Correlation between Immunoreactive Growth Hormone and Prolactin Activity in Human and Simian Pituitary Cell Cultures[1] (34247)

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(Introduced by W. F. Ganong)

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Previous investigations have failed to demonstrate conclusively whether the biological effects attributable to human growth hormone (GH) and prolactin are the property of a single protein molecule or of separate molecules (1–4). The hypothesis that both activities reside in a single protein is based primarily on the inability to disassociate these activities convinceingly into physicochemically and immunologically distinct polypeptides by purification and fractionation procedures. The contention that the activities may reside in closely related but different molecules has been supported by the observations of Brauman *et al.* (5) who demonstrated that crop sac activity of media from cell cultures of human fetal pituitary gland increased over the culture period as the immunoreactive GH levels fell.

In the present study, neonatal human and fetal monkey pituitary glands were cultured, and media from the primary cultures and subcultures were assayed for GH by radioimmunossay and for prolactin by pigeon crop sac assay. Immunoassay for GH was also performed on nutrient media from tissue cultures of a 20-week human fetal pituitary.

Method. Pituitary tissue was obtained 5 hr after death from a full-term female infant aged 24 hr (specimen 1), from 2 fetal macaque monkeys delivered on the same day by cesarean section at 120 and 135 days gestation (combined as specimen 2), and from a 20-week human fetus (specimen 3). The specimens were minced and the fragments were established in glass Carrel flasks on a chick plasma, chick embryo extract clot, with 5–6 explants in each flask. The tissues were cultured[7] in Eagle's medium with 20% fetal calf serum, 1% glutamine, and 0.3 mg of streptomycin and 650 units of penicillin/ml. The monolayer cell cultures were maintained at 36.5° in a closed system without gassing. After the cultures had become established they were fed twice weekly with 0.5–1 ml of medium. The spent medium from each flask was kept frozen until assay.

Subcultures were made when a complete monolayer had formed by digesting with 0.125% pronase (Calbiochem), washing the resulting cell suspension, and inoculating this into Carrel flasks or Leighton tubes. Explants of specimen 2 were removed from culture at 20 days; explants were not removed from specimen 1 or 3.

Samples of media were assayed for immunoreactive GH by an adaptation of the double antibody method described for insulin by Morgan and Lazarow (6). Human GH (Wilhelmi 840A) was iodinated by a modification (7) of the method of Greenwood *et al.* (8). Separation of labeled hormone and free iodide was performed on Sephadex G-75. Antihuman GH was raised against Raben GH in

[1] Supported by grants from the National Institute of Child Health and Human Development and from the National Institute of Arthritis and Metabolic Diseases, NIH, USPHS.

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[7] Method of A. Morishima and M. M. Grumbach (unpublished data).

TABLE I. Growth Hormone Concentrations (mμg/ml) in Media Removed from Tissue Cultures of a Neonatal Human Pituitary (specimen 1).

Days in culture	GH in spent media (mμg/ml)					
Flask:	A[a]		B		C	D
5	15,800		28,000		35,000	35,000
12	1250		1250		3600	2900
19	180		220		1700	1700
26	31		180		140	205
31	15		75		75	145
38	6		60		75	90
45	4		31		29	70
52	3		30		13	55
59	2		23		6	32
					1	2
62	2		23		12	96
66	2		22		5	34
	1[b]	2	1	2		
72	5	15	4	3	3	9
76	1	5	2	1	0	2
80[c]		3				

[a] Capital letters indicate primary cultures.

[b] Numbers indicate subcultures.

[c] All subculture flasks were assayed repeatedly between 83 and 104 days and no GH was detected.

guinea pigs and precipitating antiguinea pig gamma globulin in rabbits. In this assay, 0.05 mμg of GH could be measured with confidence. The GH levels in the media from monkey pituitary cultures are expressed in terms of the HGH standard. A medium sample from each specimen was assayed in multiple dilutions. A sample of human acromegalic serum was included in each assay as an internal control.

Prolactin activity was estimated by the Lyons 2-day local pigeon crop sac technique (9). This method can detect as little as 0.1 μg of highly purified ovine prolactin (NIH). The media removed on day 8 of culture from 4 flasks of specimen 1 were pooled, as were the media removed from the same 4 flasks on day 23 of culture. Each pool was lyophilized and 0.2 ml of concentrated media was injected into opposite sides of the crop sacs of 4 birds. Aliquots of unconcentrated pooled media obtained from 6 flasks of specimen 2 on day 8 (0.8 ml) and again on day 23 of culture (1.4 ml), were injected into opposite sides of the crop sacs of 3 birds. No false positive or local inflammatory reaction was observed with control samples of media.

Results. GH was found in all 15 culture flasks from specimen 1. Four of these were selected arbitrarily for longitudinal GH studies, 2 of which were subcultured after 62 days and 2 after 72 days. All 4 subcultures contained GH (Table I). The GH levels in the first subcultures from flasks A, C, and D were greater than those obtained immediately prior to subculture. This may represent a concentration effect secondary to the use of a smaller volume of media to initiate the subculture than was used for routine feeding.

GH was present in high concentration in all 6 flasks from specimen 2. Three of these were subcultured after 31 days and 2 after 37 days. GH was present in the 5 subcultures (Table II).

Washings from both flasks of the human fetal pituitary, specimen 3, contained high GH levels through primary culture (Table III). Only 1 of 3 subcultures from flask A

TABLE II. Growth Hormone Concentrations in Media Removed from Tissue Cultures of Fetal Monkey Pituitaries (specimen 2).

Days in culture	Flask:	GH in spent media (mμg/ml)											
		A[a]		B		C		D	E		F	
2		2375		3300		1000		1850	1600		1450	
10		150		190		90		300[c]			155	
13		110		80		18		95	320		120	
20		185		40		8		25	160		70	
27		115		80		3		4	80		22	
31		1[b]	2						1	2	1	2
34		5	15	42		2		2	27	39	2	2.7
37				1	2	1	2					
40		1	2	4	8	1	0	0	36	5	8	0
48		0	1	3	5	0	0	0	25	3	6	0

[a] Capital letters indicate primary cultures.
[b] Numbers indicate subcultures.
[c] On day 10 washings from flasks D and E were pooled prior to assay.

contained GH. In this subculture, GH levels remained constant for 15 days. GH was not detected in either of the 2 subcultures from flask B.

Serial dilutions of sample media from both the human and monkey pituitaries gave values which fitted the standard curve; control samples of feeding media gave consistently negative results. The mean value of the human acromegalic serum over all assays was 237 mμg/ml (SD± 32).

Two of 4 pigeons, injected with the day 8 pool from specimen 1, exhibited crop sac activity (Table IV). On same day, 3 of 8 pigeons showed positive reaction to total dose of 1 μg and 12 of 12 pigeons to total dose of 2 μg of human pituitary growth hormone. No activity was found in any of the birds injected with the concentrate made from the pooled washings on day 23. Prolactin activity was not detected in either of the pooled samples, days 18 or 23, from specimen 2. The limited volume of media available for assay restricted the number of birds used in the crop sac assay.

Discussion. The finding in this study of high GH concentrations in the media from cultured monkey and human pituitaries is similar to that of Brauman *et al.* (5) for human glands. The failure to find prolactin activity in any but the first washings from the human pituitary cultures is not in accord with their results. In this study a fall in GH levels was associated with a fall in prolactin activity in the human sample. No rise in prolactin activity was observed in either the human or the simian samples. This discrep-

TABLE III. The Growth Hormone Concentrations in Media from Tissue Cultures of a 20-week Human Fetal Pituitary (specimen 3).

Days in culture	Flask:	GH in spent media (mμg/ml)	
		A[a]	B
3		460	1240
10		400	1250
17		140	540
24		69	420
		1[bc]	
28		8	
31		100	460
35			
38		145	340
45		140	
49		74	

[a] Capital letters indicate primary cultures.
[b] Numbers indicate subcultures.
[c] Two other subcultures of flask A between 28 and 112 days and two subcultures of flask B between 52 and 112 days were negative on repeated assay.

TABLE IV. Comparison of Growth Hormone and Prolactin Values in Media from Tissue Cultures of Specimen 1 (human pituitary) and Specimen 2 (fetal monkey pituitary).

	Sample (pool of day)	GH (mμg/ml)	No. of birds used for assay	No. of birds showing positive crop sac effect
Specimen 1 (human)	8	13,000[a]	4	2
	23	500[a]	4	0
Specimen 2 (simian)	8	600	3	0
	23	80	3	0

[a] After concentration by lyophilization.

ancy may be due to differences in cell culture technique, to variation in the secretory and synthetic activity of the pituitary cells persisting in culture, or to modification by proteolysis or other factors of the HGH produced in culture (11). Further, crop sac activity in simian pituitary glands may differ from that in human pituitaries. Most purified preparations of human GH exhibit prolactin activity when tested in μg quantities (4). A positive prolactin assay was only obtained when testing media contained more than 1 μg/ml of GH. The correlation between prolactin and GH activities in the present study does not provide evidence for a separate and distinct human or simian prolactin.

The observation that GH is present in high concentration in culture media from pituitary tissue removed from a 20-week fetus is in accord with the findings of Brauman *et al.* (5). The high levels of GH found in the early washings of all 3 specimens, comparable to those reported by Kaplan and Grumbach (10) for intact pituitary glands, probably represent hormone which was present in the pituitaries at the time of culture. The persistence of immunoreactive GH activity in culture media for prolonged periods suggests continued GH synthesis.

Although GH levels remained constant in the media of a few cultures, the possible usefulness of this system in the assessment of factors regulating GH synthesis and release is limited by the tendency for GH levels to fall during culture, the difficulty of relating hormone concentration in the media to the number of hormone-producing cells and the variability between cultures.

Summary. Pituitary glands from human fetal and neonatal, and fetal monkey sources were established in tissue culture. Growth hormone (GH) was measured by immunoassay in the spent media from the tissue cultures and compared with prolactin activity of the media determined by bioassay. Both GH levels and prolactin activity declined over the first few weeks of culture, although in some flasks GH continued to be assayable in subculture for several months. These results do not support the concept that human GH and prolactin are two separate and distinctive hormones in fetal and neonatal primate pituitary glands.

We are indebted to Dr. William R. Lyons for carrying out the pigeon crop sac assays for prolactin and to Dr. Alfred Wilhelmi, Emory University; Dr. Maurice Raben, New England Medical Center; and the National Pituitary Agency for the human growth hormone used in these studies.

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Received April 7, 1969. P.S.E.B.M., 1969, Vol. 132.