

THE EFFECT OF HYPOPHYSECTOMY IN THE EARLY EMBRYO UPON THE GROWTH AND DEVELOPMENT OF THE FROG

A PRELIMINARY REPORT

P. E. SMITH

From the Anatomical Laboratory, University of California

TEN FIGURES

The extirpation of the hypophysis in the adult frog has not given uniform results. Caselli ('00) and Gaglio ('02) who reported no changes following hypophysectomies were followed by Boteano ('06) who reported a neuromuscular asthenia in the operated animals. Houssay ('10) came to the conclusion that the removal of the gland was followed by death. Adler ('14) burned out the hypophysis of a 20 mm. *Rana temporaria* larvae with the electric cautery. Out of the 1200 operated animals three were found to have been hypophysectomized, not, however, without great injury to the surrounding soft parts, particularly the brain. In not one of those three animals did hind legs develop beyond a small bud, and transformation did not take place, the specimens remaining as neotonic tadpoles.

This work was commenced in the Spring of 1914, repeated in 1915, and again in 1916, *Diemyctylus torosus*, *Rana pipiens*, and *Rana boylei* being successively used. In this paper the results obtained with the California yellow-legged frog, *R. boylei* are reported. Shortly after the closure of the medullary plate, Kopsch's stages d-e, was found to be the size in which the hypophysial invagination could be most successfully removed. About 200 larvae of this stage were operated upon. In specimens of this size the hypophysis was successfully removed in over 60 per cent of the operated animals. Approximately 30 per cent of those animals in which the gland was extirpated did

not give reliable results in the rate of growth as the mouth was wholly or partially removed thus interfering with feeding. Unoperated animals and those in which the ablation of the gland was unsuccessfully attempted were available for checks.

The operation is a simple procedure. The hypophysial invagination can be accurately determined from the pit that it early forms or from its location between the protuberance of the forebrain and the stomadeum, which is just forming. This epithelial ingrowth was removed with some neighboring epithelium. The wound healed within three hours in most cases, less than 1 per cent of the larvae disintegrating after the operation. The operated animals and checks were kept in boiled water for five days and then transferred to a frog tank where they were in an essentially normal environment.

The rate of growth in the hypophysis-free animals has been slower than in the checks. The larger hypophysectomized animals averaged smaller in size than the larger checks, the averages of the two showing a noticeable difference. On June 6 the operated but not hypophysectomized animals had an average length of 40 to 43 mm., the hypophysis-free animals averaging 33 to 35 mm., a ratio constant throughout their growth. The ratio of body to tail length is the same in the two classes, the difference in size being uniform for all parts of the animal. The tail fin did not show an increased width or pleating in the hypophysectomized animals as reported by Adler ('14).

In activity the two classes of animals showed no marked differences. The hypophysectomized specimens were perhaps slightly more alert, darted more quickly, and consequently were more difficult to capture with the pipette than were the checks.

The resistance of the hypophysectomized animals was greater than that of the checks. Towards the close of the experiment the animals were attacked by disease, none reaching the adult stage. The normal specimens succumbed more rapidly to this infection than did the hypophysectomized ones. Some of the intrinsic factors which induce growth of legs and transformation were lacking in the abnormal specimens as will be shown later. The absence of these factors may well be conducive to a greater

hardiness in an animal when compared to the normal tadpole in which the usual rapid changes are taking place.

Differences in color began to be noticeable before a length of 15 mm. was reached, and from then on the contrast in pigmentation between the hypophysectomized animals and the checks was striking. Those animals without hypophyses were characterized by a light grayish appearance; however, the dorsal side was more pigmented than the ventral (figs. 7, 10). These are referred to as albinos. The checks were a brown-black color often showing a mottling (figs. 8, 9). This color difference was more noticeable over the body than on the tail, but was evident in both regions and was the most striking feature up to the time of the appearance of the hind legs in the checks. Sections show that these pigment differences are referable chiefly, if not solely, to the condition of the epidermis. Counts of the melanophores of corresponding areas in the albinos and in the checks show that the number of these cells, in the epidermis, are reduced in the former. Further the melanophores of the albino specimens contain fewer pigment granules than do those of the checks and thus have a distinctly lighter appearance. The melanophores are equally expanded in the two types, consequently, the lighter color of the albinos cannot be due to the contracted condition of the chromatophores but must be referred, in part, to the reduced number of melanin granules in the pigment cells of the epidermis. In addition to this the free pigment granules which form a distinct zone in the superficial layer of the epidermis in the normal checks are much reduced in number in the albino specimens (figs. 5, 6). It is surprising that in the albinos the deeper or subcutaneous pigment is present in as great a quantity as in the normal animals, if not greater. The amount and distribution of the retinal pigment seem to be identical in the two.

Another important feature was the inhibition in growth of the hind legs of the operated animals. There was only a slight retardation in the time of appearance of the hind leg buds, normally, appearing when the tadpole has reached a length of 25 to 27 mm. In the albino, averages show that the hind limb buds appear when the larvae are from 26 to 28 mm. in length.

From this state on, however, the hind limbs in an hypophysectomized animal grew but little if at all, although the animal's length increased at a rate but slightly under the normal. The accompanying table shows the increase in length of the hind legs in relation to total length for the albinos and for the checks. (See also figs. 7, 8).

Average rate of growth in millimeters in terms of total length, of the hind legs of the checks and the albinos

HYPOPHYSECTOMIZED ANIMALS		CHECKS	
Total length	Hind leg length	Total length	Hind leg length
26	barely visible	25	barely visible
28	0.1	28	1.0
30	0.1	30	2.0
35	0.1	35	3.0
37	0.12	38	4.0
		40	5.0
		45	9.0

Only one exception to the rule that no hind legs grew on albinos was found. A 36 mm. albino had hind legs 4.2 mm. long when killed. The above is in accord with Adler ('14) who found that removal of the hypophysis in a 20 mm. stage inhibited the growth of the hind legs.

Examination of sections of albino and normal animals shows striking differences in the endocrine glands. The sectioned hypophysectomized animals show no trace of the anterior lobe of the hypophysis. That part of the floor of the diencephalon which normally abuts against the hypophysis, rests upon the floor of the cranium (fig. 2). This apparently demonstrates conclusively that the entoderm has not the intrinsic power to form a hypophysis. If it enters into the formation of the gland at all it must be considered as a tissue inclusion which became changed through its adaptability into glandular parenchyma, a conclusion previously drawn by the writer, Smith ('14). The infundibulum shows some structural modifications when compared to the checks, although the saccus vasculosus, as far as determined, appears to be normal. In the checks that region of the diencephalon which rests against the pars glandularis is

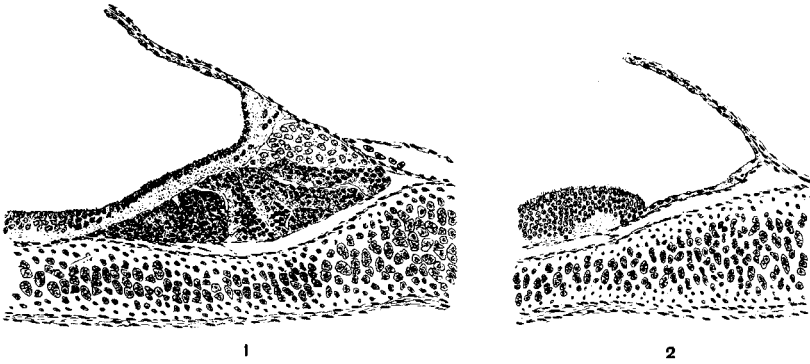


Fig. 1 A section through the hypophyseal region of a 38 mm. normal tadpole. $\times 100$.

Fig. 2 A section through the hypophyseal region of a 37 mm. albino. Note the much reduced pars nervosa. $\times 100$.

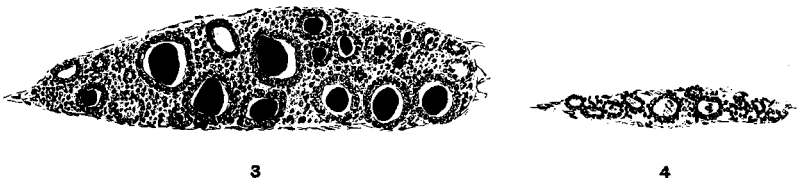


Fig. 3 A sagittal section through a lobe of the thyroid of a 38 mm. check. $\times 100$.

Fig. 4 A sagittal section through a lobe of the thyroid of a 37 mm. albino. $\times 100$.

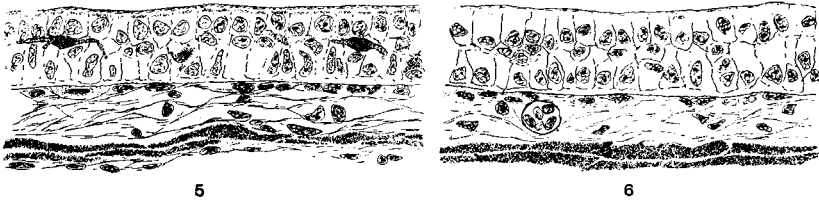


Fig. 5 A section through the epidermis, in the mid-brain region, of a normal 39 mm. check. The pigment granules are indicated by dots. $\times 200$.

Fig. 6 A section through the epidermis, in the mid-brain region, of a 38 mm. albino. A faint melanophore in the left part of the figure. $\times 200$.

of considerable thickness, that is, in addition to the ependyma there is a rudimentary pars nervosa. Caudad to this the wall is formed almost entirely of ependyma. The pars nervosa is reduced throughout most of its extent to an ependymal layer in the hypophysectomized animals. There may be a small localized thickening but nothing to correspond to the normal animal (figs. 1, 2).

The thyroid shows marked modifications in the albinos. In the accompanying table the size of one lobe of the thyroid of a normal 38 mm. tadpole with 4.0 hind legs and of a 37.0 mm. albino with 0.1 mm. hind legs is given.

Size in millimeters of one lobe of the thyroid			
38 mm. check		37 mm. albino	
Length.....	0.6	Length.....	0.21
Width.....	0.3	Width.....	0.15
Thickness.....	0.16	Thickness..	0.04

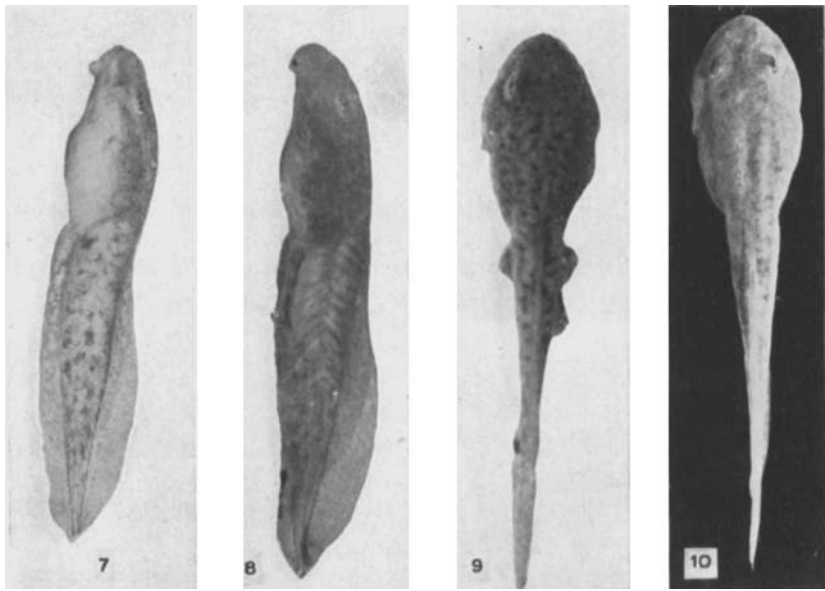


Fig. 7 Photograph of an albino. $\times 2$. Note the very small hind limb bud.
Fig. 8 Photograph of a normal tadpole. Figures 7 and 8 were photographed on the same plate' $\times 2$.
Fig. 9 Photograph of a normal tadpole. $\times 2$.
Fig. 10 Photograph of an albino. $\times 2$.

The above table shows that the thyroid of the albino is approximately one-third normal size. The contrast is even more striking when the compactness and character of the parenchyma is noted. A sagittal section through the thyroid of a 38 mm. check shows on an average 12 to 15 vesicles, many of which are largely distended with colloid, the parenchyma of the whole gland being compacted together. A sagittal section through the thyroid of a hypophysectomized 37 mm. specimen shows 6 to 8 atrophied vesicles containing but a slight amount, or no colloid, and with large spaces between the vesicles. The cells making up the vesicles of the former are cuboidal and protoplasmic-rich, in the latter little but the nuclei remain (figs. 3, 4). The results from experimental feeding of thyroid by Gudernatsch and other workers suggests that the non-development of the hind legs in the albinos is due not to the hypophysis but rather to the failure of the thyroid. In this connection the 36 mm. albino with 4.2 mm. hind legs, mentioned above, is of interest. Sections of this specimen show that the hypophysis was completely ablated but that the thyroid is normal. This specimen thus gives additional evidence that the retarded development of the hind legs must be referred to the thyroid and not to the hypophysis. Also the reduction in pigment is not due to the atrophy of the thyroids. The modifications of the thyroid obtained by Adler ('14) were similar but less striking.

An examination of a large number of male and female albinos and checks has, as yet, failed to show any constant variation from the normal in the sex glands of the hypophysectomized animals. The sex glands of the albinos although varying considerably apparently do not exceed the limit of variation met with constantly in the normal animals. This conclusion stands in contradiction to the results previously adduced by the author and to the results of Adler ('14) in the hypophysectomized tadpole and to the conclusions of Hahn ('12) in the tadpole with hypertrophied hypophysis as well to the results obtained in mammals by pituitary feeding, notably that of Goetsch ('16).

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