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Effect of Pituitary Hollow Fiber Units and Thyroid Supplementation on Growth in the Little Mouse (41949)

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Abstract. Hollow fiber units containing allogeneic pituitary cells were implanted intracranially into heterozygous (*lit/+*) and homozygous, mutant (*lit/lit*) C57BL/6J "little" weanling mice. Over the 48 days of the experiment, heterozygous mice with pituitary cell implants had a lower percentage weight gain than control mice. Homozygous, mutant mice with cell implants, however, made significant weight gains over mutant controls. Long bone lengths were lower, and organ and carcass weights were higher, in heterozygous mice receiving pituitary cell implants than in control mice, but corresponding measurements in mutant mice with and without implants were not significantly different. Supplementation of the diet with thyroid powder increased the percentage weight gain during the latter half of the 48-day period in both genotypes with and without implanted cells. Thyroid-supplemented mutant mice with pituitary cell implants had significantly higher organ and carcass weights than other mutant groups. The little mouse may serve as a model for pituitary studies and for the treatment of isolated growth hormone deficiency type 1 in man.

A recessive, autosomally inherited, ateliotic dwarfism analogous to the human condition isolated growth hormone (GH) deficiency type 1 arose among C57BL/6J inbred mice (1). The heterozygous *lit/+* adult mouse (90 day) mean body weight was 24.4 ± 0.5 g, whereas the mutant *lit/lit* adult weighed 15.2 ± 0.4 g. The mutant allele, designated little (*lit*), was shown to be located on Chromosome 6. Other studies (2, 3) showed the GH gene and somatotrophs to be present in the mutant, although the degree of gene expression, if any, remains to be established. Fresh extracts of *lit/lit* pituitary are markedly deficient in both GH and prolactin, although blood levels of TSH, LH, FSH, T_3, T_4, testosterone, and prolactin are apparently within a normal range for mice (1, 2). We have confirmed the euthyroid status of both *lit/+* and *lit/lit* mice. In mutant mice, serum corticosterone may be mildly elevated (2–3×), and serum somatomedin activity, measured by [^{3}H]thymidine incorporation into chick embryo fibroblasts, is reduced 75% below heterozygous levels (4). Whole pituitary glands from *lit/+* donors grafted under the kidney capsules of *lit/lit* recipients caused significant increases in body weight and long bone length over sham-operated controls. Similar growth-promoting effects followed repeated ip injections of ovine GH (5).

As an alternative to subcapsular, renal grafting, we have developed a method for the intracranial, ventricular implantation of pituitary cells contained within single-walled capsules made from a polyvinyl chloride acetonitrile copolymer. Spherical molecules of less than 50,000 Da penetrate the capsular membrane, but immunoglobulins and leukocytes are apparently excluded. In previous studies, we have shown that intracranial (IC) implantation of pituitary cell-filled capsules promoted growth in hypophysectomized rats and Snell (*dw/dw*) and Ames (*df/df*) dwarf mice and that the enclosed cells were alive at 110 days postimplantation (6, 7). The additive effects of GH and thyroxine on growth in *df/df* and *dw/dw* mice have also been demonstrated (7–9). The purpose of our experiments was to evaluate the effects of pituitary hollow fiber units placed within

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the brain on various growth parameters of the little mouse.

Materials and Methods. *Animals.* Initial breeder pairs of *lit/+* male and *lit/lit* female mice obtained from The Jackson Laboratory were housed in polycarbonate cages and provided with wood-shaving bedding, cellulose nesting squares, and water bottles with long sipper tubes. The mice were fed a high-energy, mouse breeder diet (Purina Mouse Chow) (17.5% protein, 11.0% fat) *ad libitum* on the cage floor. The room was on a 12:12 light cycle and maintained at 22 ± 2°C. The 23- to 25-day-old offspring of the initial and newly established breeding pairs received implants. Pituitary cell donors were 30- to 40-day-old, outbred, ICR-derived male and female mice in approximately equal numbers.

Pituitary gland dissociation and fiber loading. Donor crania and brains were removed under a low power, dissecting microscope, and the disk-shaped, white neurohypophyses were teased from the connective tissue covering the larger adenohypophyses. The adenohypophyses were pooled and dissociated into a single cell suspension using a trypsinization technique previously described (10). Cell yield was between approximately 404,000 and 511,000/gland with viability estimated >95% by trypan blue exclusion. Cells were concentrated by centrifugation and resuspended in a minimal volume of medium 199 + 0.1% BSA before loading into 6-mm-long XM-50 hollow fibers (Amicon Corp., Lexington, Mass.) with an internal diameter of 508 μm. Each fiber was loaded with 1.0 μl of either vehicle (control) or cell (experimental) suspension using a 10-μl Hamilton syringe. Open fiber ends were heat sealed followed by dipping in melted wax. Each capsule contained approximately 2.9×10^5 cells, which represents approximately two-thirds of the viable cells typically produced by trypsinization of a single mouse pituitary gland. Loaded fibers were stored in sterile medium 199 prior to implantation.

Hollow fiber implantation. Seventy host mice, *lit/+* and *lit/lit* of both sexes, were randomly taken from cages and anesthetized with ether. The skin on the crown of the head was cleaned with alcohol and opened just lateral to the median line. Fibers were implanted IC through a lateral ventricle, as described previously (6). The skin incisions were closed with a metal clip, and the mice were allowed to recover in their cages. Two mice out of the seventy died postoperatively.

Thyroid supplementation. In a pilot study, 16 *lit/+* and 6 *lit/lit* mice receiving capsules also received 25 mg desiccated thyroid powder (Sigma, 0.67% iodine) per kilogram of diet (11). The pelleted diet was powdered, half mixed with desiccated thyroid, repelleted (moisture and pressure only), and stored at 0°C until used. Thyroid-supplemented and unsupplemented control diets were prepared fresh weekly.

Weighing, bleeding, and necropsy. All mice were weighed to the nearest tenth gram immediately before surgery and every 3rd day thereafter for 48 days. Weighings were done between 8 and 9 AM. On the 48th day following implantation, the mice were anesthetized with ether and exsanguinated through a heparinized microhematocrit tube inserted into the retrobulbar venous sinus. The viscera were removed, and the heart, liver, and kidneys were stripped of fat and clots and weighed. The carcasses, cleaned of skin, fat, and remaining viscera, were weighed and radiographed using nonscreen cassettes. Lengths of long bone images were measured with calipers and a metric ruler. Packed cell volumes (PCV) were determined by the microhematocrit method.

RIA of GH. Implanted capsules were removed at necropsy, minced with sterile, fine-tipped scissors, and immersed overnight in 1 ml 0.01 *N* NaOH at 4°C. Fiber pieces were expressed of liquid and removed by forceps. The extract was centrifuged and diluted in phosphosaline buffer.

A modification of the double antibody procedure of Schalch and Reichlin (12) was used to measure immunoreactive growth hormone. Rat GH (VII-38C), with a potency of 3 USP U/mg, was used as standard (13). The anti-rGH serum was produced in Rhesus monkeys. Intraassay variation was 3.8% and interassay variation 8.3% at 21 ng/ml.

Statistical analyses. Data consisted of mouse number, genotype, sex, type of implant, and thyroid dietary supplement. The number of mice in each category is shown in the figure legends. Long bone lengths, organ and carcass weights, blood values, and

capsular GH levels (RIA) were measured at Day 48. Four mice died during the experiment, and their truncated growth data were included in the analyses. A single, thyroid-supplemented, control capsule, "mutant" male mouse (probably a misidentified heterozygote) exhibited extreme growth and was excluded from analyses.

A cumulative percentage change in body weight over weight at implantation was used as a biological index of growth. This information was subdivided into early (Days 3–21) and late (Days 24–48) growth periods.

A repeated measures analysis of covariance was used to investigate effects on growth over time. The mice were initially categorized by the four factors: genotype, sex, type of implant, and dietary supplement, each at two levels. The initial weight at implantation was used as a covariate to adjust statistically all measurements to a common starting weight. Due to significant interactions between genotype and other factors, data were analyzed separately for genotype. Organ, carcass, blood, and capsular GH level data were analyzed by analysis of variance.

Results. *Pituitary implant effects on growth.* Implantation of encapsulated pituitary cells (cell implanted) into heterozygous *lit/+* mice inhibited the average percentage change in weight gain compared with controls (Table I; Fig. 1). This inhibition was significant during both periods and was evident as early as 3 days postimplantation. Male *lit/+* mice had significantly higher gains than females ($P < 0.001$), but analysis of covariance indicated no significant interactions between sex and treatment effect, and data were combined with respect to sex.

In contrast to *lit/+* mice, homozygous *lit/lit* mice receiving cell-filled capsules consistently had higher percentage weight gains than control counterparts (Table I; Fig. 2). Growth stimulation was significant during both periods. Sex differences in *lit/lit* mice were not significant.

Thyroid-supplementation effects on growth. Heterozygous *lit/+* mice receiving dietary thyroid had increased, average percentage weight gains over nonsupplemented mice, but differences were significant only during the 24- to 48-day period (Fig. 1). Thyroid-supplemented, *lit/+* mice with empty capsules gained significantly more than cell-implanted mice ($\bar{X} = 23.2\%$; $P < 0.002$) and more than controls ($\bar{X} = 11.1\%$; $P < 0.05$). Gains of thyroid-supplemented mice over mice receiving both thyroid and implanted cells were not significant ($\bar{X} = 14.5\%$; $P < 0.13$).

Homozygous *lit/lit* mice receiving thyroid and pituitary cell implants had significant gains in the 24- to 48-day period over mice receiving cells alone ($\bar{X} = 59.5\%$; $P < 0.02$) and a large ($\bar{X} = 47.9\%$) but not significant percentage gain over mice receiving thyroid alone (Fig. 2). Homozygous mice receiving thyroid alone outgained controls, but the difference was not significant ($\bar{X} = 37.1\%$; $P < 0.27$).

Effects on body components. Analysis of variance conducted on long bone lengths, organ and carcass weights, PCVs, and capsular GH levels in both *lit/+* and *lit/lit* mice showed that, except for capsular GH, values in heterozygous mice were significantly ($P < 0.01$) higher than corresponding values in mutant mice (Table II).

Long bones in *lit/+* mice receiving im-

TABLE I. MEAN PERCENTAGE WEIGHT GAIN COMPARED WITH WEIGHT AT IMPLICATION USING INITIAL WEIGHT AS A COVARIANT IN MICE NOT RECEIVING THYROID SUPPLEMENT

Mice	Day	Control	Experimental[a]	% Change	*P*
lit/+	3–21	95.3 ± 2.4 (13)[b]	84.3 ± 3.0 (9)	−11.0	0.01
	24–48	168.0 ± 3.0 (12)	154.0 ± 3.7 (9)	−8.4	0.01
lit/lit	3–21	39.6 ± 4.5 (8)	55.1 ± 3.2 (15)	29.1	0.01
	24–48	75.9 ± 9.8 (7)	93.0 ± 6.3 (14)	19.4	0.01

[a] Twenty-three to twenty-five-day-old, mixed sex mice received approximately 2.9×10^5 pituitary cells IC from 5-week-old, ICR-derived, donor mice.

[b] Values are given as means ± SEM with the numbers of mice in parentheses.

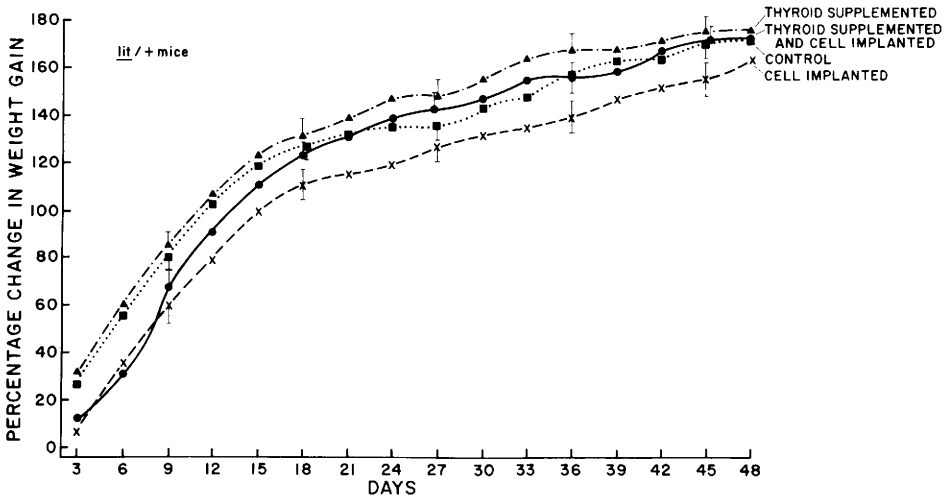

FIG. 1. Effect of four different treatments on mean percentage weight gain in heterozygous (*lit*/+) mice. Values are adjusted means ± SEM and represent percentage weight gain over adjusted initial weights (8.6 g for mice with cells; 8.3 g for control mice). Triangles = thyroid supplemented only (N = 7). Circles = thyroid supplemented and cell implanted (N = 9). Squares = control mice (N = 13 for Days 3–12 and N = 12 for Days 15–48). Crosses = cell implanted only (N = 9). See text for various levels of significance.

planted cells were significantly shorter ($P < 0.01$) and organ and carcass weights higher ($P < 0.01$, except for kidney, $P < 0.05$) than in mice receiving empty capsules (no cells). Thyroid supplementation had no significant bone effects, but thyroid with and without implanted cells produced significant ($P < 0.01$) gains in heart and kidney weights.

There were no significant differences between data from unsupplemented *lit*/*lit* mice with and without implanted pituitary cells. Thyroid supplementation, however, variably

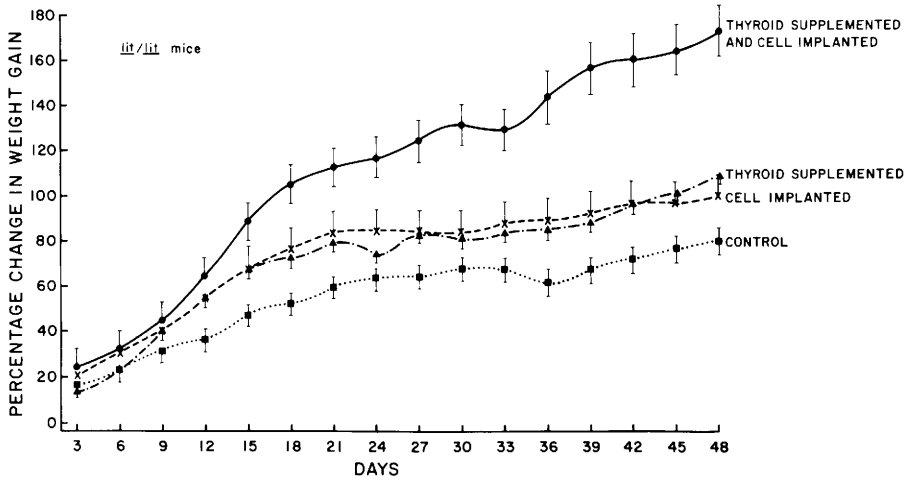

FIG. 2. Effect of four different treatments on mean percentage weight gain in homozygous (*lit*/*lit*) mice. Values are adjusted means ± SEM and represent percentage weight gains over adjusted initial weights (5.8 g for mice with cells; 6.2 g for control mice). Circles, thyroid supplemented and cell implanted (N = 2). Triangles, thyroid supplemented only (N = 4 for Days 3–30 and N = 3 for Days 33–48). Crosses, cell implanted only (N = 15 for Days 3–21 and N = 14 for Days 24–48). Squares, control mice (N = 8 for Days 3–24 and N = 7 for Days 27–48). See text for various levels of significance.

TABLE II. LONG BONE LENGTHS, ORGAN AND CARCASS WEIGHTS, AND CAPSULAR GROWTH HORMONE LEVELS IN HETEROZYGOUS (*lit/+*) AND HOMOZYGOUS (*lit/lit*) MICE RECEIVING NO IMPLANTED CELLS, IMPLANTED PITUITARY CELLS, THYROID SUPPLEMENTATION, OR IMPLANTED CELLS AND THYROID SUPPLEMENTATION

Source	No cells (12)	Cells (9)	Thyroid (7)	Cells + T (9)
		lit/+		
Tibia length (mm)	17.5 ± 0.3^{a}	16.2 ± 0.3^{b}	$17.1 \pm 0.4^{a,b}$	$16.8 \pm 0.3^{a,b}$
Radius length (mm)	12.6 ± 0.3^{a}	11.4 ± 0.3^{b}	11.4 ± 0.4^{b}	10.9 ± 0.3^{b}
Heart weight (g)	0.10 ± 0.01^{a}	0.13 ± 0.01^{b}	0.17 ± 0.01^{c}	0.18 ± 0.01^{c}
Liver weight (g)	0.88 ± 0.06^{a}	1.19 ± 0.07^{b}	1.29 ± 0.08^{b}	1.23 ± 0.07^{b}
Kidney weight (g)	0.23 ± 0.02^{a}	0.28 ± 0.02^{b}	0.36 ± 0.02^{c}	0.35 ± 0.02^{c}
Carcass weight (g)	8.1 ± 0.5^{a}	10.4 ± 0.6^{b}	10.1 ± 0.7^{b}	10.4 ± 1.8^{b}
GH(RIA) (ng/capsule)	0.30 ± 0.26^{a}	204.72 ± 112.12^{b}	0.66 ± 0.52^{a}	134.93 ± 57.32^{b}
%PCV	46.4 ± 0.6^{a}	46.9 ± 0.7^{a}	45.9 ± 0.8^{a}	46.4 ± 0.6^{a}
		lit/lit		
Tibia length (mm)	$14.3 \pm 0.4^{a,b}$	14.3 ± 0.3^{a}	15.7 ± 0.6^{b}	$14.8 \pm 0.5^{a,b}$
Radius length (mm)	10.4 ± 0.3^{a}	10.1 ± 0.2^{a}	10.0 ± 0.5^{a}	10.0 ± 0.5^{a}
Heart weight (g)	$0.06 \pm 0.01^{a,b}$	0.06 ± 0.01^{a}	0.09 ± 0.02^{b}	0.09 ± 0.01^{b}
Liver weight (g)	0.52 ± 0.08^{a}	0.51 ± 0.06^{a}	$0.50 \pm 0.12^{a,b}$	0.78 ± 0.10^{b}
Kidney weight (g)	0.12 ± 0.02^{a}	0.13 ± 0.01^{a}	$0.17 \pm 0.03^{a,b}$	0.20 ± 0.03^{b}
Carcass weight (g)	4.7 ± 0.7^{a}	5.2 ± 0.5^{a}	4.9 ± 1.1^{a}	8.0 ± 1.0^{b}
GH(RIA) (ng/capsule)	0.77 ± 0.32^{a}	243.20 ± 79.53^{b}	2.60 ± 1.70^{c}	113.68 ± 60.57^{b}
%PCV	44.3 ± 0.8^{a}	45.3 ± 0.6^{a}	46.3 ± 0.9^{a}	46.3 ± 1.6^{a}

Note. Measures taken at 48-days postimplantation. Number of observations in parentheses in headings. Values include SEM. T, thyroid supplementation. Different superscripts within genotype indicate significant differences at $P < 0.05$ using analysis of variance.

caused increases in heart, liver, kidney, and carcass weights. Cell-filled capsules contained significant levels of GH(RIA) at 48 days postimplantation.

Although *lit/+* mice tended to have higher PCVs than *lit/lit* mice, all PCVs were within the normal range for mice. Also, neither implanted cells nor thyroid supplementation had a significant effect on the PCV.

Discussion. From the results of previous studies (5, 7, 14), the growth-promoting effects of encapsulated, allogeneic pituitary cells placed intracerebrally were anticipated, as was the depression of gain seen in intact animals. The rationale for the use of cell-filled capsules includes protection of the contents from immunologic attack and retrievability of implanted cells and hormone. The intracerebral location was selected because of proximity to the cerebral ventricles and the hypothalamic and hypophyseal areas, ease of implantation, and the precedent of obvious growth following IC cell implantation compared with other sites (6, 15). Moderate to severe neuronal lesions accompany capsular implantation (6), and the possible effects of these lesions on behavior and physiology must be considered.

The growth stimulation in *lit/lit* mice apparently caused by the encapsulated cells may reflect increased host tissue sensitivity to the exogenous hormones, low levels of brain somatostatin, refractiveness of the pituitary gland to inhibition by exogenous hormones, or an overwhelming growth promoting effect of the implanted cells. The inhibition of weight gain in *lit/+* mice may be due to selective inhibition of the host's pituitary gland. Capsular GH and other hormones may have crossed from the ventricles into the portal system and exerted a direct effect on the host's pituitary, or the exogenous GH may have stimulated somatostatin activity. In the case of organ effects, exogenous and endogenous hormonal effects may have been additive rather than antagonistic.

Previous reports (7, 8) noted that thyroid hormone modified the response to GH. The data here suggest that thyroid hormone supplementation promotes growth in dwarf mice both with and without pituitary cell implants (Figs. 1 and 2), and that thyroid apparently

acts synergistically with cell implants in *lit/lit* mice.

The viscerotropic effects seen in this study are consistent with previous findings (7), with the important exception of the lack of accelerated long bone growth in mice receiving pituitary cell implants. At this time, we have no explanation for this finding.

From the data in Table II, it is evident that growth responses, as monitored by weight gain or loss, can be deceptive. For example, *lit/+* mice receiving cell implants gained less than controls but had significant increases over controls in organ and carcass weights. The net loss, therefore, probably occurred in the fat, skin, or gut, which were not weighed. The *lit/lit* mice receiving cell implants, on the other hand, had significant weight gains over controls, but carcass and organ weights at 48 days postimplantation were not significantly different between the two groups. Again, the specific tissues exhibiting growth changes were undetermined. Also, the nature of the growth promoting (or inhibiting) factors remains uncertain. In a previous study (6), rats receiving implanted pituitary cells had enlarged adrenal and thyroid glands at necropsy, indicating possible stimulation. On the other hand, recent work in this laboratory has shown that an encapsulated preparation of 90% somatotrophs promoted growth in GH-deficient rats, and the suppression of growth following GH administration to intact animals is well documented (14).

The utility of the hollow fiber approach to the study of cell function is confirmed by the present study. It was not possible, however, to assess the interaction between implanted and host pituitaries or the growth-promoting products secreted by encapsulated cells in this protocol. Further experiments with purified cell preparations can be expected to elucidate these interactions.

1. Eicher EM, Beamer WG. Inherited ateliotic dwarfism in mice. J Hered **67**:87–91, 1976.
2. Phillips JA III, Beamer WG, Bartke A. Analysis of growth hormone genes in mice with genetic defects of growth hormone expression. J Endocrinol **92**:405–407, 1982.
3. Cheng TC, Beamer WG, Phillips JA III, Bartke A, Mallonee RL, Dowling C. Etiology of growth hormone deficiency in Little, Ames, and Snell dwarf mice. Endocrinology **113**:1669–1678, 1983.
4. Nissley SP, Knazek RA, Wolff GL. Somatomedin activity in sera of genetically small mice. Horm Metab Res **12**:158–164, 1980.
5. Beamer WG, Eicher EM. Stimulation of growth in the little mouse. J Endocrinol **71**:37–45, 1976.
6. Hymer WC, Wilbur DL, Page R, Hibbard E, Kelsey RC, Hatfield JM. Pituitary hollow fiber units in vivo and in vitro. Neuroendocrinology **32**:339–349, 1981a.
7. Hymer WC, Harkness J, Bartke A, Wilbur D, Hatfield JM, Page R, Hibbard E. Pituitary hollow fiber units in the dwarf mouse. Neuroendocrinology **32**:350–354, 1981b.
8. Bartke A. The response of two types of dwarf mice to growth hormone, thyrotropin, and thyroxine. Gen Comp Endocrinol **5**:418–426, 1965.
9. Holder AT, Wallis M, Biggs P, Preece MA. Effects of growth hormone, prolactin and thyroxine on body weight, somatomedin-like activity and in vivo sulphation of cartilage in hypopituitary dwarf mice. J Endocrinol **85**:35–47, 1980.
10. Hymer WC, Snyder J, Wilfinger W, Swanson N, Davis JA. Separation of pituitary mammotrophs from the female rat by velocity sedimentation at unit gravity. Endocrinology **95**:107–122, 1974.
11. Beamer WG, Eicher EM, Maltais LJ, Southard JL. Inherited primary hypothyroidism in mice. Science (Washington, DC) **212**:61–63, 1981.
12. Schalch DS, Reichlin S. Plasma growth hormone concentrations in the rat determined by radioimmunoassay: Influence of sex, pregnancy, lactation, anesthesia, hypophysectomy and extrasellar pituitary transplants. Endocrinology **79**:275–280, 1966.
13. Ellis S, Grindeland RE, Nuenke J, Callahan PX. Isolation and properties of rat and rabbit growth hormones. Ann NY Acad Sci **148**:328–342, 1978.
14. Kelsey RC, Hymer WC, Page R. Pituitary cell transplants to the cerebral ventricle suppress the pituitary of the recipient host. Neuroendocrinology **33**:312–316, 1981.
15. Weiss S, Bergland R, Page R, Turpen C, Hymer WC. Pituitary cell transplants to the cerebral ventricles promote growth of hypophysectomized rats. Proc Soc Exp Biol Med **159**:409–413, 1978.

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