Fig. 2a). The findings of the mitochondria and the endoplasmic reticulum are considered in detail in view of their importance in steroid hormone production.

Various types of *mitochondria* could be distinguished:

1. Predominantly, large round to oval forms occured with light appearing matrix and relatively sparse lamellar cristae (Fig. 3a).
2. Less often oval to enlongated mitochondria were found, with numerous lamellar and singular tubular cristae as well as a moderately dense matrix.
3. Rarely, mitochondria were present in single cells with a very dark matrix and well preserved lamellar cristae.
4. Finally, numerous mitochondria were found with electrondense intramitochondrial granules (Fig. 3b).

The smooth *endoplasmic reticulum* was rather poorly developed in many cells which contained large numbers of mitochondria (Fig. 2a). This was espicially true in cells with numerous intra-mitochondrial granules. However smooth endoplasmic reticulum was often present in large amounts in the form of tubular and vesicular formations in mitochondria-rich cells with lamellar cristae. *Granular endoplasmic reticulum* was arranged in short parallel tubules (Fig. 2b). In addition, a Golgi apparatus could be demonstrated consisting of dilated and tubular cisternae. Only rarely were *liposomes* seen in the form of small, membrane-bound vesicles with a faint osmiophilic content (Fig. 2b). Ribosomes were diffusely distributed. Numerous microbodies were conspicuous, defined by a membrane with homogenous varying electron density (Fig. 2b). Lysosomes and lipofuscin pigment were only rarely demonstrable. A basal membrane was clearly seen beneath the endothelium and around tumor cell complexes.

Discussion

Only few authors consider it possible to differentiate between adrenocortical tumors with feminization and other endocrine syndromes by light microscopy

(Landau et al., 1954). Generally a similar histological picture is present in different types (Symington, 1969). The findings of Goormaghtigh (1940), who reported a high lipofuscin content in feminizing tumors and the absence of this pigment in virilizing tumors, could not be confirmed in our case.

In attempting a correlation between functional states and the morphological picture, special attention should be given to the steroid hormone-producing organelles. As ultrastructural examinations of adrenocortical feminizing tumors have not yet been reported, comparisons can only be made with other adrenal endocrine syndromes.

Aldosteronomas share, as a rule, an abundance of lipids. The mitochondrial structure is variable; elongated mitochondria with lamellar cristae are present as in normal glomerulosa cells, and in addition, hybrid forms with tubulovesicular internal structures are present (Cervos-Navarro et al., 1965; Kovacs et al., 1974; Mackay, 1969; Propst, 1965; Reidborn and Fisher, 1969).

Most of the cortisol-producing tumors contain lamellar cristae in contrast to the normal tubulovesicular internal mitochondrial structures (Bahu et al., 1974; Kano and Sato, 1977; Mackay, 1969; Mitschke et al., 1973; Mitschke and Saeger, 1975; Tannenbaum, 1973). Intramitochondrial granules have been demonstrated in cortisol-producing as well as in virilizing tumors (Gorgas et al., 1976; Kano and Sato, 1977; Tannenbaum, 1973). Variability in size and internal structures of mitochondria is very common in adrenal tumors. One is tempted to suggest a relationship between these mitochondrial alterations and the suspected enzyme defects, but it was not possible to establish a specific ultrastructural basis for the estrogen production in this case. One might speculate that the partial reduction of mitochondrial membranes might indicate a decreased steroidogenesis. In this case, however, the enzyme supply must have been adequate for the very high oestrogen and the increased cortisol levels. The intramitochondrial granules probably represent a form of glycoproteids (Gorgas et al., 1976). These mitochondria should be looked upon as degenerate forms.

The altered steroid hormone production probably proceeds by the stages Δ^5-pregnenolone—dehydroepiandrosterone (DHEA)—Δ^4-androstendione to oestrone in feminizing tumors (Bodansky, 1975). It is equivalent to a conversion from androgens to oestrogens (Gabrilove et al., 1970). If we suppose that oestrogen production occurs with an androgen-active intermediate step this could explain why there are many ultrastructural parallels between virilizing and feminizing adrenal tumors.

The functional significance of the many microbodies remains questionable. In Leydig cells they could play a part in cholesterol metabolism and androgen synthesis (Reddy, 1973), providing a correlation with some virilizing tumors.

The decrease of liposomes is a characteristic of high cholesterol metabolism. This can be demonstrated in particular in adrenal compact cell tumors with hypercortisolism (Mitschke et al., 1973). In comparison to normal adrenocortical cells the paucity of lipofuscin granules and the reduced numbers of lysosomes are notable.

The nuclear changes, with deep invaginations and pseudoinclusions, have been reported in adrenal carcinomas (Mackay, 1969; Thiele, 1974); they have

also been observed in virilizing tumors (Fisher and Danowski, 1973; Gorgas et al., 1976). These are, however, probably not a criterion for malignancy but rather an expression of degeneration. More suggestive of the proliferative capacity of these tumors is the considerable enlargement of the nucleoli which are found more often in large, round and euchromatin-rich nuclei.

The ultrastructural findings thus suggest compensatory processes of adaptation to altered steroidogenesis and degenerative changes, and signs of increased proliferation.

Interruptions of the basement membranes in adrenal carcinomas (Mackay, 1969) could not be demonstrated on the ultrathin sections. By electron microscopy, no additional signs of malignancy were found, except for the nuclear alterations. By light microscopy, however, vascular invasion could be demonstrated. The significance of this change is disputable as a sign of malignancy, according to the WHO tumor classification.

In most adrenal tumors causing feminization the interpretation of malignancy has been no problem. The diagnosis of a carcinoma can be made when numerous mitoses, capsular and extensive vascular invasion are present. These changes are found in 80% of feminizing adrenal tumors. 50% of these cases were already palpable by clinical examination; they had reached a large size (up to 2000 g). Survival times of more than three years are rare in these tumors (Solomon et al., 1968). An uncertain prognostic criterion is the level of oestrogen secretion; very high levels are assumed to indicate a malignant and lower levels a benign tumor (Wotiz et al., 1968). A correlation is supposed to exist between a high degree of tumor differentiation as expressed by a multihormonal steroidogenic activity and the rate of survival (Solomon et al., 1968), and the more diverse the steroid hormone pattern, the more favorable seems to be the prognosis.

Longer follow-up periods, however, have shown that even eight years after tumor excision a local recurrance or distant metastases can appear in regional lymph nodes, liver, lungs or the skeletal system (Bacon et al., 1965; Dempsey et al., cit. in Gabrilove et al., 1970). Mitoses or atypical cells may be very rare or even absent in feminizing adrenal tumors (Gabrilove et al., 1970). One should therefore avoid the term "adenoma" for these tumors. As a rule, adrenal feminization is caused by an adrenocortical carcinoma.

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