

### A Thyrotropic Field Surrounding the Immature Pituitary of the Tadpole.\*

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It was previously reported that pituitary primordia when grafted are capable of developing thyrotropic activity in the tadpole, though such activity is generally delayed in appearance and much less than that of the normal gland.<sup>1, 2</sup> In the course of a series of investigations on the development of function in such grafts, it was found that multiple grafts placed under the adhesive discs and into the eye cup of hypophysectomized tailbud embryos of *Rana sylvatica* induced precocious metamorphosis in the host. Since single grafts similarly placed did not induce precocity, it was considered that the excess pituitary tissue was responsible for the precocity.<sup>3</sup> No precocity was observed by Blount in *Amblystoma* with excess pituitary grafts.<sup>5</sup>

In attempting to confirm these observations in *R. pipiens*, a series of normal animals with extra pituitary primordia inserted into the eye, near the otocyst and under the adhesive discs was prepared. Only those animals which had extra pituitaries placed under the adhesive discs showed precocity. In another experiment one animal which had its own pituitary primordium placed under the adhesive discs, displayed unmistakable metamorphic precocity.

It was thought that the explanation of these results might lie in some effect of the proximity between the pituitary and thyroid, for when grafts are placed under the adhesive discs, they develop in the neighborhood of the thyroid gland. The high proportion of precocity observed in tadpoles with several grafts in this region as compared to those with one, may be accounted for by the greater depth to which the grafts must be pushed when several are inserted, for embryological considerations indicated that the more deeply placed the grafts are, the closer they approach to the place of origin of the thyroid. The following experiment was conducted to test this hypothesis.

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<sup>1</sup> Etkin, William, *Anat. Rec.*, 1934, **60**, Suppl. 1; 1935, **64**, Suppl. 1.

<sup>2</sup> Atwell, Wayne J., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 224.

<sup>3</sup> Etkin, William, *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1653.

<sup>5</sup> Blount, Raymond F., *Proc. Nat. Acad. Sci.*, 1930, **16**, 218.

Experiment 1936—3. *Rana pipiens*—All animals from one batch of eggs. *Operations*:—Type A—175 tailbud embryos were hypophysectomized and their own pituitary primordia inserted under the adhesive discs and pushed forward to rest near the place where the thyroid is to form.

Type B—50 normal tailbud stages with thyroid primordia which had been dissected out of embryos 1-2 days older and inserted near the pituitary primordium. Such animals retained their own thyroids in the normal situation.

Type C—40 normal control animals.

All the animals were set out into fingerbowls, 4 animals to a dish with about 100 cc. of water, and fed spinach and oatmeal. They were kept on a water table surrounded by running water regulated to 26°-29°C. Of the Type A animals only those were set out which showed the darkening which is the effect of a successful take of the pars intermedia of the pituitary.

At 20 days after the operation most of the animals had reached a total length of 20-30 mm. A selection of these animals considered to be of interest were then fixed in Bouin's fluid and the appropriate regions sectioned serially, stained with Mallory's connective tissue stain and examined.

*Results*:—Controls—The data on the 40 control animals of this experiment were combined with 131 available in all other experiments with this species under the same conditions. The hind leg length was plotted as ordinates against the total length as abscissæ. This graph showed the range of variation in normal controls. In this way standards were established for the observation of early precocity in the hind leg growth and, (since hind leg growth depends upon the thyroid hormone) for estimating the degree of activity of the thyroids under the influence of the pituitary. Those animals in which the hind leg length was twice or greater the maximum size found in the 171 normals at a like total length were deemed definitely precocious, those showing an increase of 25% to 100% over the maximum in the controls were considered as probably precocious, and the rest regarded as not precocious (Fig. 1). Several animals of each operated type were allowed to continue their metamorphosis, which they did. These will not be considered in the present report.

Type A—Hypophysectomized animals with own pituitary placed near thyroid. Of 60 of these which were set out in the experiment, 14 were definitely precocious in hind leg growth, 6 were

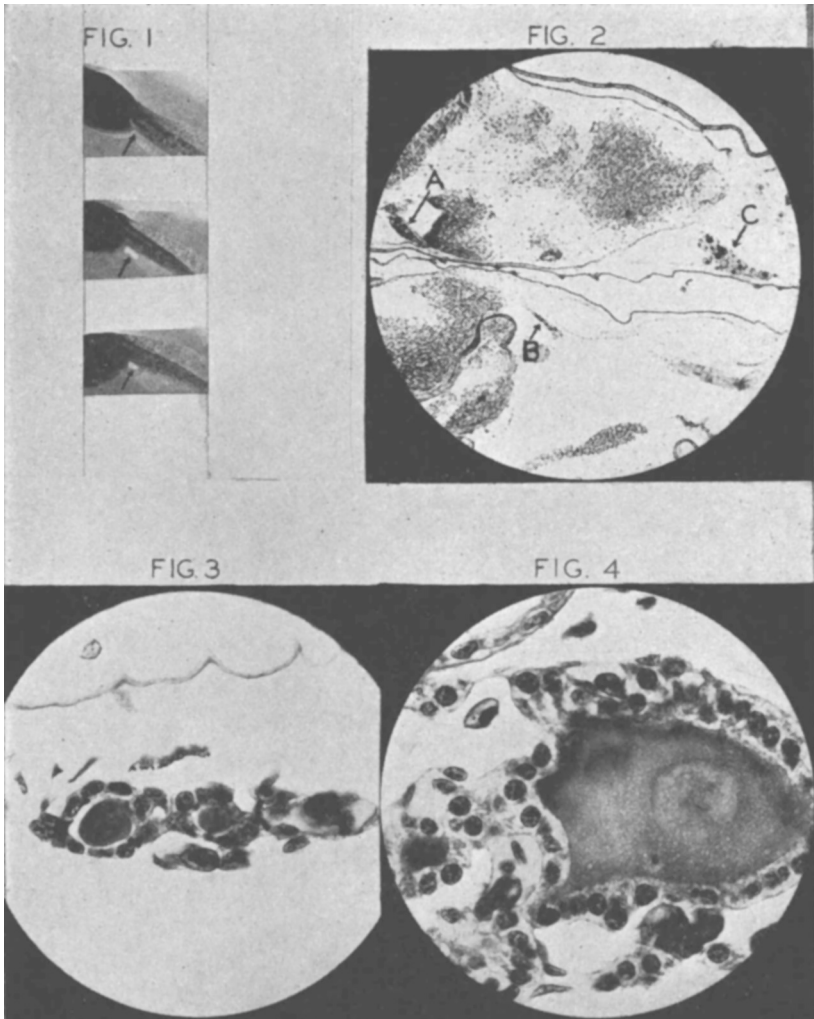


FIG. 1.

Above—Normal control—Total length, 26 mm. Hind leg length, 0.2 mm. Arrow points to limb bud showing normal size, appears as white dot.

Center—Hypophysectomized with own pituitary implanted near thyroid. T. L., 25; H. L. L., 2.0.

Lower—Normal animal with extra thyroid primordium implanted near hypophysis. T. L., 26; H. L. L., 1.5.

FIG. 2.

Sagittal section of head of Type B showing normal hypophysis at "A," normal-site thyroid at "B" and thyroid graft at "C." Section selected to show the maximal cross-section size of normal thyroid. T. L., 27; H. L. L., 0.6. Mag. about 29 $\times$ . Note that the mouth cavity separates "A" from "B".

FIG. 3.

Largest section of normal thyroid of Type B animal. T. L., 27; H. L. L., 1.4, Mag. about 420 $\times$

FIG. 4.

Section through grafted thyroid of same animals as Fig. 3. Entire section not shown. Mag. about 420 $\times$ .

probably so, and in the rest the hind leg length lay within the normal range of variation.

Type B—One extra thyroid placed near the pituitary. Of 49 of these set out, 8 were definitely precocious, 5 were probably so, and the rest were normal.

*Histological Findings:*—The development of the thyroid in this and related species was studied previously. It was shown that before metamorphosis, the gland is extremely small, the follicles being but a few times larger than the diameter of a nucleus, and with very flat epithelium, poor in cytoplasm (Fig. 3). Such glands were found in 3 normal controls and, most significantly, in all the normal-site glands in the Type B animals. In the Type A animals, 7, classified as definitely precocious, showed thyroids greatly enlarged in total size, most follicles much larger than normal. In some cases very small colloid vacuoles were shown. Often one follicle presented more area than the largest x-section of an entire normal gland. The epithelium was decidedly higher than in controls, varying from low cuboidal through columnar. The gland was characteristically highly vascularized. The picture resembled that of mid-prometamorphosis in normal development, but the gland did not attain the extreme activity shown by normal thyroids at the climax of metamorphosis. Figure 4 shows the type of epithelium which characterizes such glands. These will be designated as activated glands. In 2 classified as probably precocious, one activated gland, and one about normal were found. Of 5 classified as not precocious, 2 activated, one intermediate and 2 small normal glands were found.

In all cases the graft hypophysis was found in the neighborhood of the thyroid, sometimes in contact with it. It is not apparent that the cases with non-activated thyroid showed any greater separation of the glands than did the others, though this factor might contribute. In several cases where the thyroid was diffuse in structure, it appeared that the part of the gland closest to the graft seemed to have the most highly activated epithelium, though quantitative distinctions cannot be relied upon to any great extent.

In the Type B animals sections are available of 4 specimens in each classification, precocious, probably precocious, and not precocious. With the exception of one animal in the last group all these showed that the graft thyroids placed near the hypophysis were decidedly enlarged as compared to the glands in the normal position (Figs. 2, 3, 4). The epithelium in all except 3 cases in the

latter 2 classifications was clearly activated (Figs. 3, 4). In one non-precocious animal no graft thyroid was found. Thus the normal thyroid glands of these animals showed the inactive condition characteristic of the gland in the normal tadpoles of this age whereas the grafted thyroid is clearly increased in size and activity. That the mere grafting of thyroids does not result in activation has already been shown by Allen<sup>4</sup> and others.

The graft thyroids were rather widely distributed, several lying in contact with the anterior part of the forebrain, some in the basicranial cartilage, but in all cases touching or lying in a space that borders the meninges lining the cranial cavity (Fig. 2).

These results are interpreted as indicating that approximation of these glands brought about by the transplantation of either one induces a precocious activation of the thyroid. This activation was morphologically apparent in nearly all cases and physiologically demonstrated by precocious hind leg growth in most cases. The absence of metamorphic activity in some instances where the thyroid appeared activated morphologically may be attributed to the early stage at which the animals were sacrificed.

It is thought that this experiment demonstrates that the pituitary of an immature animal produces and releases into its neighborhood effective concentrations of thyrotropic hormone. Such secretions are somehow rendered ineffective for the normal thyroid whether by reason of dilution in the blood stream or otherwise.

It would appear from this experiment that the precocity observed with multiple pituitaries as previously reported<sup>8</sup> was due to the position of the grafts and not to the excess tissue *per se*.

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#### Effect of Various Amino Acids on Motility of Excised Segments of the Small Intestine.

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In the course of some unpublished work by Weinstein and Rettger on the effects of alkaline bacterial cultures it was deemed ad-

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<sup>4</sup>Allen, B. M., *Anat. Rec.*, 1929, **44**, 207; *Contributions to Marine Biology*, Stanford U. Press, 1930.