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# Pituitary Cell Transplants to the Cerebral Ventricles Promote Growth of Hypophysectomized Rats[1] (40359)

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Surgical removal of the adenohypophysis in young animals results in retarded growth as well as decline in peripheral endocrine organ function. Attempts at demonstrating recovery of growth by means of heterotopic hypophysial transplants have met with only limited success (1–5). For example, Halasz and associates reported that transplantation of the whole pituitary gland into the hypophysiotropic area of the brain resulted in partial restoration of growth (3). Growth restoration of a smaller magnitude was observed after transplantation of pituitary glands to such remote sites as the renal capsule (2) or anterior chamber of the eye (1), as well as intramuscular (5) or subcutaneous (4) placements. Gittes and Kastin (5) observed a log dose relationship between growth and number of intramuscular glands, and by extrapolation concluded that 750 glands would be required for restoration to normal growth. Meites and Kragt (4), on the other hand, reported partial restoration of growth (46%) in young (37-day-old) hypophysectomized (hypox) ♀ rats bearing a single subcutaneous pituitary for 30 days.

The ease with which pituitary glands can be enzymatically dispersed to yield suspensions of single viable cells is now widely appreciated. In addition to their usefulness for *in vitro* studies, these single cell suspensions have also been implanted into the kidney capsule or hypophysiotropic area of hypox rats (6, 7). On the basis of morphological criteria it was suggested that such transplanted cells retained functionality *in vivo*.

There is increasing evidence that cerebrospinal fluid (CSF) contains (hypothalamic) neurohormones (8) which may participate in the regulation of pituitary function (9). In the present study, the ventricular system of the brain of hypox rats was therefore chosen as the implantation site for dispersed pituitary cells, and the restoration of body growth was used as an index of functionality of the implanted cells.

*Materials and methods.* In the usual experiment, hypox Sprague Dawley male rats weighing 80–100 g (~30 days old) were purchased from Charles River Breeding Laboratories, Inc., (CD Strain (Outbred Albino), Wilmington, MA) and permitted one week of postoperative recovery. In some cases, sham–hypox littermates were also used. Twenty-gauge hypodermic needles, filed to an unbevelled end 3.25 mm in length and filled with silastic, were stereotaxically implanted into the left ventricle and anchored with acrylic cement according to the procedures of Severs *et al.* (10). Animals were maintained one additional week prior to cell implantation. During this period, animals showing increases in body weight of >5% over initial postoperative levels, suggestive of incomplete hypophysectomy, were discarded from the experiment. Anterior pituitaries from donor males of the same strain (CD, 250–400 g, >70 days) were dispersed in trypsin (11), counted, and resuspended in "mock CSF", consisting of 16 mg dextrose, 176 mg $NaHCO_3$, 15 mg KCl, 14.0 mg $CaCl_2$ (anhydrous), 8.1 mg $NaH_2PO_4 \cdot H_2O$, 23.5 mg $MgCl_2 \cdot 6H_2O$, 13 mg urea, 91 mg NaCl in 100 ml double-distilled water. Each animal received a single injection of 10–20 μl either "mock CSF" (control) or 1–3 × $10^6$ cells prepared in CSF vehicle (experimental), delivered via the needle of a microliter syringe

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through the silastic plug of the indwelling cannula. The quantity of cells delivered was equivalent to approximately ¼–¾ of a whole pituitary gland. Three to 6 animals were used per group. The animals were maintained with 5% glucose in their drinking water and allowed lab chow *ad libitum,* under a 12-hr light (0600–1800) cycle, for periods up to 3 months. They were weighed 3 times per week.

In one experimental series, body composition analysis was done according to the procedure of Hartsook and Hershberger (12). The experimental protocol involved analysis of 12 hypox rats (80–120 g) 2 weeks postsurgery (group A) and 12 hypox littermates which had received either "CSF" or $2 \times 10^6$ cells 2 weeks postsurgery followed by a 30-day growth period (group B). Regression analysis of the body composition data from group A gave the following equations for prediction of initial body compositions of animals in group B: Dry matter = 0.34 (BW) − 4.77 [$r^2$ = .95]; Lipid = 0.1 (BW) − 4.07 [$r^2$ = .79]; Ash = .04 (BW) − .31 [$r^2$ = .90]; Protein = 0.2 (BW) − .71 [$r^2$ = .94]. This protocol permitted evaluation of changes in body composition over the growth period.

Growth hormone (GH) was measured with a double antibody radioimmunoassay procedure (sensitivity, 3 ng/ml) using materials provided by the NIAMDD (Rat Pituitary Program). Protein content of brain homogenates was estimated by the Lowry procedure (13).

Growth curves were analyzed by the variance ratio test on double reciprocal plots of log weight gain vs. log time. This transformation yielded linear graphs and randomly scattered residual variance plots. Comparative growth responses at 30 days postimplantation, as well as other data (bone lengths, body composition and hormone levels) were analyzed by ANOVA or, when appropriate, Student's *t* test.

*Results. Growth response.* During the first 3-week postimplantation period, growth of hypox animals bearing $1 \times 10^6$ cells, expressed as % weight gain, was similar to that of sham–hypophysectomized littermates (Fig. 1). After this time growth tended to plateau (see Fig. 1, Exp. #1 and #2, $1 \times 10^6$ cells). The growth response was related to the number of cells implanted. At no time did total growth exceed that of the animal with an intact pituitary; however, animals receiving more cells tended to plateau later. A single injection of $3 \times 10^6$ cells resulted in a doubling of the animals' body weight over a period of 3 months (Fig. 1, insert). Implantation of $1 \times 10^6$ cells into the ventricles of *nonhypophysectomized* rats resulted in slightly but significantly ($P < .05$) depressed growth curves.

There was an increase in tibial and femoral

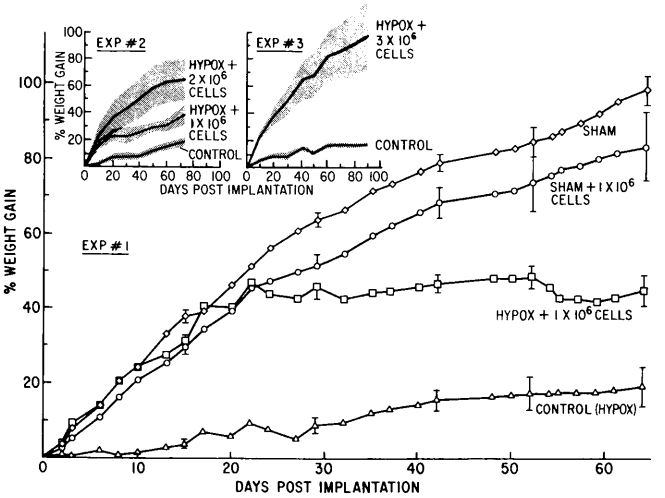


FIG. 1. *Exp. #1,* percent increase in body weight of ~30-day male hypox rats receiving a 10 μl intraventricular injection of either "mock" cerebral spinal fluid (CSF control) or $1 \times 10^6$ single pituitary cells from 70-day-old donors (bottom two lines) or sham hypophysectomized littermates ± $1 \times 10^6$ pituitary cells (top two lines). Each line corresponds to the weight gain of 4 animals; error bars and shading represent ± SEM. Effect of implantation of 1×, 2× (Exp. #2) or $3 \times 10^6$ cells (Exp. #3) on weight gain is shown in the inserts.

bone lengths measured either radiographically or on bones dissected from the rats at autopsy (see Fig. 2). In both cases bone lengths were significantly ($P < .05$) longer in the experimental group. There was a positive correlation between the two methods of measurement. Actual tibial, femoral, and pelvic lengths were 31.5 ± (SEM) .29, 26.0 ± .33, 29.6 ± .24 mm respectively for controls and 33.8 ± .88, 28.9 ± .24, 33.3 ± .44 mm for experimentals ($1 \times 10^6$ cells). Correlations with x-rays were r = .77 (tibia), r = .84 (fibula), r = .97 (pelvis).

*Body composition.* In a separate experiment, 30-day-old hypox ♂ rats receiving $2 \times 10^6$ cells intraventricularily showed weight gains over 30 days of 22.9 ± 0.5 g (SEM) (~34% increase in body weight) vs. 7.5 ± 1.4 g (~11% increase in body weight) for those receiving "CSF". The increase in the experimental group represented 14.1 ± 3.5 g dry matter of which 5.0 ± 1.5 g were protein, 8.5 ± 1.9 g were lipid, and 1.1 ± 0.5 g were ash. The increase in the control group represented 2.8 ± 0.5 g dry matter of which 0.1 ± 0.3 g were protein, 2.6 ± 0.7 g were lipid and 0.4 ± 0.1 g were ash. These results clearly show that significant ($P < .05$) increases in both protein and fat account for the weight gain in the experimental animals.

*Age and sex.* Younger recipients showed a better growth response than the older ones (Fig. 3, top). Pituitary cells from older donor animals gave better responses than cells from young animals (Fig. 3, middle). Cells from male donors of different ages gave consistently inferior responses when implanted into young hypox females (Fig. 3, middle vs. bottom). This result is consistent with the observation that male rats grow larger than females. Cells from >70-day-old female donors were as effective as their male counterparts when transplanted into hypox males (data not shown).

*Somatotroph implantation.* Intraventricular implantation of 630,000 somatotrophs purified to 90% by the method of Snyder *et al.* (14) resulted in a weight gain at 30 days of

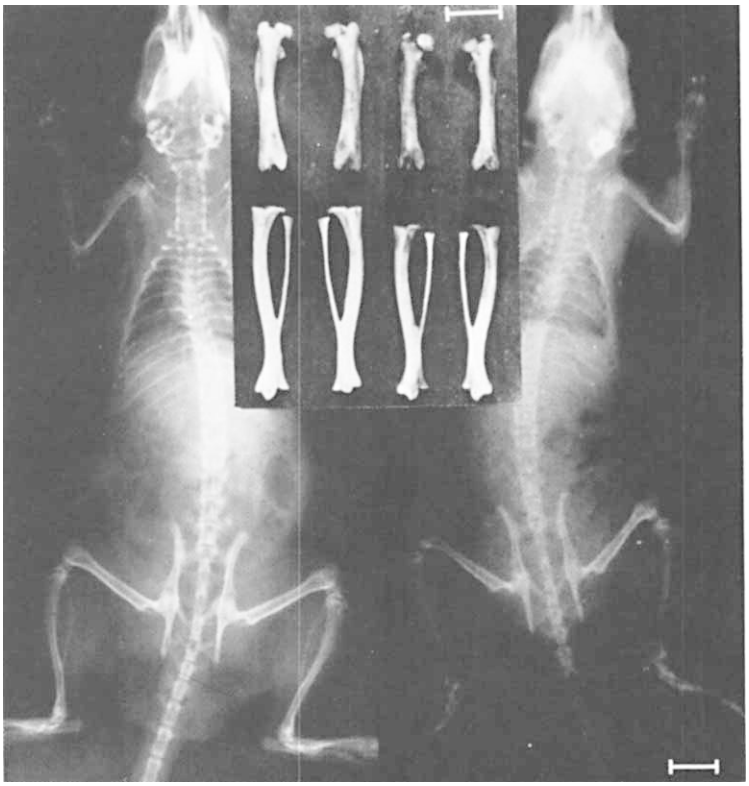

FIG. 2. Radiographs and bones (tibia-lower, femur-upper) from two hypox animals 30 days after intraventricular implantation of either $2 \times 10^6$ cells (left) or "mock" CSF vehicle (right). Scale bar equals 1 cm.

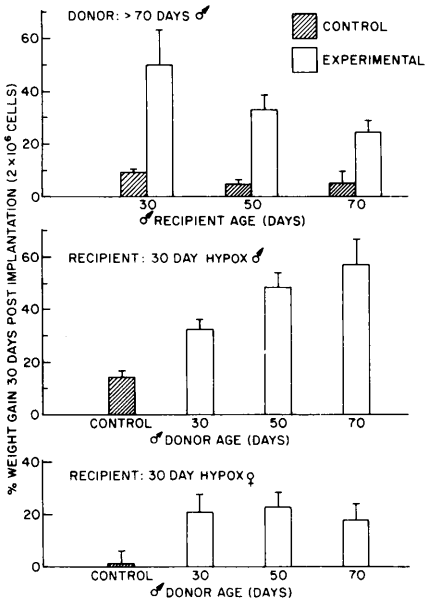


FIG. 3. Effect of age of recipient at hypophysectomy (top), and age of donor pituitary cells in ♂ recipients (middle) or ♀ recipients (bottom) on weight gain. Statistical analysis: top panel: one animal in the 30-day experimental group grew 3× more than the other three. Analysis of variance (ANOVA) on these data excluding this single animal resulted in significant ($P < 0.05$) elevations in the experimental groups in all cases. Middle panel: by ANOVA 50 and 70-day old donor cells caused significant ($P < 0.05$) growth. Bottom panel: growth, although apparently elevated, was not statistically significant.

17.4 ± 3.6% vs. −1.5 ± 4.3% for those injected with vehicle ($P < .05$).

*Castration.* Four groups ($n = 5$ each group) of hypox recipients, two of which were castrated at the time of pituitary removal, received either $2 \times 10^6$ pituitary cells or vehicle. Growth (% wt. gain) 30 days postimplantation was as follows: (a) castrated animals with cells 45.8 ± 10.8%; (b) castrated animals with vehicle 5.3 ± 1.5%; (c) noncastrated animals with cells 69.1 ± 15.0% and (d) noncastrated animals with vehicle 9.1 ± 2.7%. Growth of animals at 30 days in both experimental groups was significantly greater than in controls ($P < .05$), but not significantly different between experimental groups.

*Brain and blood growth hormone (GH).* The levels of GH in homogenates of brains prepared from animals receiving either $1 \times 10^6$ pituitary cells or vehicle is given in Table I. The data reveal detectable hormone in the brains of the experimental group 30 days

TABLE I. GROWTH HORMONE LEVELS (ng GH/mg PROTEIN) IN BRAIN HOMOGENATES PREPARED FROM HYPOX RATS PREVIOUSLY IMPLANTED WITH $1 \times 10^6$ PITUITARY CELLS (EXPERIMENTALS) OR CSF VEHICLE (CONTROLS).

| | Days postimplantation | | |
|---|---|---|---|
| Treatment | 12 | 20 | 30 |
| Experimentals[a] | 53.4 ± 9.0[b] | 16.1 ± 4.1 | 13.6 ± 2.3 |
| Controls | 3.2 ± 3.2 | 0[c] | 0 ± 0 |

[a] 30 day old hypox ♂ rats received $1 \times 10^6$ pituitary cells from 70-day-old ♂ rats.
[b] SEM.
[c] One animal; all other groups had three to four animals.

postimplantation, but at ¼ the level detected 12 days postimplantation.

GH levels in the sera of each of the animals in Table I were, in every case, undetectable. Possible reasons for this result are currently under study.

*Cell placement and viability.* In four separate experiments designed to assess requirements of cell placement and viability in relation to the growth response, the following data were collected (% wt. gain in 30-day-old hypox males one month postimplantation): (a) $1 \times 10^6$ cells − intraperitoneally, 13 ± 4%; (b) $1 \times 10^6$ cells-anterior chamber of the eye, 7.8 ± 2.4%; (c) heat-killed (56°, 30 min) cells-intraventricularity, 4.7%; and (d) a 100,000*g* particle fraction (prepared from $1 \times 10^6$ cells) 5.4 ± 0.3%. None of these responses were significantly different from vehicle-injected controls, but all were significantly lower ($P < .01$) than the response obtained by implanting $1 \times 10^6$ cells intraventricularily (40.6 ± 4.0%, mean of the four experiments).

*Histology.* Serial sections of the entire brains of several experimental animals revealed epithelial cells in the 3rd ventricle, lateral ventricles, and subarachnoid space. Since such cells were not found in the sections of the brains of a control animal, it is tentatively concluded that the pituitary cells spread throughout the entire ventricular system.

*Discussion.* The key finding in this study is that implantation of pituitary cells into the ventricular system of hypophysectomized rats results in animal growth. This growth is reflected both in increased bone length as well as deposition of total body protein. Our data

show that intact cells placed in the ventricles are required to obtain this response since neither cells placed in the anterior chamber of the eye or peritoneal cavity nor heat-killed cells or pituitary organelles gave a positive growth response.

The growth response can probably be attributed to somatotrophs in the pituitary cell suspensions for the following reasons: first, implantation of purified somatotrophs gave a positive response; second, the response was obtained in a castrated animal in which influences of anabolic steroids were not present; and third, GH was detected in the brains of rats 30 days postimplantation of cells, but not in vehicle-injected controls (Table I).

The results show that the CSF of the hypox rat provides a suitable functional milieu for maintainence of somatotrophs for at least 3 weeks postimplantation.

*Summary.* Implantation of acutely dispersed adenohypophysial cells into the lateral ventricles of hypophysectomized rats resulted in partial growth restoration for periods of one to three months. Weight gain by experimental animals was consistently 20%–60% greater than among hypophysectomized control rats; the response was related to the number of cells implanted. The weight gain reflected increases of both protein and fat in body composition. A significant increase in long bone lengths was also observed among rats bearing intraventricular cells. Intraventricular implantation of either heat-killed anterior pituitary cells or subcellular organelles, or implantation of pituitary cells into the peritoneal cavity or anterior chamber of the eye, did not promote significant growth in hypophysectomized recipients. The results suggest that transplanted growth hormone-secreting cells are provided with a suitable functional milieu by the cerebrospinal fluid of the hypophysectomized rat.

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