Human growth hormone in cultures of human pituitary tumors

ULRICH BATZDORF, M.D., VIVIAN GOLD, B.S., NANCY MATTHEWS, B.A., AND JOSIAH BROWN, M.D.
Departments of Surgery/Neurosurgery, and Medicine/Endocrinology, UCLA School of Medicine, and the Brain Research Institute, Los Angeles, California

Tissue culture medium of 14 human pituitary adenomas removed from acromegalic and hypopituitary patients was analyzed for human growth hormone (HGH). High HGH levels were detected in the culture medium of all adenomas removed from acromegalic patients. HGH was undetectable in the tissue culture medium of six of eight adenomas removed from hypopituitary patients. Elevated HGH levels were also found in the culture medium of pituitary tissue obtained from a juvenile diabetic patient.

KEY WORDS · pituitary adenoma · human growth hormone · tissue culture · acromegaly · hypopituitarism

Human pituitary tumors may be separated, on clinical grounds, into those that are hormonally active and those that are hormonally inactive, the latter being capable of reducing or abolishing function of the normal pituitary gland. Hormonally active tumors produce the clinical states of acromegaly and pituitary gigantism as well as the rare states of pituitary-induced Cushing's disease. Hormonally inactive tumors produce their effects by compression and ultimate obliteration of the normal pituitary gland, resulting in various degrees of hypopituitarism. The histological correlates of these clinical states are the eosinophilic tumors associated with acromegaly and pituitary gigantism, the rare basophilic adenomas associated with Cushings disease, and chromophobe adenomas associated with hypopituitarism. In addition, there are mixed chromophobe-eosinophilic tumors which exhibit features of acromegaly or of a hypopituitary state occasionally associated with fugitive acromegaly. Hypopituitarism associated with chromophobe adenoma is the most common of these entities followed in frequency by acromegaly occurring with eosinophilic or mixed adenomas.

In general, the clinical condition of acromegaly is correlated with the histological diagnosis of eosinophilic or mixed adenoma. The histological diagnosis of chromophobe adenoma, however, is not uncommon in patients with acromegaly. In a recent survey of 64 patients with pituitary tumors, a histological diagnosis of chromophobe adenoma was made in five of 12 acromegalic patients whereas the remaining seven had eosinophilic or mixed tumors, as anticipated. The hypopituitary patients, without exception, had chromophobe adenomas.

Human pituitary tumors grow in tissue culture under favorable conditions. Growth hormone has been identified in cultures of human pituitary glands, although other studies have not corroborated this finding. Elaboration of growth hormone has been reported to occur in cultures of a transplantable rat pituitary tumor and in incubated normal rat pituitary gland. The de-
velopment of more accurate assay techniques for human growth hormone (HGH) has made possible analysis of the growth medium of pituitary tissue cultures and has greatly facilitated this research. The recent report of Kohler and associates demonstrates the synthesis of human growth hormone, as well as luteinizing hormone (LH) and thyroid-stimulating hormone (TSH) in tissue cultures of pituitary tumors from acromegalic patients.

**Method**

Fragments of pituitary tissue from 19 patients (18 tumors and one non-neoplastic gland from a juvenile diabetic patient) were cultured in Rose chambers and Pyrex tissue culture ("T") flasks. Tissue obtained at operation was freshly minced for explant cultures and disaggregated in 0.25% trypsin for suspension cultures. Estimates of cell concentration were made in the most recent experiments by performing hemocytometer counts. The culture medium used was glucose-enriched (600 mg/liter) Eagles's medium containing 20% fetal bovine serum, penicillin, and streptomycin. Cultures were incubated at 37°C in a moisturized atmosphere of 5% CO$_2$ and 95% air.

The cultures were observed and photographed under the phase microscope. The culture medium was removed after various periods of growth of the cells, frozen, and then analyzed for HGH by the technique of Glick and associates as modified by Boden and Soeldner. Medium derived from cultures of nonendocrine brain tumors was used as a control, and freshly prepared culture medium was used as a blank. Cultures were fixed in formalin after different periods of growth and were stained with standard histological stains. A small number of cultures were stained with PAS-orange G, which shows an affinity for the secretory granules of eosinophilic cells.

In the earlier studies in this series, samples of culture medium withdrawn from one tumor culture at different times were pooled and analyzed as a single sample. After these pilot studies were completed, samples were obtained at weekly intervals and were analyzed separately for their content of HGH. Four samples were not included in the final analysis because the culture medium was sampled after a significantly longer period of time than was true for the remaining cultures.

The diagnosis of acromegaly and hypopituitarism was established by the usual clinical criteria. Characteristic changes in appearance were present in all acromegalic patients, although they were more pronounced in some than in others. Hypopituitary states ranged from mild to severe.

All patients underwent detailed medical and neurological evaluation, including examination of their visual system. Radiological examination included contrast studies in every patient. A laboratory endocrine survey was also performed on every patient. Plasma determinations of HGH were obtained on four acromegalic patients and on four hypopituitary patients as part of their preoperative endocrinological evaluation. The same assay technique used for tissue culture medium was also used for the plasma HGH determination.

**Results**

The growth in all cultures of pituitary tissue in the present study was good to excellent (Fig. 1). Cell counts in suspension cultures ranged from 35,000 to 100,000 cells per cubic millimeter initially and then declined. In our experience, pituitary tumor cultures characteristically become overgrown by mesenchymal cells after approximately 2 weeks in culture (Fig. 2). Growth hormone values were maximal between the 7th and 14th day in culture (Table 1), during which time all cultures were healthy in appearance. Values for preoperative plasma HGH are also listed whenever obtained. The fluctuation in culture medium HGH of two tumors was recorded over a 4-week period (Fig. 3). Culture medium HGH values from tissue obtained from the hypopituitary patient declined quickly, whereas tissue from an acromegalic patient shows peak levels of HGH at 2 weeks. Following this the culture is gradually overgrown by mesenchymal cells.

Fasting plasma HGH values were elevated above normal in all four patients with acromegaly and were within or close to the normal range (0–5 mU/ml) in three of four patients who were clinically regarded as hypopituitary. The fourth patient (Case 303)
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was in the first trimester of pregnancy at the
time of diagnosis and at the time of opera-
tion for pituitary tumor. Fasting preopera-
tive plasma HGH values of the diabetic pa-
tient were normal.

Culture medium HGH levels during the 7-
to 14-day growth period were similarly ele-
vated in all six patients diagnosed clinically
as acromegalic and were too low to be de-
tectable in six of eight patients shown to ex-
hibit various degrees of hypopituitarism. The
elevation in one hypopituitary patient (Case
220) will be discussed. The other is the
same patient (Case 303) who exhibited
slightly elevated plasma HGH levels during
the first trimester of pregnancy. Culture me-
dium derived from tissue obtained from a
24-year-old woman who underwent hypo-

physectomy for severe diabetic retinopathy
showed elevated HGH levels. Freshly pre-
pared culture medium and medium obtained
from cultures of nonendocrine brain tumors
did not show any detectable HGH.

The histological diagnosis of eosinophilic
adenoma was made in four of the six
acromegalic patients (Table 1). One patient
(Case 100), however, was thought to have a
chromophobe adenoma with focal eosino-
philic hyperplasia (mixed adenoma) while

another tumor (Case 305) was interpreted
as a classical chromophobe adenoma (Fig.
4). The clinical diagnosis of acromegaly was
beyond controversy in both of these patients
(Fig. 5) and was confirmed by elevated heel
pad measurements and, in Case 305, by ele-
vated preoperative serum HGH levels. All of

![Fig. 1. Photomicrograph of pituitary chromo-
phobe adenoma showing typical rounded cells
forming a colony. In many cultures such clusters
do not attach readily to glass. 13-day-old culture.
Phase contrast, × 500.](image1)

![Fig. 2. Pituitary chromophobe adenoma cells
form darkly staining colonies surrounded by faintly
staining mesenchymal cells. Numerous gland struc-
tures are visible in this 90-day-old culture. Nissl
stain, × 125.](image2)
the tumors removed from patients who were hypopituitary were identified as typical chromophobe adenomas. The tissue obtained from the diabetic patient was normal adenohypophysis. Details of the growth characteristics of pituitary adenomas in vitro will be presented in a separate report.

Discussion

The validity of serum HGH assays has been established by numerous studies. Such determinations are now recognized as a valuable diagnostic tool in the evaluation of patients suspected of having acromegaly and other endocrine disorders.6,9,14

Growth hormone (GH) content has been measured in the extirpated pituitary glands of rats8,19 and in a transplantable rat mammosomatotropic pituitary tumor.5,13,15 Blood studies have been carried out on tumor-bearing rats and have shown elevated hormone content.2 This tumor could be cultured,7 but sustained hormone secretion was not achieved until the tumor cells were cloned.

TABLE 1

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Histological Diagnosis</th>
<th>HGH mug/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Preop. plasma</td>
</tr>
<tr>
<td>Acromegalic Patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>eosinophilic</td>
<td>48</td>
</tr>
<tr>
<td>100</td>
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<td>—</td>
</tr>
<tr>
<td>117</td>
<td>eosinophilic</td>
<td>27</td>
</tr>
<tr>
<td>258</td>
<td>eosinophilic</td>
<td>—</td>
</tr>
<tr>
<td>284</td>
<td>eosinophilic</td>
<td>&gt;32</td>
</tr>
<tr>
<td>305</td>
<td>chromophobe</td>
<td>25</td>
</tr>
<tr>
<td>Hypopituitary Patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>chromophobe</td>
<td>—</td>
</tr>
<tr>
<td>82</td>
<td>chromophobe</td>
<td>—</td>
</tr>
<tr>
<td>144</td>
<td>chromophobe</td>
<td>—</td>
</tr>
<tr>
<td>180</td>
<td>chromophobe</td>
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</tr>
<tr>
<td>208</td>
<td>chromophobe</td>
<td>&lt;4.0</td>
</tr>
<tr>
<td>226</td>
<td>chromophobe</td>
<td>—</td>
</tr>
<tr>
<td>220</td>
<td>chromophobe</td>
<td>5.4</td>
</tr>
<tr>
<td>303</td>
<td>chromophobe</td>
<td>8.0</td>
</tr>
<tr>
<td>Diabetic Patient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>326</td>
<td>normal adenohypophysis</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

Clonal strains of the rat mammosomatotropic tumor produced GH in vitro, thus establishing that these tumor cells were able to maintain their organ-specific function.25,27

Culture of Adenomas from Acromegalic Patients

The present study confirms the release of HGH in tissue culture by pituitary adenomas surgically removed from acromegalic patients. High levels of HGH were detected in the tissue culture medium of every pituitary adenoma. Our short-term cultures do not permit us to conclude with certainty that HGH is actively produced in culture, but we do have evidence to support such a hypothesis. Kohler, et al.,8 in recently published studies of long-term cultures, demonstrated in vitro HGH production from cultured normal pituitaries for approximately 4 months. Cultured adenomas from acromegalic patients secreted HGH for as long as 1 year. The configuration of the curves for levels of HGH in culture medium

![Fig. 3. Graph showing the levels of human growth hormone (HGH) detectable in the culture medium of two pituitary tumors. Samples obtained at weekly intervals; 303 A and B represent duplicate cultures of the same tumor.](image-url)
over a 4-week period (Fig. 3) shows a peak level of HGH at 2 weeks. It can be surmised that if HGH were merely released from storage or carried over into the culture vessel, rather than being actively secreted, levels of HGH would show a steady decline soon after the tumor was brought into culture. Thus, our investigation confirms the findings of Kohler, et al., that secretion of HGH takes place in cultures of adenomas removed from acromegalic patients.

Just as sustained GH secretion was not demonstrable in the rat mammosomatotropic tumor until a clonal strain was established, we suspect that the prevention of early fibroblastic overgrowth by Kohler, et al., with the resultant establishment of a purer culture, permitted detection of HGH secretion for longer periods of time. Mesenchymal cell overgrowth visibly restricts the growth of pituitary tumor cells (Fig. 2). Unfortunately, the elegant cloning technique developed by Yasumura and others, which was highly successful for rat pituitary tumors, is not applicable to human pituitary adenomas. Direct cloning techniques of human pituitary tumors so far have proved unsuccessful in our experience.

The unexpected histological diagnosis of "chromophobe" adenoma in the case of surgically removed tumors of acromegalic patients has long been a source of confusion, although this finding is by no means rare. An excellent discussion of this problem is presented by Russfield. In our own experience almost half of a group of 12 acromegalic patients was shown to have chromophobe adenomas. The detection of large quantities of HGH in the culture medium of an acromegalic patient whose tumor was diagnosed as a typical chromophobe adenoma points out that this biological test correlates more accurately with the clinical diagnosis than does the histological diagnosis by light
microscopy and hematoxylin and eosin stain. It is possible that the secretory granules in some mixed tumors do not stain in the usual manner, for yet unknown reasons, thus leading to the diagnosis of a chromophobe adenoma. Degranulation from intense secretory activity may also lead to the inaccurate diagnosis of an agranular, or chromophobe, adenoma.\textsuperscript{22}

Kohler, \textit{et al.},\textsuperscript{16} showed that adenomas from patients who had no clinical evidence of hormone hypersecretion did produce hormones in culture. One such adenoma produced HGH for 66 days while several other such tumors produced LH and TSH in culture. These tumors were regarded as "chromophobe" adenomas and the possibility that such tumors can produce hormones \textit{in situ} in small quantities has thus been raised. Abortion gland formation has been seen in a culture of a typical chromophobe adenoma (Fig. 2), which strengthens the concept that these tumors may have a secretory capacity. Evidence from electron microscopic studies, cited by Russfield,\textsuperscript{22} may provide yet another explanation for the disparity between the clinical picture and the histological diagnosis.

It is difficult to ascertain whether the adenoma in tissue culture releases and produces HGH by the same mechanism as it does when the adenoma, \textit{in situ}, is under the influence of hypothalamic releasing factor. Indeed, the adenoma may be independent of such hypothalamic control.

\textit{Culture of Adenomas from Hypopituitary Patients}

Failure to demonstrate elaboration of growth hormone by adenomas removed from six of eight patients with mild to severe hypopituitarism is not surprising. Plasma HGH levels, determined in two of these patients, were within limits of normal. The detection of HGH in plasma at normal levels may be attributed to the presence of some residual normal pituitary gland. All eight of these patients were shown to have chromophobe adenomas.

Detection of HGH in the tissue culture medium of two chromophobe adenomas from patients with no clinical evidence of pathologic HGH hypersecretion is of considerable interest. One of these patients (Case 303) was the pregnant patient mentioned before. While it is recognized that the pituitary gland normally enlarges during pregnancy, this patient had visual field defects and radiologically demonstrated suprasellar tumor extension. It is now believed that maternal HGH levels remain normal throughout most of pregnancy, whereas chorionic growth hormone prolactin (CGP) levels rise, beginning early in the first trimester.\textsuperscript{15,13} The CGP, which may cross-react immunologically with HGH, is said to be undetectable in adult pituitaries,\textsuperscript{22} which would imply that growth hormone activity in the culture medium of the adenoma is due to HGH and not to CGP. Our observations would thus seem to be in conflict with the concept that maternal HGH levels do not rise during pregnancy. Other interpretations, however, may account for the presence of HGH in the tissue culture fluid of these two adenomas, as well as the very slight (Case 220) and moderate (Case 303) elevation of plasma HGH observed in the two patients preoperatively.

The simplest interpretation would be that we are dealing with remnants of normal pituitary gland, some of which were contained in the sample taken for tissue culture. The value of HGH in the tissue culture medium of one chromophobe adenoma, however, was in the acromegalic range. It is thus possible that these two tumors represented mixed adenomas whose eosinophilic components were not recognized for reasons mentioned above. The plasma HGH levels are nearly normal in one patient and the elevation in the second patient may not have been sufficient to produce clinical evidence of acromegaly. The possibility that immunological cross reaction between HGH and CGP might account for the elevated plasma HGH levels in Case 303 cannot be excluded. True adenomas requiring surgery during pregnancy are so rare that confirmation of our findings cannot be expected in the near future.

Sampling of the tissue culture medium of one of these chromophobe adenomas which produced detectable quantities of HGH (Case 303) showed rapid decline in the quantity of HGH over the 4-week culture
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period (Fig. 3). This is in sharp contrast to the elaboration of HGH by a culture of tumor from an acromegalic patient.

Culture of Pituitary Gland from a Diabetic Patient

Unexpected high values of HGH were detected in the culture medium of a pituitary gland from one patient with juvenile-onset diabetes who underwent hypophysectomy. This finding correlates with a recent report of elevated serum HGH levels in insulin-treated juvenile-onset diabetic patients. Further studies are planned to confirm this observation and hopefully to produce a long-term culture of such tissue. The depression of blood sugar levels in the insulin-treated patient with juvenile-onset diabetes is thought to account for the elevated serum HGH levels observed in these patients. Evidence of increased HGH secretion by cultured pituitary cells of such patients would be of greatest interest. Preoperative serum HGH levels in this patient were normal, as was the response to arginine stimulation. The lack of agreement between plasma and tissue culture fluid HGH values in this patient might be explained by the wide fluctuations in plasma HGH levels that are seen in such individuals.

Summary

The tissue culture medium of adenomas removed from six acromegalic and eight mildly or severely hypopituitary patients was analyzed for HGH content. High HGH levels were found in the media from all adenomas removed from acromegalic patients, including one mixed and one chromophobe adenoma. These findings suggest that the biological test correlates more accurately with the clinical diagnosis of acromegaly than does the histological diagnosis by light microscopy. The HGH was undetectable in the tissue culture medium of tumors from six of eight hypopituitary patients. The two exceptions included a pregnant patient. Elevated HGH levels were also found in the culture medium of pituitary tissue obtained from a patient with insulin-treated juvenile-onset diabetes who underwent hypophysectomy.

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References


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Address reprint requests to: Ulrich Batzdorf, M.D., Department of Surgery, Division of Neurosurgery, UCLA School of Medicine, Los Angeles, California 90024.