Growth Characteristics of Human Pituitary Adenomas in Tissue and Cell Cultures

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Summary. Pituitary adenomas of 120 patients were investigated in tissue and in cell cultures. Under different conditions of culture, the biopsies revealed different rates of growth. In tissue cultures 66% of the samples could be propagated while tumour specimens explanted in cell suspensions proliferated in 80% of the cases. Attempts to establish subcultures were also more successful when specimens were put into cell cultures primarily. Further differences of growth characteristics were observed in connection with the histological types of the parent tissues. According to the old terminology cells of mixed type adenomas had the longest life-span both in tissue and in cell cultures. Considering the proliferative activity, this type of tumour could be most frequently subcultured.

Concerning the relationship of different cell components of the biopsies it was proven that survival and proliferation of adenoma cells may occur without fibroblasts. The appearance of fibroblast-like cells in older cultures is a morphological manifestation of the senescence of the specific cell types. Qualitative features of cultured adenoma cells showed that on the basis of cytomorphological properties "chromophobe" samples could be separated from the other types. Cells of acidophil and mixed type growths had the capacity of developing in vitro various migratory shapes, while "chromophobe" cells did not possess this ability. The histological diagnoses made independently from cultures confirmed the tissue culture findings. Differences of in vitro characteristics correlated also with the ultrastructural features of the cultured adenoma cells.

Key words: Human pituitary adenomas - Tissue culture - Cell culture - Ultrastructure

Our aim was to study the growth properties of surgically removed pituitary adenomas. The observations were made on tissue and cell cultures and were undertaken primarily to determine the life span of the adenoma cells. It was also of interest to ascertain how long pituitary tumour cells could preserve their healthy structure under in vitro circumstances.

From quantitative data of cell growth we wished to obtain parameters which would provide a basis for further experiments, aiming at the investigation of hormone production and secretion of cultured adenoma cells.

As regards the qualitative characteristics of the tumour cells, we wanted to ascertain whether pituitary adenomas of different histological types could be distinguished from one another on the basis of their in vitro behaviour.

Materials and Methods

Primary Cultures

Pituitary adenomas of 120 patients were submitted for culture immediately after surgery. Biopsies were explanted in two ways. For purposes of tissue cultures, tumour specimens were cut into fragments of about 1 mm³ placed in Leighton tubes on coverslips previously coated with plasma-embryonic extract. Biopsies explanted as cell cultures were mechanically dissociated and cell suspensions were inoculated into Leighton tubes containing coverslips. The fluid nutrient medium consisted of an 8:2 mixture of TC-199 and foetal calf serum with an addition of 40 μg/ml Gentamycin. As a rule, from all tumour samples five culture tubes were prepared with seven to eight explants or having a density of 1 - 3 x 10⁵ cells in each tube. The culture medium was changed after 48 h, then bi-weekly. The cultures were examined daily under the microscope and changes in the cells were studied until growth terminated. Appropriate cultures were fixed in absolute methanol and stained by the May-Grünwald-Giemsa method at varying intervals.

Subcultures

From the more rapidly growing biopsies confluent monolayers developed and the coverslips became completely covered. These primary cultures were subcultured. Cells were mechanically removed from the coverslips and resuspended in culture medium. Harvested cells were seeded into fresh culture tubes in the same way as the
primary cell cultures. Cell growth was controlled continuously and representative samples were fixed and stained or subcultured.

Electron Microscopy of Cultures
Cultures were fixed for 2 h at 4°C in 2.5% purified glutaraldehyde buffered to pH 7.4 with 0.1 M cacodylate, washed for 24 h in the same buffer, and postfixed for 1 h in 1% osmium tetroxide buffered at pH 7.4 with 0.1 M cacodylate then rinsed for a short time in the same buffer and dehydrated and embedded in Durcupan ACM (Fluka AG Buchs, Switzerland). The blocks were sectioned on a Reichert Om U2 ultramicrotome. The ultrathin sections were stained with 20% uranyl acetate in methanol and then with lead citrate by the method of Reynolds, and examined in a JEOL 100 C electron microscope, at 80 kV.

Histology
Histological diagnoses of the tumours were made at the Pathological Department of the Neurosurgical Institute, independent of the cultures. Their light- and submicroscopical characteristics are reported by Slowik et al.

Results

Primary Cultures
Tissue Cultures. Explants of tumour tissue 1 mm³ remained in one piece, or fragmented into groups of cells. Explants of 20 biopsies gradually disintegrated, while in 40 samples, cell growth started both from the main explants, and from the satellite fragments within 2–3 days (Table 1). The tissue pieces flattened on the bottom of the tube, and growth zones developed around their edges. Initially, the closely packed cells retained their original rounded shapes and later assumed polygonal shapes. The round cell nuclei were central or eccentric. The cytoplasm of the smaller cells stained uniformly basophilic, while the bigger polygonal cells presented intense basophil staining at the periphery only. The cell bodies were often foamy due to vacuoles, which sometimes contained eosinophilic inclusions. Besides the growth of adenoma cells, in some cultures a varying number of fibroblasts was also seen.

The outgrowth of fibroblasts did not precede the migration of parenchymal cells but the fibroblasts proliferated more actively than the epithelial cells so that fibroblasts were more numerous in older cultures. Explants, free from stromal elements developed pavement-like growth zones of adenoma cells without any fibroblasts (Fig. 1). In these cultures the life span of the tumour cells was not shorter than in growths with stromal and parenchymal elements.

The type of growth, outlined above, was characteristic of 31 biopsies. Histologically, these adenomas were “chromophobe” in 29 cases, while in the other two, classification of their nature was not possible (Table 1).

From solid explants of nine biopsies migration of elongated cells with cytoplasmic processes occurred. The adenoma cell could be observed elongating from their original rounded shape to a bipolar spindle form.

Table 1. Growth capacity of pituitary adenomas of different histological types 120 cases

<table>
<thead>
<tr>
<th>Histological diagnoses</th>
<th>Tissue cultures 60 cases</th>
<th>Cell cultures 60 cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grown</td>
<td>Not grown</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>“Chromophobe”</td>
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<td>9</td>
</tr>
<tr>
<td>Acidophil</td>
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<td>1</td>
</tr>
<tr>
<td>Mixed type</td>
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<tr>
<td>Not classifiable</td>
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<td>5</td>
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</tbody>
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Fig. 1. Pavement-like growth zone in a tissue culture of 10 days of a “chromophobe” adenoma. MGG. ×71

Fig. 2. Tapering cells in a tissue culture of a mixed type adenoma 5 days after explantation. MGG. ×70