THE EFFECTS OF EARLY HYPOPHYSECTOMY AND HORMONE THERAPY ON BRAIN DEVELOPMENT

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INTRODUCTION

The high mortality rate encountered after removing the pituitary from very young rats has been a major obstacle in obtaining information on the early development of various systems in an hormonally deprived animal. In our laboratory out of 554 male rats hypophysectomized at 5 days of age, only 18 survived for 27 days, a mere 3.2%. At 6 days of age, the survival rate was slightly higher; of 369 animals operated upon, 53 survived for 27 days, 14%. Interest in pursuing the problem in spite of the odds stemmed from the work of Walker *et al.*[16], and Asling *et al.*[1], who reported that early hypophysectomy did not depress brain development in a manner comparable to the depression of somatic and visceral growth.

That early thyroidectomy will cause retardation of brain growth, measured by brain weight and linear dimensions, as well as body growth, has been substantiated[8,14]. In fact, detailed morphological studies of the somesthetic area of the cerebral cortex after early thyroidectomy have shown that the cortical neurons are smaller, development of the cell processes is impaired, myelination of the axons is retarded, and neurons are more closely aggregated (cited from Eayrs and Taylor[8]). Now if the brain weight decreases and the somesthetic morphological picture is impaired in the early thyroidectomized rat, the question can be raised as to whether or not the brain of the early hypophysectomized animal actually grows at a normal rate, as reported from the observations of Walker *et al.*[16].

In an attempt to answer this question a series of histological and chemical experiments have been carried out. These experiments are concerned with the effects of early hypophysectomy on total brain weights, the histology of the brain, hormone replacement studies, enzymatic activity measures, body weights, and endocrine organ weights. This presentation, the first of the series, will deal with some of the histological examinations after hypophysectomy of 6-day-old rats, namely the depth measurements in the somesthetic and visual cortices, diencephalic measurements and the

results of hormone replacement therapy upon these cortical and diencephalic measurements.

METHODS

Littermate pairs of Long–Evans male rats were used for this study. The experimental methods can be divided into four parts: (1) surgical procedures, (2) hormonal treatments, (3) histological techniques, and (4) brain measurements.

Surgical procedures

The surgical technique involved the removal of the pituitary from 6-day-old rats. Prior to surgery the animals were weighed, placed in gauze and paper lined containers, and anesthetized in a deep freeze (0°C) for 15–30 min. Both the experimental animals and their controls were anesthetized in this manner. As soon as spontaneous motor activity ceased, the experimental rats were hypophysectomized by a parapharyngeal approach under a binocular microscope. The occipital-sphenoid suture was bored with a dental drill, and the pituitary was removed by slight aspiration with a glass cannula, taking care not to rupture the diaphragma sellae. After surgery the animals were placed in an oxygen-enriched incubator (32–33°C) for 18–20 h.

The control rats were transferred directly from the freezer to the oxygen-enriched, warm environment. Various attempts were made to determine the most adequate control. Sham operated controls included one or a combination of the following conditions: a neckline incision, an approach to the spheno-occipital bone, drilling through the bone but not removing the pituitary. None of these conditions affected the body weight or survival of the rats any differently from subjecting the animals to the hypothermic conditions alone[11]. In addition the brains from animals with the spheno-occipital bone drilled at 6 days, without removing the pituitary completely, were examined at 27 days of age. These brains showed no changes in the diencephalic measures from the normal, intact control. Therefore, the hypothermic, intact animal was considered to be an adequate control. The brains from 22 uninjected, hypophysectomized animals and their littermate controls were studied.

Post operative observations were made on the general health of the animals, and body weights were recorded weekly.

Hormonal treatment

Three sets of experiments dealt with hormone supplements. (1) Thyroxine: for 3 weeks, single, graded doses, 5 μg the first week, 10 μg the second week and 15 μg the third week, of L-thyroxine* were administered subcutaneously to 21 male rats, hypophysectomized at 6 days of age. For each injected animal there was an intact, littermate control. (2) Thyroxine: 17 normal, intact males also received the same

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dosage of L-thyroxine for the same period as mentioned in (1). The intact, thyroxine injected animals and their uninjected littermates were not exposed to the hypothermic conditions, as were all the other animals in these experiments. (3) Growth hormone: three single, weekly graded doses, 3.5 μg, 7 μg, and 10.5 μg, of growth hormone** were injected subcutaneously into 9 male rats hypophysectomized at 6 days of age. For every injected, hypophysectomized animal, there was an uninjected, littermate control.

Histological techniques

All animals were autopsied at 27 days of age. The rats were anesthetized with ether and perfused through the left ventricle of the heart with 10% formol–saline. The brains were carefully removed from the skull after peeling off the dura and were placed in 10% formol–saline. The testes, the thyroid, and the adrenals were removed and weighed, the weights being used to evaluate the completeness of hypophysectomy. Also, at this time the sella turcica was examined under the binocular microscope for pituitary remnants. No data from brains were used from animals containing visible pituitary fragments.

From the brains, transverse, frozen sections were cut at 20 μ, utilizing subcortical landmarks to obtain identical overlying cortical samples. Tissues representing the somesthetic cortex were cut at the crossing of the anterior commissure. The posterior commissure served as a guide for sections from the visual cortex (Diamond[4], Fig. 1, sections 6 and 9). All brain sections were stained with Windle's modified thionin, Nissl stain[17].

Brain measurements

Cortex. From previous experiments we have learned that cortical depth measurements are useful indicators for brain changes and that several methods can be used interchangeably for obtaining cortical depths[4]. In the present experiment both microslide projection readings and ocular micrometer measures were taken. With the microslide projector, the cortical and subcortical outlines were projected (enlarged $\times$ 22.5) and drawn. Beginning lateral to the elevation on the corpus callosum, five cortical depths were read with a millimeter rule extending from the white matter to the pial surface. Both right and left hemispheres were read, and the results are presented as the means of both hemispheres. With the ocular micrometer, eight readings were taken directly from the slide in the identical position as measured with the microslide projector. The means of these readings on the right and left hemispheres were also recorded. A conversion factor was used to equate the final readings obtained from the two methods.

Diencephalon. With the microslide projector, enlarged images of the diencephalon were reproduced directly from the thionin stained sections at the level of the

** Fully active Bovine Growth Hormone, L 3020A, Hormone Research Laboratory, University of California, San Francisco Campus.

anterior and posterior commissures. Two linear readings with a millimeter rule were taken on the dimensions of the diencephalon: (1) a vertical, midline, depth reading extending from the dorsal surface of the diencephalon to the ventral surface, and (2) a horizontal measure between two points demarcating the widest lateral distance in the diencephalon[3].

RESULTS

Cortical depths

There were no significant differences between the depths of the somesthetic cortex and the visual cortex from animals hypophysectomized at 6 days of age and sacrificed at 27 days of age when compared with the cortical depths of the unoperated controls (see Table I, part A). In the thyroxine injected, hypophysectomized animals, the visual cortex showed significantly greater depths than other groups whether they were uninjected, intact littermates, 4.2% ($P < 0.01$)*; uninjected, hypophysectomized controls, 3.5% ($P < 0.01$)**; or normal, thyroxine injected animals, 3.5% ($P < 0.01$)** (Table I, parts B, E, and F, respectively). The somesthetic cortex does not show these differences found in the visual cortex. If anything, the administration of thyroxine to the control animals inhibits the development of the somesthetic cortex when compared with the uninjected, intact control, C + Tx < C by 3.1% ($P < 0.02$) (see Table I, part C). Growth hormone does not alter the cortical depths in the hypophysectomized animals when compared to the uninjected, intact controls (Table I, part D).

Diencephalon measures

Hypophysectomy at 6 days of age affects the depth of the diencephalon to a greater extent than it does the width. The percentage increase in depth in the hypophysectomized rat is about the same when compared to the control whether the hypophysectomized animals are uninjected or injected with thyroxine or with growth hormone. In other words, thyroxine and growth hormone do not change the depth increase found in the early hypophysectomized animals. Under all experimental conditions, the increase in the depth of the diencephalon of the hypophysectomized rats amounts to 7–8% ($P < 0.001$) at the anterior commissure level, and 5–7% (the P ranging from < 0.02 to < 0.001) at the posterior commissure level (see Table II).

In contrast to the diencephalic depth measurements, which showed similar changes at both the anterior and posterior commissure level, the diencephalic width shows variations depending upon the level of the section and the hormone treatment (see Table II). With 6 day hypophysectomy and no hormonal supplement, the diencephalic width is greater than the uninjected control by 3.6% ($P < 0.001$), at the anterior commissure level, but there are no significant differences in the measures at the posterior

* A correlated t test for matched pairs was used.
** A t test for unmatched pairs was used.

TABLE I

COMPARISONS AMONG CORTICAL DEPTH MEASUREMENTS (μ) OF MALE RATS WITH THE FOLLOWING TREATMENTS: UNINJECTED CONTROLS (C), 6 DAY HYPOPHYSECTOMIZED ($\bar{H}$), THYROXINE INJECTED (T_4), GROWTH HORMONE INJECTED (GH)

A. $\bar{H}$ *vs. C (N = 22 pairs)*

	$\bar{H}$		C		*% diff.**	*P*
	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*		
Somesthetic area	2143	170	2200	88	−2.6	NS
Visual area	1573	75	1585	99	−0.8	NS

B. $\bar{H} + T_4$ *vs. C (N = 21 pairs)*

	$\bar{H} + T_4$		C		*% diff.*	*P*
	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*		
Somesthetic area	2189	91	2166	63	1.0	NS
Visual area	1630	68	1562	61	4.2	< 0.01

C. $C + T_4$ *vs. C (N = 17 pairs)*

	$C + T_4$		C		*% diff.*	*P*
	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*		
Somesthetic area	2189	66	2257	76	−3.1	< 0.02
Visual area	1573	59	1596	65	−1.5	NS

D. $\bar{H}$ + *GH vs. C (N = 9 pairs)*

	$\bar{H}$ + *GH*		C		*% diff.*	*P*
	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*		
Somesthetic area	2200	91	2223	70	−1.0	NS
Visual area	1607	91	1573	71	2.1	NS

E. $\bar{H} + T_4$ *(N = 21) vs.* $\bar{H}$ *(N = 22)*

	$\bar{H} + T_4$		$\bar{H}$		*% diff.*	*P*
	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*		
Somesthetic area	2189	91	2143	170	2.1	NS
Visual area	1630	68	1573	75	3.5	< 0.01

F. $\bar{H} + T_4$ *(N = 21) vs.* $C + T_4$ *(N = 17)*

	$\bar{H} + T_4$		$C + T_4$		*% diff.*	*P*
	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*		
Somesthetic area	2189	91	2189	66	0	NS
Visual area	1630	68	1573	59	3.5	< 0.01

* The percentage differences were found by subtracting the second condition from the first condition and dividing by the first condition, *e.g.*, A.

$$\frac{\bar{H} - C}{\bar{H}} \times 100.$$

commissure level. If thyroxine is injected into the hypophysectomized rat, the widths of the diencephalon are not significantly different from the uninjected control. However, the width of the diencephalon in the hypophysectomized animal injected with growth hormone is greater than the uninjected control by 4.1% ($P < 0.01$) at the anterior commissure level, and by 2.8% ($P < 0.02$) at the posterior commissure level (see Table II).

TABLE II

COMPARISONS AMONG DIENCEPHALIC MEASUREMENTS IN μ OF MALE RATS WITH THE FOLLOWING TREATMENTS: UNINJECTED CONTROLS (C), 6-DAY HYPOPHYSECTOMIZED ($\bar{H}$), THYROXINE INJECTED (T_4), GROWTH HORMONE INJECTED (GH)

A. *$\bar{H}$ vs. C (N = 19 pairs)*

	$\bar{H}$		C		% diff.*	P
	$\bar{x}$	S.D.	$\bar{x}$	S.D.		
Width						
Anterior comm.	10,977	471	10,577	329	3.6	< 0.001
Posterior comm.	9510	280	9332	338	1.9	NS
Depth						
Anterior comm.	5644	249	5244	218	7.1	< 0.001
Posterior comm.	6177	342	5733	400	7.2	< 0.01

B. *$\bar{H} + T_4$ vs. C (N = 20 pairs)*

	$\bar{H} + T_4$		C		% diff.	P
	$\bar{x}$	S.D.	$\bar{x}$	S.D.		
Width						
Anterior comm.	10,843	283	10,666	271	1.6	NS
Posterior comm.	9421	212	9288	213	1.4	NS
Depth						
Anterior comm.	5644	249	5199	271	7.9	< 0.001
Posterior comm.	5955	182	5599	222	6.0	< 0.001

C. *$\bar{H}$ + GH vs. C (N = 9 pairs)*

	$\bar{H}$ + GH		C		% diff.	P
	$\bar{x}$	S.D.	$\bar{x}$	S.D.		
Width						
Anterior comm.	11,243	204	10,799	240	3.9	< 0.01
Posterior comm.	9688	258	9421	280	2.8	< 0.05
Depth						
Anterior comm.	5688	151	5288	124	7.0	< 0.001
Posterior comm.	6133	151	5777	244	5.8	< 0.02

* The percentage differences were found by subtracting the second condition from the first condition and dividing by the first condition, *e.g.*, A.

$$\frac{\bar{H} - C}{\bar{H}} \times 100.$$

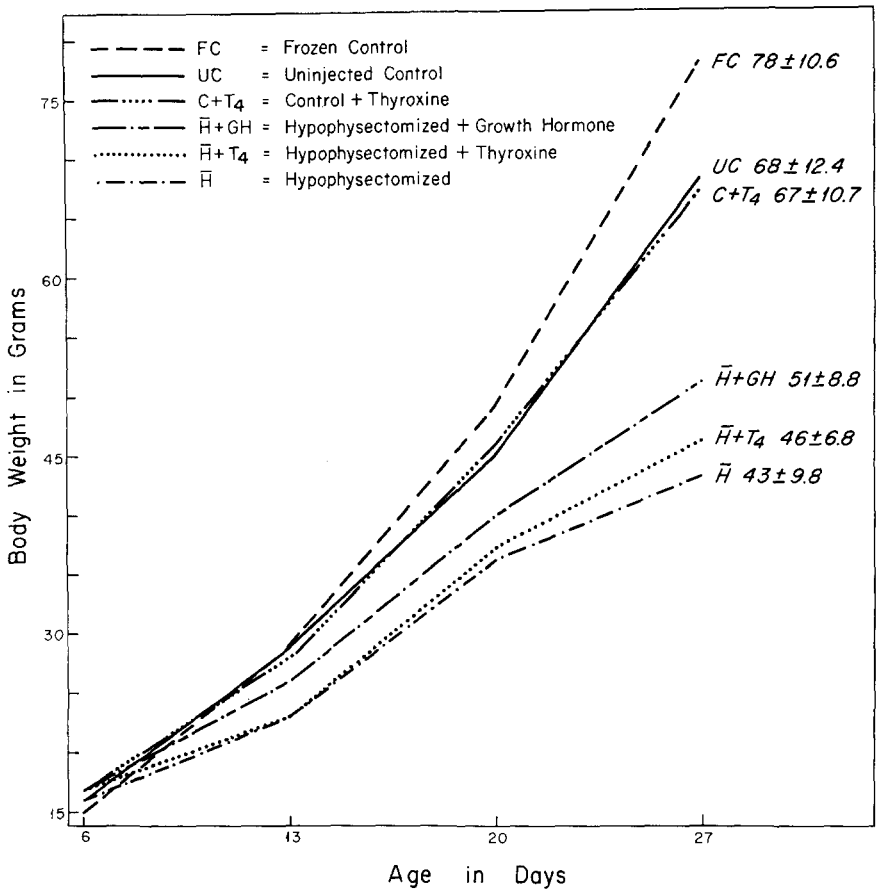

Fig. 1. Body weight gains (GMS) of male rats from 6 days of age to 27 days of age (± S.D.).

Body weights

Fig. 1 presents the body weight gains from 6 to 27 days in the hormone deficient animals, in the hormone replacement studies, and in the intact controls. Table III presents and compares the body weights of the four groups at 27 days of age. The hypophysectomized animals have a mean body weight which is 81.4% less than that of the unoperated controls ($P < 0.001$, Table III, part A). The body weight of the uninjected hypophysectomized animals is only 7.0% (NS) less than the thyroxine injected hypophysectomized animals (Table III, part B). Thyroxine given to normal animals does not produce a body weight significantly different from the normal, uninjected control weight (Fig. 1).

The uninjected, hypophysectomized animals have a body weight which is 18.6% ($P < 0.05$) less than that of hypophysectomized animals receiving growth hormone (Table III, part C). There are no significant differences between the body weights of the animals receiving thyroxine and those receiving growth hormone (10.9% (NS), Table III, part D).

TABLE III

COMPARISONS AMONG BODY WEIGHTS (g) OF MALE RATS AT 27 DAYS OF AGE WITH THE FOLLOWING TREATMENTS: UNINJECTED CONTROLS (C), 6-DAY HYPOPHYSECTOMIZED ($\bar{H}$), THYROXINE INJECTED (T_4), GROWTH HORMONE INJECTED (GH)

A. $\bar{H}$ *vs.* *C* (*N* = 21 *pairs*)

$\bar{H}$		*C*		*% diff.**	*P*
$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*		
43	9.8	78	10.6	−81.4	< 0.001

B. $\bar{H}$ *vs.* $\bar{H} + T_4$ (*N* = 21 *pairs*)

$\bar{H}$		$\bar{H} + T_4$		*% diff.*	*P*
$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*		
43	9.8	46	6.8	−7.0	NS

C. $\bar{H}$ (*N* = 21) *vs.* $\bar{H}$ + *GH* (*N* = 10)

$\bar{H}$		$\bar{H}$ + *GH*		*% diff.*	*P*
$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*		
43	9.8	51	8.8	−18.6	< 0.05

D. $\bar{H} + T_4$ (*N* = 21) *vs.* $\bar{H}$ + *GH* (*N* = 9)

$\bar{H} + T_4$		$\bar{H}$ + *GH*		*% diff.*	*P*
$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*		
46	6.8	51	8.8	−10.9	NS

* The percentage differences were found by subtracting the second condition from the first condition and dividing by the first condition, *e.g.*, A.

$$\frac{\bar{H} - C}{\bar{H}} \times 100.$$

There is always the problem of whether these results will be replicable, and since there is such difficulty in obtaining these animals, and replication is unlikely at present, we have examined the data by another approach. These experiments were carried out over a considerable period of time, so we compared the results for the first half period with those of the second half period, in essence, considering the second half a replication experiment. The results obtained from the two halves were similar, giving us greater assurance of the reliability of these data.

DISCUSSION

In animals hypophysectomized at 6 days of age the body weights are 55% of the weights of the intact controls at 27 days of age. It is apparent that the lack of

growth hormone in the early hypophysectomized animal is responsible for the retarded body growth. The cortical depths between the two groups are not significantly different, though there may be internal deficiencies which do not affect cortical depth. The cortical growth, as measured by cortical depth, is not evidently dependent on growth hormone, as suggested in the earlier reports from the gross brain observations of Walker *et al.*[16], and Asling *et al.*[1].

That thyroxine deficiency will bring about certain brain defects at early ages is well established[5–7,9,10,12,15] and with hypophysectomy the thyroid stimulating hormone has been removed. We then ask the question, why does not the lack of thyroid stimulation after early hypophysectomy cause measurable deficiencies in brain development? Evidently, there is sufficient thyroxine released from the thyroid to promote and regulate brain growth and development during the early stages, at least up until 27 days of age. It will be of interest to see the long term effects of hormone deprivation induced by early hypophysectomy.

The histological sections from the visual cortex of the thyroxine injected, 6-day hypophysectomized animals showed significantly greater cortical depths than other groups studied. That this region should be more responsive to hormone replacement is of interest, for our investigations dealing with the effects of an enriched environment on brain chemistry and anatomy[2–4] have shown the sections through the visual cortex to have greater increases in enzymes and in structure than those from other cortical areas. Thus, we now have additional information to support the hypothesis that the visual area may possess qualities which allow this region to respond to various 'non-specific' stimuli.

Walker *et al.*[16] and Asling *et al.*[1] have reported that the skull of the 6-day hypophysectomized rat is characterized by a prominent convexity of the calvarium and by an accentuated width, which nearly equalled the length. The diencephalon measures of the 6-day hypophysectomized rat appear to be altered in similar directions, at least with regard to width and height. We find the depth of the diencephalon has increased markedly in the hypophysectomized rat, and this depth increase may be caused by the foreshortening of the skull. The possible concomitant brain pressures which may occur from the diencephalic depth increase may, in turn, account for the 'domed' calvarium. Eayrs and Taylor[8] have reported that the hypothyroid rat possessed a brain disproportionately wide in relation to length. The increase in depth of the diencephalon in the hypophysectomized rat appears to be unaffected with either growth hormone or thyroxine administration. In the case of the diencephalic widths, thyroxine is able to correct the changes found in the uninjected, hypophysectomized animals.

In the introduction, the extremely low survival rate after early hypophysectomy was mentioned. With hormone therapy, particularly the administration of thyroxine, the loss was not as severe. For example, from 58 animals hypophysectomized and injected with thyroxine (a total of 30 μg) 25 animals survived, 43%, in contrast to 14% surviving without thyroxine. Thus, it is apparent that the thyroid hormone is important for the survival of the early hypophysectomized animal, and the high mortality rate is not just due to surgical trauma. (The difference in the number of

animals presented here in the text *versus* the number of animals in the tables is due to the reduction in number during the histological processing of the tissues.)

The growth hormone effects on survival were evident, but not as marked as those brought about with thyroxine. In 38 animals injected with growth hormone (a total of 21 μg) 11 survived, 29%. Rosenberg[13] has given as much as 100 μg/day for 21 days to rats hypophysectomized at 6 days of age. With this much larger dose of growth hormone, 8 hypophysectomized animals out of 28 survived, again but 28%, a similar increase to that found with the smaller dose.

CONCLUSIONS

1. A study was carried out to compare the brains of rats hypophysectomized at 6 days of age with their littermate, intact controls. The effects of growth hormone and thyroxine on these animals was also investigated.

2. At 27 days of age no significant differences were found in the visual and somesthetic cortical depths between animals hypophysectomized at 6 days and their littermate controls.

3. The visual cortices from thyroxine injected, hypophysectomized animals were deeper than uninjected, intact littermates; uninjected hypophysectomized controls; and normal, thyroxine injected animals. The somesthetic area did not show these differences.

4. Growth hormone had no significant effect on the cortical depth of the hypophysectomized animal when compared with the uninjected control.

5. The diencephalon depth was greater in the hypophysectomized animal than in the uninjected, littermate control. This depth increase was not altered with the administration of thyroxine or growth hormone.

6. The width of the diencephalon in the hypophysectomized rats was greater than the controls at the anterior commissure level but not at the posterior commissure level. The width increase was reduced with the administration of thyroxine, but not with growth hormone.

7. The body weights of the hypophysectomized animals were 81% less than the littermate controls. Growth hormone increased the body weights of the hypophysectomized animals more than did thyroxine, but the differences in body weight between the two hormone groups were not significant.

8. Thyroxine administration to the early hypophysectomized animals promoted the survival rate to a greater extent than did the injections of growth hormone.

SUMMARY

Littermate, male, Long–Evans rats were paired, with one of each pair being hypophysectomized at 6 days of age by a surgical parapharyngeal approach. Histological examinations were made of brains from hypophysectomized rats and their intact controls, from hypophysectomized rats injected with 30 μg of thyroxine and their uninjected controls, and from hypophysectomized rats injected with 21 μg of growth hormone and their uninjected controls. Body weights were recorded weekly.

All animals were autopsied at 27 days of age under ether anesthesia. The brains were perfused with formol–saline. Utilizing subcortical landmarks to obtain identical overlying cortical samples, frozen, transverse sections were cut (20 μ) and stained with thionin.

Cortical depth and diencephalic measures were taken on sections cut at the anterior and posterior commissure levels. Results showed that even though the body weights of the hypophysectomized animals were 81% less than the controls, there were no significant differences in the visual and somesthetic cortical depths between the two groups. The visual cortex increased in depth with thyroxine, whereas the somesthetic cortex did not. Growth hormone had no significant effect on the cortical depth. The depth of the diencephalon increased more with hypophysectomy than did the width. Thyroxine increased the survival rate of the hypophysectomized animals to a greater extent than did growth hormone.

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