Changes in the Fine Structure and Function of a Hormone-Secreting Adrenocortical Tumour Investigated in Tissue Culture

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Summary. Tissue cultures of a surgically removed adrenocortical tumour causing Cushing's syndrome, and tissue cultures from the attached, tumour-free adrenal were studied. There were two cell types characteristic of tumour tissue. The cell type occurring most frequently had pronounced hypertrophied agranular endoplasmic reticulum. A fewer number of lipid-rich cells containing many electron-dense granules could also be found. The ratio of cells changed during cultivation. In the 17 days tumour culture, a higher percentage of lipid-rich cells could be observed. In spite of continuous ACTH treatment, the initially high hydrocortisone level decreased, gradually. It may be assumed that the lipid-rich cells are of reduced ability as regards hydrocortisone production.

Functioning adrenocortical tumour frequently gives rise to Cushing's syndrome. The atrophy of the attached, tumour-free, and of the contralateral cortex is a known concomitant symptom. Temporary clinical improvement can be achieved with steroid inhibitors, mainly with aminogluthethimide (Schteingart et al., 1966; Fishman et al., 1967; Smilo et al., 1967).

The ultrastructure of adrenocortical hyperplasia and tumours, underlying Cushing's syndrome, has already been described by a fair number of authors (Holzmann and Lange, 1966; Luse, 1967; Reidbord and Fisher, 1968; Mackay, 1969; Symington, 1969; Hashida et al., 1970; Macadam, 1970; Mitschke et al., 1971; Urushibata, 1971; Neville and Mackay, 1972; Mitschke et al., 1973; Tazaki et al., 1974). The aim of the present investigation has been to find out whether or not the tumour retains its characteristic morphological and functional features in tissue culture.

Material and Methods

The right adrenocortical tumour and adrenal were removed by surgery from a 38 year-old woman. Prior to surgical intervention, the patient was clinically diagnosed to suffer from Cushing's syndrome. In addition to the conventional treatment, she was given daily doses of 0.5 g of aminogluthethimide for 6 weeks. This treatment was withdrawn 14 days prior to the operation. Immediately after extirpation, small pieces were cut both from the tumour and the attached adrenal cortex. For polarization microscopy, sections were cut in cryostat, fixed in 4% buffered formalin. The pieces for cultivation were rinsed several times with chemically defined TC 199 medium, containing antibiotics, and then cut into pieces of about 1 mm³.
Five separate plastic Falcon flasks, of 250 ml each, were taken and 100 to 150 such pieces were placed into each of them on a coagulate of plasma and embryonic extract. After 24 hours, the explants attached to the coagulate and began to grow. The outgrowing cells showed an epithelial-like character. Mitosis was common in the outgrowing cells. After the fifth day, the explants began to die off. The cuts were made from the outgrowing cells surrounding the explants. For electron microscopy, some pieces were placed into plastic Falcon Petri-dishes and treated in the same way as the Falcon flasks. Twenty-four hours later, the 8:2 mixture of TC 199 medium and pooled human sera were added to the cultures. In one of the Falcon flasks pieces from the attached adrenal cortex, in the other four, tissue pieces from the adrenocortical tumour were cultivated. The cultures were washed every 48 and 72 hours, resp. On the 5th day of cultivation, 100 mU/ml of ACTH (Organon, Oss.) were added to each culture. In the subsequent days nutrient medium, alternately with or without ACTH, was added to the cultures. Hydrocortisone production in the medium was determined by paper chromatography (Stark et al., 1963). On the 17th day of cultivation the cultures in the Petri-dishes were processed for electron microscopy. These cultures were fixed *in situ* in 2.5% glutaraldehyde for 2 hours, post-fixed in 1% osmium tetroxide for further 2 hours. 0.1 M sodium cacodylate-HCl buffer was used to adjust the fixatives to pH 7.4. Dehydration in graded ethanol followed and the cultures in the Petri-dishes were embedded into Durcupan ACM. The adrenal pieces, both from the tumour and the attached uninvolved cortex, were also fixed and embedded in a similar way. Sections approximately 1 \(\mu\)m thick were mounted on glass slides, stained with toluidine blue and examined with light microscope. Ultrathin sections were cut on a Reichert Om U2 ultramicrotome and mounted on copper grids coated with carbon-stabilized Formvar film. The sections were counterstained first with 20% uranyl acetate, dissolved in methanol, then with lead citrate according to Reynolds (1963). The sections were studied in a JEM-6AS electron microscope, operating with 80 kV accelerating voltage.

**Observations**

**Light Microscopic Findings.** With polarization microscope, significantly more birefringent lipid was seen in the tumour-free adrenal cells than in the tumour (Figs. 1 and 2). In the zero-time tumour tissue haemorrhagic, necrotic and lobular areas occurred intermittently. The cells in the lobular areas were mainly of the fascicular type forming alveoli and cords. The tumour cells had vesicular and pleomorphic nuclei, vacuolated cytoplasm with finely dispersed lipid droplets. Sporadic mitotic activity was observed. The tumour was considered to represent an adrenocortical carcinoma (Fig. 3). In the tumour tissue cultivated for 17 days, many cells were rich in lipid. The nuclei were enlarged and vesicular in appearance (Fig. 4).

**Electron Microscopic Findings.** In the adrenal cortex adjacent to the tumour, the cells of the zona fasciculata were atrophied, the lipid droplets became larger and increased in number.

The morphology of the zero-time tumour tissue was highly variable. The cell type occurring most frequently had enlarged nuclei with multiple inclusions. Hypertrophy of the agranular endoplasmic reticulum (AER) was conspicuous. Some of the AER vesicles were dilated to such an extent that they became cyst-like structures. Lysosomes in the cells were less abundant. Many abnormal mitochondria can be found, they have relatively few intramitochondrial structures. The number of lipid droplets varied from cell to cell. In general, they were less numerous than normally (Fig. 5). The other characteristic cell-type observed, besides containing lipids varying in size and density, had numerous electron-dense granules mainly lysosomes. Some of these granules frequently showed a close