TISSUE CULTURE STUDIES ON HUMAN PITUITARY TUMOURS: LONG TERM RELEASE OF ANTERIOR PITUITARY HORMONES INTO THE CULTURE MEDIUM

By

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ABSTRACT

A study was undertaken to determine the length of time that human pituitary tumours are capable of releasing anterior pituitary polypeptide hormones in vitro under basal conditions and to study the spectrum of hormone release by functioning and "non-functioning" pituitary neoplasms. Fragments from the pituitary tumours of 10 patients in the following categories: 1 Cushing's disease, 2 with amenorrhoea-galactorrhoea, 3 with acromegaly, and 4 with "non-functioning" pituitary tumours and from 2 normal human anterior pituitary glands were placed in primary culture immediately after surgery. The in vitro release of human growth hormone

Supported in part by grants from the U.S. Public Health Service, National Institutes of Health, Bethesda, Maryland C.A. – 07368, H.D. – 07843 and 07196, and A.M. – 04501; an Institutional General Research Support Grant R.R. – 05486–12 to the Massachusetts General Hospital, the Vincent Research Appropriation Fund of the Massachusetts General Hospital and by the Medical Research Council of Canada (M.T. – 1824).

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(hGH), prolactin (Prl), thyrotrophin (TSH), adrenocorticotrophin (ACTH), luteinizing hormone (LH), and follicle stimulating hormone (FSH) was measured by specific radioimmunoassays at the end of each week in culture. Hormone release was surveyed from 6 weeks to 6 months depending upon the survival of the culture. Hormone release patterns were compared with clinical and pathological data.

In the initial week of the study, all 6 anterior pituitary polypeptides were detected in the media from the 2 control pituitaries and from 4 of the tumours (1 amenorrhoea-galactorrhoea and 3 acromegaly) in concentrations up to 100 ng/ml of medium while 5 of the 6 hormones were readily detectable in the media from 2 additional tumour samples (Cushing's disease and 1 "non-functioning" pituitary tumour). The media of the remaining 4 tumours contained at least 3 of the 6 hormones (1 amenorrheoa-galactorrhoea and 3 "non-functioning" pituitary tumours). After 6 months in culture, the 6 hormones were readily detectable in at least 1 of the 5 surviving cultures and hGH (up to 800 ng/ml) and LH were each detectable in the media from 2 cultures. Although most of the hormone concentrations in the media decreased with length of time in culture, there were 2 exceptions. First, in the media from 5 of the 12 cultures from both controls and tumours, Prl concentrations increased after 50 to 80 days culture. This increase usually lasted for several weeks before Prl levels again began to decline. The second unusual finding occurred in a tumour from a patient with acromegaly in the media of which hGH levels rose from 60 ng/ml to 800 ng/ml between days 125 and 174. These findings of prolonged hormone release in vitro give promise of future usefulness of tissue culture methods for study of polypeptide hormone releasing mechanisms and long-term production of human anterior pituitary hormones for use in research and possible therapy.

The direct analysis of human pituitary function is difficult. Reports have included the results of measurement of hormone content of abortuses, cadavers or surgical specimens (Zimmerman et al. 1974; Pasteels et al. 1977); cavernous sinus catheterization during pituitary surgery (Conway et al. 1969); and tissue culture studies of foetal pituitaries (Gitlin & Biasucci 1969; Pasteels 1972; Pierson et al. 1973) or adult pituitaries and pituitary tumours removed at surgery (Kohler et al. 1969; Gala 1971; Batzdorf et al. 1971; Teraoka 1972; Peillon et al. 1973; Crowell et al. 1974; Skyler et al. 1977; Lipson et al. 1978).

Foetal pituitary cells have been shown to synthesize and release polypeptide hormones and their subunits in vitro as well as to respond to the presence of exogenous hormones and releasing factors (Groom et al. 1971; Groom & Boyns 1973; Siler-Khodr et al. 1974; Pasteels et al. 1977). Normal and adenomatous adult human pituitaries release as many as 6 hormones from a single specimen in vitro (Lipson et al. 1978), synthesize hGH de novo (Kohler et al. 1971; Peillon et al. 1973), and release subunits of LH and FSH into the culture medium (Beitins et al. 1977). Furthermore, in a report of a large series of human pituitary tumours studied in tissue culture, all 30 of the "non-func-
tining" pituitary tumours released one or more of the 6 pituitary hormones assayed in the medium in easily detectable concentrations (Lipson et al. 1978).

We have extended our previous observations of anterior pituitary hormone release over a one-week period in vitro to periods lasting as long as 6 months. Thus, the long-term in vitro release of hGH, Prl, ACTH, TSH, LH, and FSH from 2 adult pituitary glands and 10 pituitary adenomas removed at surgery has been determined. The purpose of this investigation was to ascertain the length of time primary pituitary cultures could release anterior pituitary hormones and to determine the feasibility of using the techniques of tissue culture for long-term hormone production.

METHODS

Patient selection for surgery

Seven women and 5 men, ages 20 to 68, requiring hypophysectomy were included in this study. Criteria for surgery included one or more of the following: decreased visual fields, enlarged or irregularly shaped sella turcica with suprasellar extension of the tumour mass, and excessive hormone secretion. It should be emphasized that this study was retrospective and that a uniform protocol was not employed. In most instances, only those clinical studies were performed that were essential for establishment of the diagnosis.

Based upon the clinical appearance, radiological findings, and hormone determinations, the patients were classified as follows: 1 Cushing’s disease, 2 with amenorrhoeagalactorrhoea, 3 with acromegaly, and 4 with "non-functioning" pituitary tumours. Two patients with breast or prostate carcinoma, who had previously undergone gonadectomy, were subjected to palliative total hypophysectomy.

Surgery

The tumour mass was approached by the transsphenoidal route. Microsurgical dissection was performed on the sellar contents and tissue fragments were removed under sterile conditions. The tissue was divided in the operating room so as to yield homogeneous portions for pathological examination and tissue culture.

Pathology

The pituitary fragments were fixed in 10% formalin, dehydrated, sectioned and stained with haematoxylin and eosin. In selected cases, PAS-orange G staining was also used. The criteria for the pathological diagnoses included the absence of neural tissue and the presence of eosinophilic or basophilic staining of the pituitary cells. If the cells failed to accumulate stain they were classified as chromophobe.

Tissue culture studies

After weighing, the pituitary fragments were gently agitated in 3 changes of Ham’s F 10 nutrient medium (Ham & Puck 1962) containing 15% foetal calf serum. The tissue was finely diced into cubes of 1 mm³. Ten of these were placed into a 25 cm² tissue culture flask and 1 ml of medium was added per 10 mg wet weight of pituitary tissue. Each flask was then placed in a water-jacketed tissue culture incubator at 37°C. Cellular growth was observed with a phase contrast light microscope. At the end of
the first week in culture and after each subsequent 7 days, the medium was removed from each flask, centrifuged at 2000 × g for 5 min to remove particulate matter, divided into aliquots and stored frozen at –20°C for subsequent hormonal assays.

**Hormonal measurements of culture media**

Hormonal determinations of hGH, PRL, ACTH, TSH, LH, and FSH were performed by the specific radioimmunoassay techniques as modified for tissue culture studies by Lipson et al. (1978). The sensitivities of these assays, inter- and intra-assay variabilities and standards used have been previously reported (Lipson et al. 1978). None of the 6 hormones was detectable in control culture medium. All samples of media from each pituitary specimen were assayed for each hormone at the same time.

**RESULTS AND DISCUSSION**

**Light microscopy studies of pituitary cells grown in culture**

Cellular growth was slow during the first 3 weeks *in vitro*. Initial morphological changes included the outgrowth of bipolar “fibroblastoid” cells from the periphery of the explants followed by the appearance of epitheloid cells adjacent to the explanted tissue. These nests of epitheloid cells were less firmly attached to the culture flask than the bipolar cells and were easily dislodged.

By the end of the first month in culture, sheets of bipolar cells were noted around each explant area, in some cases surrounding the frequently seen nests of the larger pituitary epitheloid cells (Thompson et al. 1959; Batzdorf et al. 1971; Teraoka 1972; Tixier-Vidal 1975; Lipson et al. 1978). The tendency of these epitheloid pituitary cells to form clusters, some of which are free floating, is similar to the behaviour of epithelial cells from other endocrine glands *in vitro* (Lissitzky et al. 1971).

At the end of 3 months *in vitro* the surviving cultures were confluent and in most cases there was layering of cells. The major cell type was the elongated bipolar cell and the number of clusters of epitheloid cells had decreased. A new cell type was noted which had extensive filmy cytoplasm and a small nucleus. The appearance of these cells was most compatible with that of cells of mesenchymal origin.

After 4½ months the cultures were distinguished by increasing density of the bipolar cells with both packing and stacking of cells, an increase in the mesenchymal-type cell population and a reduction or disappearance of the epitheloid cells.

There was no relationship between tumour type and length of survival *in vitro*. Pituitary tissue from the patient with Cushing’s disease (case 3, Table 1) and from one patient with a “non-functioning” pituitary tumour (case 11) remained viable *in vitro* for 6 weeks. The cultures from one of the controls (normal pituitary, case 1) and from one acromegalic patient (case 7) remained viable for 3 months. Tissue from case 4 (amenorrhoea-galactorrhoea) and case 8
### Table 1.

Pre-operative laboratory values and pathological diagnoses.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Clinical diag.</th>
<th>Path. diag.</th>
<th>hGH (ng/ml)</th>
<th>Cortisol 8 AM (µg/100 ml)</th>
<th>17-OHCS (mg/24 h)</th>
<th>LH (mIU/ml)</th>
<th>FSH (mIU/ml)</th>
<th>T (µg/100 ml)</th>
<th>TSH (µU/ml)</th>
<th>T&lt;sub&gt;4&lt;/sub&gt; (µg/100 ml)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>46</td>
<td>Ca of breast</td>
<td>Normal</td>
<td>–</td>
<td>&lt;1</td>
<td>1.8</td>
<td>9.8</td>
<td>3.9</td>
<td>0.09</td>
<td>3.3</td>
<td>8.0</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>68</td>
<td>Ca of prostate</td>
<td>Normal</td>
<td>&lt;1</td>
<td>0</td>
<td>2.2</td>
<td>2.5</td>
<td>0.09</td>
<td>3.3</td>
<td>5.4</td>
<td>6.0</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>28</td>
<td>Cushing's (early)</td>
<td>C. A.</td>
<td>1</td>
<td>22</td>
<td>42.0</td>
<td>2.2</td>
<td>2.5</td>
<td>0.09</td>
<td>3.3</td>
<td>5.4</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>20</td>
<td>Amenorrhoeagalactorrhoea</td>
<td>C. A.</td>
<td>&lt;1</td>
<td>18</td>
<td>3.3</td>
<td>2.9</td>
<td>1.6</td>
<td>2.1</td>
<td>2.1</td>
<td>6.0</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>23</td>
<td>Amenorrhoeagalactorrhoea</td>
<td>C. A.</td>
<td>–</td>
<td>15</td>
<td>12.0</td>
<td>9.1</td>
<td>2.8</td>
<td>–</td>
<td>–</td>
<td>9.5</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>52</td>
<td>Acromegaly (early)</td>
<td>E. A.</td>
<td>14</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>0.06</td>
<td>1.6</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>37</td>
<td>Acromegaly</td>
<td>Mixed</td>
<td>43</td>
<td>10</td>
<td>4.0</td>
<td>–</td>
<td>5.7</td>
<td>&lt;1.0</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>40</td>
<td>Acromegaly</td>
<td>E. A.</td>
<td>&gt;25</td>
<td>5</td>
<td>4.5</td>
<td>–</td>
<td>2.5</td>
<td>0.33</td>
<td>–</td>
<td>9.4</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>49</td>
<td>N. F. P. T.</td>
<td>C. A.</td>
<td>&lt;1</td>
<td>27</td>
<td>–</td>
<td>5.2</td>
<td>3.9</td>
<td>0.03</td>
<td>–</td>
<td>4.5</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>57</td>
<td>N. F. P. T.</td>
<td>C. A.</td>
<td>–</td>
<td>32</td>
<td>3.8</td>
<td>30.0</td>
<td>57.5</td>
<td>&gt;50</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>67</td>
<td>N. F. P. T.</td>
<td>C. A.</td>
<td>–</td>
<td>12</td>
<td>18.2</td>
<td>75.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>7.1</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>64</td>
<td>N. F. P. T.</td>
<td>C. A.</td>
<td>–</td>
<td>21</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.5</td>
</tr>
</tbody>
</table>

**Abbreviations:** C. A. = Chromophobe adenoma; E. A. = Eosinophilic adenoma; Mixed = Mixed chromophobe and eosinophilic adenoma; N. F. P. T. = “Non-functioning” pituitary tumour; T = Testosterone.

**Normal values:** hGH < 5–10 ng/ml; 8 a.m. Cortisol > 10 µg/100 ml; 17 OHCS (urine) 3–7.5 mg/24 h; T > 0.3 µg/100 ml in males; TSH 0–3.5 µU/ml; T<sub>4</sub> 4–11 µg/100 ml; male follicular LH 5–40 mIU/ml FSH 5–40 mIU/ml mid-cycle LH 20–22 mIU/ml FSH 15–30 mIU/ml luteal LH 50–250 mIU/ml FSH 28–94 mIU/ml post-menopause or castrate LH 15–30 mIU/ml FSH 50–200 mIU/ml
(acromegaly) survived in vitro for 4 months while case 10 ("non-functioning" pituitary tumour) survived for an additional month. The remaining 5 cultures consisting of tissue from normal pituitary (case 2), amenorrhoea-galactorrhoea (case 5), acromegalic tumour (case 6) and 2 "non-functioning" pituitary tumours (cases 9 and 12) were viable for at least 6 months. Prolonged survival of primary pituitary cultures in vitro has been reported for both foetal and adult pituitaries and pituitary tumours. Foetal glands have been grown in culture for 150 days (Gailani et al. 1970) and up to 350 days (Siler-Khodr et al. 1974). Adult glands and tumours have remained viable in vitro for 150 days (Teraoka 1972), 250 days (Thompson et al. 1959), 350 days (Kohler et al. 1969) and up to 405 days (Taskjian 1969). In all cases of prolonged growth, the cultures eventually become overgrown with cells of fibroblastic type.

Radioimmunoassayable hormone concentrations in the culture media from human pituitary cells

After each week in culture, the medium was removed and frozen at –20°C. When a culture was observed to be no longer viable, all samples of its medium were subjected to specific radioimmunoassays to determine the concentrations of the following hormones: hGH, Prl, ACTH, TSH, LH, and FSH. It is recognized that the hormone concentrations in the medium represent synthesis and release from the pituitary cells, release of preformed hormone, and hormone leakage from cells as well as hormonal degradation within the medium.

Carcinoma of the breast and prostate

The control pituitaries came from a woman with carcinoma of the breast (case 1) and a man with prostatic carcinoma (case 2, Table 1) who underwent palliative transsphenoidal hypophysectomy after previous gonadectomy had failed to alter the course of their disease. Both pituitaries were classified as having normal morphology on pathological evaluation. These specimens released substantial quantities of hGH, Prl, ACTH, TSH, LH, and FSH at the end of the first 7 days in vitro. In semilogarithmic plots of hormone release per ml of medium versus days in culture (Fig. 1), both pituitaries showed a progressive decrease in hormone release with length of time in culture. In case 1, however, the culture was no longer growing actively after 3 months (day 87) at a time when release of Prl (131 ng/ml), hGH (25 ng/ml) and LH (49 ng/ml) was still readily detectable. The culture from case 2 survived for 190 days but hormone release was undetectable after 132 days. It is notable that in these controls there appeared to be 2 distinct groupings of hormones by rate of release: those which declined rapidly with time in culture (TSH, ACTH and FSH) and those which continued to be secreted for a longer period of time and which declined in concentration less quickly (hGH, Prl and LH). The persistence
Fig. 1.
Semilogarithmic plots of anterior pituitary polypeptide hormones released in vitro per ml of medium versus days in culture. The pituitaries from cases 1 and 2 were normal on pathological evaluation and were removed for palliation of advanced breast or prostate carcinoma. All hormone concentrations are in ng/ml except for TSH which is in μU/ml.

of this latter group of 3 hormones implies de novo hormone synthesis in vitro from these pituitary cells. The few long-term studies of normal adult human pituitary glands in culture have involved measurements of a maximum of 3 hormones from any one sample. These glands were likewise derived from patients with advanced carcinoma or severe diabetic retinopathy. Prl was found to be released for 27 days (Gala 1971), hGH for 405 days (Tashjian 1969), hGH and LH for 30 days (Teraoka 1972), TSH for 60 days and LH and hGH for 125 days (Kohler et al. 1969). The concentrations of these hormones in the media fell rapidly as the time in culture increased. Normal foetal pituitaries have been cultivated up to 1 year and have been shown to release hGH for 80 days (Solomon et al. 1969), TSH for 25 days and hGH for 150 days (Gailani et al. 1970); hGH, ACTH, TSH and FSH for about 20 days and LH for 68 days, and Prl for 60 days (Siler-Khodr et al. 1974); LH, FSH, TSH and β-subunits and a-subunit for at least 42 days (Pasteels et al. 1977). All hormone concentrations dropped rapidly with time in culture except for Prl (Siler-Khodr et al. 1974) where an initial decline was followed by an increase in concentration by the third week. During the sixth week of culture, levels of Prl ex-
ceeded those released during the first week (possibly reflecting, removal of hypothalamic restraint). In a study of gonadotrophins and their subunits released by cultured foetal pituitaries, Pasteels et al. (1977) demonstrated an increase in FSH secretion and continued α-subunit release despite the rapid fall of LH, βLH and TSH. Thus, there are no comparable studies of adult pituitaries and pituitary tumours.

Cushing's disease

The pituitary tumour from the patient with Cushing's disease (case 3) released mainly ACTH, hGH and Prl after the first week in vitro. The ACTH concentration was 11 ng/ml, which was in the range of the control pituitaries. hGH concentration was 1.350 ng/ml which was only 2% of that observed in controls. After 3 weeks in culture, these hormones were barely detectable. Prl, however, was present in high concentration at the end of the first week in culture (65 000 ng/ml). This value was increased 30-fold over controls. The Prl concentration declined to 16 500 ng/ml after 3 weeks in vitro and to 2 300 ng/ml after 6 weeks. This high Prl concentration occurred in a male patient who had neither gynaecomastia nor galactorrhoea, but who pre-operatively had decreased levels of gonadotrophins, and testosterone, and an increased plasma prolactin concentration (greater than 70 ng/ml), Table 1. The relationship be-

Table 2.
The Prl content of the culture medium from a tumour removed from a patient with the amenorrhea-galactorrhoea syndrome (Case 4).

<table>
<thead>
<tr>
<th>Days in culture</th>
<th>Prl concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>102 000</td>
</tr>
<tr>
<td>14</td>
<td>59 000</td>
</tr>
<tr>
<td>21</td>
<td>4 600</td>
</tr>
<tr>
<td>28</td>
<td>4 400</td>
</tr>
<tr>
<td>35</td>
<td>2 200</td>
</tr>
<tr>
<td>42</td>
<td>2 100</td>
</tr>
<tr>
<td>49</td>
<td>2 800</td>
</tr>
<tr>
<td>63</td>
<td>3 400</td>
</tr>
<tr>
<td>70</td>
<td>2 800</td>
</tr>
<tr>
<td>84</td>
<td>1 800</td>
</tr>
<tr>
<td>105</td>
<td>138</td>
</tr>
</tbody>
</table>

Amenorrhoea-galactorrhoea syndrome

The tumours from 2 patients with amenorrhoea-galactorrhoea syndrome were studied in vitro (cases 4 and 5). Pre-operatively both patients had serum PRL levels greater than 360 ng/ml and low gonadotrophin concentrations, but normal indices of thyroid and adrenal function. In vitro the 2 tumours behaved very differently. Case 4 released only trace amounts of hGH and LH after the first week in vitro. PRL release was very high (Table 2) and remained substantial for 3 months in culture. Of interest is the findings that between day 21 and 42 in vitro PRL secretion fell slowly from 4600 to 2100 ng/ml. A significant rise in PRL release then occurred which lasted from day 49 through at least day 70. A similar increase in PRL release was seen in several of the other pituitary cell cultures and occurred between days 50 to 80. Other pituitaries exhibiting this phenomenon were case 1 (control), cases 6 and 7 (acromegaly) and case 9 ("non-functioning" pituitary tumour). An increase of PRL release in vitro may represent a loss of hypothalamic control of PRL synthesis and release and has been reported only in cultures from human foetal pituitaries (Siler-Khodr et al. 1974). Case 5 was unusual in that the tumour released large quantities of all 6 hormones tested after one week in culture. hGH was 120000 ng/ml, PRL was 62500 ng/ml, ACTH was greater than 40 ng/ml, TSH greater than 700 µU/ml, LH was 4400 ng/ml and FSH was 1700 ng/ml. These elevated values were all well above those from the control pituitaries and they persisted with slow decline through the first 56 days in culture (hGH 16500 ng/ml, PRL 2600 ng/ml, ACTH 26 ng/ml, TSH 150 µU/ml, LH 182 ng/ml and FSH 35 ng/ml at 56 days). After 178 days in vitro hGH, ACTH, TSH, LH and FSH were still detectable. Cases of this type hold promise for high yield production of various human anterior pituitary hormones, provided that the hormones possess biological activity.

Acromegaly

Cases 6, 7 and 8 were patients with acromegaly diagnosed by clinical and radiological criteria and by elevated serum hGH levels (Table 1). One man with early acromegaly (case 6) had hypoadrenocorticism, hypogonadism and hypothyroidism. Cases 7 and 8 had normal basal adrenal and thyroid function. The first week in culture, the tumour from case 7 released low levels of ACTH, TSH, LH and FSH. The concentration of PRL was 346 ng/ml and that of hGH was 8500 ng/ml (approximately 12% of that released from control cultures - perhaps reflecting higher hGH turnover and decreased storage in the tumour.
Semilogarithmic plots of anterior pituitary hormones released in vitro per ml of medium versus days in culture. The pituitary tumour from case 6 was removed from a patient with acromegaly and was identified as an eosinophilic adenoma on pathological evaluation. All hormone concentrations are in ng/ml except for TSH which is in µU/ml.

cells as compared with the normal gland). By 3 weeks in vitro only Prl and hGH persisted, but at greatly reduced levels. The hormone release from case 6 is shown in Fig. 2. As in case 7, gonadotrophin secretion was low and did not long continue. Although the TSH concentration was initially 200 µU/ml and that of ACTH was 11 ng/ml, both had declined to the lower limits of detection by days 60 and 30, respectively. Case 6 is interesting by virtue of an increase in Prl release between days 58 and 100 and an even more striking increase in hGH secretion between day 125 (60 ng/ml) and day 174 (800 ng/ml). Just prior to termination of the culture, hGH release was still 250 ng/ml (day 184). Tumours from patients with acromegaly have been studied in vitro by several investigators. Kohler et al. (1971) and Peillon et al. (1973) demonstrated de novo synthesis of hGH in cultures of these tumours for up to 60 days (production decreased with time in culture). These tumours have been shown to release up to 3 hormones for nearly 1 year (hGH, LH and TSH by Kohler et al. (1969) and Tashjian (1969)). Initial concentrations of hGH were lower in these tumours than in controls, while TSH and LH concentrations were variable. No previous study has demonstrated an increase in hGH release during time in culture.
Case 8 is unusual in that high concentrations of the 6 hormones assayed were present at the end of the first week of culture (hGH 60 000 ng/ml, Prl 7500 ng/ml, ACTH 800 ng/ml, TSH 460 μU/ml, LH 2235 ng/ml and FSH 292 ng/ml). These high levels persisted for over 49 days for 3 of these hormones (hGH 6000 ng/ml, Prl 3150 ng/ml, and LH 158 ng/ml). The release pattern exhibited by case 8 is very similar to that from case 5 in that high levels of several hormones were released from the tumour over a prolonged period.

"Non-functioning" pituitary tumours

Four specimens were cultured from patients with “non-functioning” pituitary tumours (cases 9–12, Table 1). Pre-operatively these patients had normal 8 a.m. plasma cortisol levels. The only male had hypogonadism (case 9, plasma testosterone 30 ng/ml). One woman had a low T₄ (2.5 μg/100 ml) and an associated high TSH (greater than 50 μU/ml). All women were post-menopausal with elevated FSH and lower than expected LH levels were measured (cases 10 and 11). The media from the first week of culture of the tumours from cases 10, 11 and 12 contained mainly LH and FSH with scarcely detectable levels of any of the other hormones. Initial concentrations of LH ranged from 39 (case 12) to 330 ng/ml (case 10) and of FSH from 25 (case 11) to 340 ng/ml (case 10). Gonadotrophin release continued at a low level throughout the life

Case 9

Semilogarithmic plots of anterior pituitary hormones released in vitro per ml of medium versus days in culture. The pituitary tumour from case 9 was identified as a chromophobe adenoma on pathological evaluation.
of each of these cultures. Case 9 was different in that moderate levels of hGH (95 ng/ml) and Prl (325 ng/ml) were present initially in the culture besides LH and FSH (Fig. 3). Although the concentration of all 4 hormones fell with time in vitro, an increase in both Prl and hGH release occurred simultaneously between 45 and 95 days. In a long-term study of 5 "non-functioning" pituitary tumours, Kohler et al. (1969) studied the release of LH, TSH and hGH. These investigators found that a rapid decline in hormone release occurred from these tumours in vitro, but that hGH was detectable up to 66 days, LH to 113 days and TSH 184 days. It is paradoxical that these so-called "non-functioning" pituitary tumours are capable of releasing 2 or more hormones up to 6 months in vitro. Among possible explanations for this finding are: a) the tumours may contain nests of normal pituitary cells that continue to function and release hormones, or b) the chromophobe-appearing cells per se may be capable of secreting hormones at a low to moderate level but, because of lack of hormone granule storage, fail to stain. (Lipson et al. 1978).

These studies demonstrate that normal adult human pituitaries and pituitary tumours are capable not only of surviving longer than 6 months in culture, but also of releasing one or more anterior pituitary hormones into the medium even after this length of time in vitro. High concentrations of hGH, Prl, ACTH, TSH, LH and FSH (up to 0.1 mg/ml) were secreted by these cultures initially and even after 7 weeks. Normal pituitaries and tumours have been shown capable of releasing numerous hormones into the culture medium, sometimes several in high concentration. LH, Prl and hGH tend to be secreted longer in vitro than do FSH, ACTH and TSH. Release of Prl and hGH has been shown to increase under the culture conditions employed. Thus it seems possible that by modifying this tissue culture system, it may be possible to enhance hormone output sufficiently to render tumours and normal pituitaries a source of human anterior pituitary polypeptide hormones. Such modifications might include: a) addition to the medium of growth factors, cyclic nucleotides or hypothalamic releasing factors; b) the use of monolayer cultures with viral transformation to impart greater survival potential to these cells; c) the use of Sendai virus to fuse such isolated cells with more malignant lines so that survival for long periods of time is assured and d) cloning of these transformed or fused cells (Ishikawa et al. 1977; Steinberger et al. 1973). In this manner long-term populations of specific cell types could be produced for synthesis of large quantities of these hormones and also for dissection of the processes of anterior pituitary hormone biosynthesis and release.

ACKNOWLEDGMENTS

We wish to extend our gratitude to members of the Department of Neurosurgery, Drs. Nicholas Zervas, Robert Ojemann, Charles Poletti, Henry Schmidek, William Sweet

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and James Wepsic for contributing many of the pituitary tumour tissues further characterized in these studies. We also thank Mrs. Kathleen O'Loughlin, Mrs. Leslie Johnson, Mr. Alan Maltz, Mr. Theodore M. Liszezak and Ms. Beverly O. Whitman for their excellent technical assistance.

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Received on September 5th, 1978.