A RETARDATION IN THE RATE OF METAMORPHOSIS OF THE COLORADO AXOLOTL BY INJECTION OF ANTERIOR HYPOPHYSEAL FLUID.*

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WITH PLATE.

In 1922, the author with I. P. Smith, reported in a brief abstract that the intraperitoneal injection of fresh anterior hypophyseal fluid slowed decisively the environmentally-produced metamorphosis of the Colorado axolotl (A. tigrinum). In a second abstract (Smith and Smith, 1923), it was shown that the injection of fresh anterior lobe fluid also diminished the metamorphic stimulus afforded by thyroid administration.

Since the appearance of these abstracts, Hogben (1923) has reported opposite effects, namely, that the intraperitoneal injection of a commercial anterior lobe extract caused a rapid metamorphosis of the normal unoperated Mexican axolotl (A. tigrinum) and even of thyroidectomized individuals of this species. Spaul (1924 a), using the same commercial preparation as Hogben, confirmed the results of this investigator in the unoperated axolotl and found in addition that the metamorphic rate of the tadpole was greatly accelerated. More recently, Spaul (1924 b) tested some twelve commercial anterior pituitary preparations for the presence or absence of a principle which would metamorphose the axolotl. He found that only two of these produced what he terms "the appropriate response," namely, metamorphosis; the other ten gave negative results. He assumed that these ten preparations contained some inhibitory substance which prevented the principle inducing metamorphosis from expressing itself. The opposite assumption might seem as reasonable, namely, that the ten preparations,

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because of their great numerical majority, gave “the appropriate
response,” and that the two preparations inducing metamorphosis
contained some unusual substance giving an atypical response.

This thyroid-like effect secured by Hogben, and from certain
preparations by Spaul, by the injection of anterior lobe extract
is thus not in harmony with our findings in the axolotl (Smith
and Smith, 1922) nor with earlier work on the tadpole (Smith
and Cheney, 1921).*

It appears important to clear up these discordant findings
and to definitely determine the response of the axolotl to the
injection of anterior lobe fluid. Such a determination is of
considerable significance in understanding the function of the
thyroid, of the hypophysis, and of the inter-relationship obtaining
between these glands. It involves the broad question as to
whether the anterior hypophysis can substitute functionally for
the thyroid, whether, in other words, there is a vicarious
relationship between these glands. I thus feel justified in
presenting in some detail my findings and in pointing out the
error of the conclusions into which Hogben and Spaul have
been led through their willingness to accept the response secured
from administering a commercial preparation of a gland as
indicating the activity of that gland.

The form of axolotl which Hogben and Spaul used differs
somewhat in its normal behaviour from the form which I used.
The Mexican (European) form used by Hogben and Spaul is
usually permanently larval and fails to metamorphose even when
removed from its native habitat. There is consequently an
“all-or-none” response as Hogben has pointed out. The
Colorado (New Mexican) axolotl, on the other hand, although
it may remain permanently neotenous in its native habitat, does
not invariably do so. It almost always undergoes metamor-
phosis, independently of the size reached, when transported
to this region. This has been shown to be also the case
when removed to the Eastern seaboard (Swingle, Uhlenhuth).
The speed with which the metamorphic changes progress in
this region varies with the time of year; those received in

* Swingle (1922) states that he believes that the injection of fresh pituitary
substance induces axolotl metamorphosis. This opinion was based on results
obtained from a series of heteroplastic pituitary transplants. He does not appear
to be very confident of the results obtained in his series.
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the spring and summer months metamorphose within a few weeks; those received in the autumn months exhibit but slight metamorphic changes until the following February or March when they rather rapidly assume adult characteristics.

It is possible to hasten greatly in the Colorado axolotl this environmentally-produced metamorphosis by the administration of thyroid substance as has repeatedly been shown. Thus this form responds readily to agents which usually induce metamorphosis, and would consequently appear to be as useful in determining the activity of such an agent as is the Mexican strain. It has, moreover, an additional advantage since it can also be used in determining the activity of any agent which prolongs or slows the metamorphic process, a use that is not possible with the Mexican axolotl, for in that form, as pointed out, no metamorphic changes take place under ordinary laboratory conditions.*

Three series of axolotls have been run in order to ascertain the effect of fresh anterior pituitary injections upon the rate of the environmentally-induced metamorphosis of the axolotl. Each series consisted of one group injected with fresh anterior lobe fluid, one with muscle extract, and a muscle-fed uninjected group. One of these series, in addition, contained a group injected with Ringer's solution and one with posterior pituitary extract.

Three series of axolotls were run in order to determine the effect of anterior lobe injections when a thyroid stimulus was superimposed upon the environmentally-induced metamorphosis. Each series consisted of one group injected with thyroid extract, one with anterior lobe fluid, and one injected with both thyroid and anterior lobe fluids, the amount of each substance injected being identical with that administered to the other two groups respectively. Each series contained a muscle-fed control group.

The fresh anterior lobe fluid was made according to the method of Evans and Long (1921). The thyroid fluid (extract)

* The view taken by Swingle (1922) that the instability of the larval condition in the Colorado (New Mexican) strain makes this form undesirable or even useless for experimental work is not concurred in by the writer. Effects which transcend this variability in metamorphosis surely are significant. Only pronounced effects which transcend all variability are presented here.
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was made from fresh sheep glands which after being sterilised in 50 per cent. alcohol were ground, diluted with 3 c.c. of saline for each gram of gland substance and centrifuged. The posterior lobe and the muscle extracts were prepared in the same way as the thyroid extract.

The extract of Armour's anterior pituitary tablets (purchased on the open market) was made by grinding one 2-gr. tablet in 1 c.c. of saline.

The size of the dosages and length of the treatment is given in the protocols.

That the additional handling incurred in the injection of the various fluids did not alter the rate of metamorphosis was early determined by our saline injections. Likewise the protein content of the injected substances produced no distinguishable effects as shown by the muscle injections. Ineffective also, as a metamorphic retardant or stimulant, were the injections of the bovine posterior lobe. The injection of this substance produced a pronounced darkening. Attention has already been called to the fact that metamorphosis was slower in the autumn and winter than in the summer. It would be expected, consequently, that the effect of any agent slowing metamorphosis would be magnified as regards the time element during these months of slow metamorphosis. Such proved to be the case with our hypophyseal injections. In the summer, our injections, although postponing for a time the appearance of the metamorphic changes, and slowing them after they had appeared, still did not exhibit a sufficient inhibition to postpone the completion of metamorphosis for any considerable time. In the autumn and winter months the retarding effect of anterior lobe was pronounced.

There was noted another response from our anterior lobe injections which it seems may be of considerable significance in determining the reason why these injections retard metamorphosis. They produced a pronounced increase in the width of the ventral and more especially of the dorsal tail fin. Associated with this growth phenomenon was noted the further interesting fact that the resorption of the tail fins in these specimens was delayed more than any other external metamorphic change. It was not an unusual occurrence for the
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tail fins to remain of considerable width for some time after all other external larval characteristics had been lost. The orderly sequence of the metamorphic changes were thus disturbed.

Protocols of one of the series showing the typical response of the axolotl to the injections of anterior hypophysseal fluid as compared with un.injected and muscle injected controls.

Series VIII.—Axolotls received 9th October 1922.—The series was composed of three groups of five specimens each: Group 1, controls, no injection; group 2, controls, injected with muscle extract; group 3, injected with anterior lobe fluid, $\frac{1}{4}$ c.c., three times a week. All specimens fed daily with muscle. Kept in container measuring 12 in. x 12 in. in slowly running water, approximately 2 in. deep.

18th November.—All specimens typical larvae. The specimens measured from 26 cm. to 28 cm. in total length. Injections commenced.

18th December.—No specimens show metamorphic changes. The members of group 3 (anterior lobe injected) are jet black, the others are a yellowish-brown varying somewhat in depth of colour. The tail fins of anterior lobe group have distinctly broadened and are slightly fluted transversely due to the fact that the length of the fin somewhat exceeds the length of the central stalk, a condition not shown by the other specimens.

7th January 1923.—No metamorphic changes evident.

17th February.—No metamorphic changes evident.

27th February.—The specimens receiving anterior lobe injections have tail fins about one and a half times as wide as the specimens of the control groups.

15th March.—Three of the specimens of the muscle injected controls have metamorphosed, the other two specimens have considerably reduced gill filaments and tail fins. The specimens of the muscle fed controls are in approximately the same stage as those of the muscle injected controls. The specimens receiving anterior pituitary fluid show no metamorphic changes.

21st March.—Injections stopped. All specimens save those receiving anterior hypophysis are metamorphosed; those receiving anterior lobe injections are typical larvae.
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5th April.—The specimens of the anterior lobe injected group show a slight shortening of the gill filaments. No other signs of metamorphosis.

12th April.—Two of the anterior lobe injected specimens show a distinct shortening of their gill filaments and roughening of the skin. The three other members of the group are typical larvae.

3rd May.—Two of the anterior lobe treated specimens have metamorphosed; the other three show definite metamorphic changes.

This same definite retardation of metamorphosis from the injection of fresh anterior lobe fluid was also secured in our other series, the protocols of which it seems unnecessary to present here. It was just the opposite effect from what we had anticipated would result, and consequently was not accepted until it had been repeatedly secured and found to be a uniform and invariable response.

An experiment which would more severely test this inhibiting effect of anterior lobe injections upon metamorphosis was suggested by Dr Evans. This consisted in the administration of both thyroid and anterior lobe to the same specimen. Several investigators have shown that thyroid will speedily induce metamorphosis in the axolotl as in most amphibia. The next phase of the work then was to determine whether the stimulus to metamorphosis afforded by thyroid administration could be aborted or lessened by the pituitary injections.

In the two preliminary series in which this test was tried, negative results were obtained, that is, no inhibition of the rapid thyroid-induced metamorphosis appeared to result from anterior lobe injections. Metamorphosis took place with surprising rapidity (two to three weeks). It seemed probable from this extremely rapid change that the stimulus to metamorphosis from the dosage (1 c.c. on alternate days until six to eight injections had been given) was so great that no inhibiting effect from the anterior lobe injections could express itself. Further, these preliminary tests were given during the summer months when the normal rate of metamorphosis was at its peak, consequently, there was superimposed upon a rapidly proceeding metamorphosis the metamorphic stimulus afforded
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by the heavy thyroid dosage. As subsequent experiments, carried out in the winter months, have shown, the injection of anterior lobe substance diminishes the stimulus to metamorphosis which results from a moderate thyroid dosage.

As shown by the protocols given below, the effect of a thyroid dosage which metamorphosed the axolotl in a period not exceeding five weeks was almost entirely aborted by injections three times a week of $\frac{1}{4}$ c.c. of fresh anterior hypophyseal fluid.

**Series IX.**—*Axolotls received 28th November 1922.*—This series was composed of three groups of five specimens each, varying considerably in size: Group 1, uninjected controls; group 2, injected twice with $\frac{1}{4}$ c.c. of thyroid; group 3, given the same thyroid dosage as the specimens of group 2, and in addition to this $\frac{1}{4}$ c.c. of anterior lobe fluid three times a week. Reared as were the specimens of Series VIII.

9th January 1923.—Injections commenced. All specimens are typical larvae.

16th January.—The second and last injection of thyroid given.

3rd February.—The specimens of group 2 (thyroid injected only) show a definite absorption of their tail fins and gill filaments, and cutaneous changes. They will not eat. The controls and the thyroid-anterior lobe injected specimens show no metamorphic changes and eat voraciously. The specimens of the latter group are black.

17th February.—All specimens of the thyroid injected group are metamorphosed. The specimens of the other two groups are typical larvae.

27th February.—The injection of anterior lobe was stopped. The three groups were photographed. The specimens of the control group show no metamorphic changes. Those of the thyroid-anterior lobe injected group show possibly a slight curling and shortening of the gills.

15th March.—The thyroid-anterior lobe injected specimens are metamorphosing somewhat more rapidly than the controls.

21st March.—All specimens of the thyroid-anterior lobe injected group are metamorphosed. Three specimens of the controls are metamorphosed and the other two are nearly so.
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It would seem that no better evidence of the inhibiting effect of injected fresh anterior hypophyseal substance upon the metamorphosis of the axolotl could be presented than is given in the foregoing protocol. The specimens of both the injected groups received identical amounts of thyroid extract. Those receiving the thyroid only, showed definite metamorphic changes within three weeks and became adults in five and a half weeks. Those receiving this same thyroid dosage, but, in addition, being injected with anterior lobe, showed, as in the case of the uninjected controls, no definite signs of metamorphosis at the end of this five and a half week period and only finally completed metamorphosis after a period of ten weeks, nearly concurrently with the uninjected controls.

It will be recalled that earlier in the paper, reference was made to papers by Hogben and by Spaul in which they secured the metamorphosis of the permanently neotenous Mexican axolotl by the intraperitoneal injection of an extract of a commercial anterior pituitary preparation, the first author even metamorphosing the thyroidless larvae with this treatment. Spaul, in addition to the axolotl, also induced precocious metamorphosis in the normal tadpole. It seemed wise to repeat this work upon the Colorado axolotl, using the identical preparation (anterior pituitary tablets, Armour & Company) employed by these investigators.

The results which they obtained were immediately confirmed with this additional significant finding, namely, that the feeding of these tablets was fully as effective in producing precocious metamorphosis as was the intraperitoneal injection of an extract made from the tablets. A protocol of this experiment follows.

Series II.—Axolotls received 19th August 1924.—The series was composed of five groups of three specimens each: Group 1, fed three times a week with $\frac{1}{2}$ of a 2-gr. anterior pituitary tablet; group 2, injected three times a week with $\frac{1}{4}$ c.c. of an extract made from the pituitary tablets; group 3, fed with fresh anterior lobe; group 4, injected with fresh anterior lobe fluid; group 5, uninjected controls. All animals supplied with a muscle diet.
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21st August.—Injections and special feeding started. All specimens larval.

10th September.—The specimens fed with the pituitary tablets are nearly metamorphosed. Those injected with the extract made from these tablets are definitely metamorphosing. The specimens of the other groups are larval.

15th September.—The tablet-fed specimens are metamorphosed. The tablet-injected animals are nearly metamorphosed. The specimens of the other groups are unchanged except one specimen of the control group which shows a slight reduction in its gill filaments and tail fins.

27th September.—The pituitary tablet injected animals are metamorphosed. Two animals of the control group and one of the anterior lobe fed group have their tail fins and gill filaments about one-half resorbed, the other specimens are typical larvae.

The specimens receiving the anterior pituitary tablets, either orally or intraperitoneally as shown by the above protocol, metamorphosed rapidly, a result obtained by Hogben and by Spaul in their injections of this identical commercial pituitary preparation. Those, however, which were given the fresh gland either by mouth or by injection showed none of this accelerating action upon metamorphosis. Had Hogben and Spaul taken the precaution to feed as well as to inject the tablets they would have found that the feeding of the pituitary tablets did not give results which harmonised with earlier work of Huxley and Hogben (1922), in which it was shown that the feeding of fresh anterior lobe does not produce metamorphic changes in the Mexican axolotl. Their error in concluding that the metamorphosis which they secured from the injection of the anterior pituitary tablets was due to the action of anterior lobe substance could also have been avoided had cognisance been taken of the papers of Hoskins and Hoskins (1920) and of Smith and Cheney (1921). Their results are identical with those secured by Hoskins and Hoskins in the normal and thyroidless tadpole, results which Smith and Cheney showed were atypical and could not be secured by the use of other commercial pituitary preparations, by the dried gland prepared in the laboratory, nor by the fresh gland. Smith and
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Cheney duplicated, however, the anomalous results obtained by Hoskins and Hoskins from the tablet feeding when to dried anterior lobe substance they added thyroxin iodin in amounts sufficient to make its iodin content identical with that of the tablets.*

It seems unsafe to substitute a commercial glandular preparation for one prepared in the laboratory under known conditions.

We are not chiefly concerned here, however, with the fact that the administration of a particular commercial anterior pituitary preparation gives effects which cannot be secured with the fresh fluid; we are concerned with the finding that the injection of fresh anterior lobe substance slows and postpones the environmentally-produced metamorphosis in the Colorado axolotl and can neutralise in this form the activity of as powerful a metamorphic agent as is thyroid, even though the thyroid extract be given in sufficient amounts to greatly accelerate metamorphosis. This action was unexpected and appears paradoxical since it has been shown (1) that homoplastic anterior lobe grafts causes an enlargement of the thyroid and initiates metamorphic changes in the hypophysectomised tadpole, a permanently larval form (Allen, 1920); (2) that the injection of bovine fresh anterior lobe substance in the hypophysectomised tadpole carries the animal through complete metamorphosis, repairs the thyroid and may even produce a profoundly hyperplastic gland (Smith and Smith, 1922). This opposite effect which appears at first to be disconcerting casts light upon the function of the pituitary and of the thyroid, and will be discussed more at length in a subsequent paper.

For the present it will be sufficient to state that the physiological activity of the anterior hypophysis as ascertained in this and other work appears to be very dissimilar to that of the thyroid. One gland, the anterior hypophysis, has to do with the constructive phase of development and growth; the other gland, the thyroid, has to do with changes characterised by Swingle and by Uhlenhuth as catabolic.

* Analyses by Dr E. C. Kendall revealed the significant fact that the iodin content of the tablets causing this precocious metamorphosis exceeded by 120 times the iodin content of the other dried anterior lobe preparations.
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Summary.

1. The intraperitoneal injection of fresh anterior hypophyseal fluid retards the environmentally-induced metamorphosis of the Colorado (New Mexican) axolotl.

2. The metamorphic stimulus afforded by a small but effective thyroid dosage can be neutralised, physiologically, in the axolotl by the injection of fresh anterior pituitary fluid.

References.


Smith, P. E. (1923), "The Response of the Colorado Axolotl to the Intraperitoneal Injection of the Thyroid, Anterior Hypophysis, and combined Injections of these two Substances," Anat. Rec., 28, 151.


Smith, P. E., and Cheney, G. (1921), "Does the Administration of the Anterior Lobe of the Hypophysis to the Tadpole produce an effect similar to that obtained by Thyroid Feeding?" Endocrinology, 6, 448-460.


Description of Plate.

Photographs of the living axolotls (Colorado strain of *A. tigrinum*) of Series IX., 1922. Photographs taken 27th February 1923.

**Fig. 1.**—The specimens of group 1. These are normal uninjected controls.

**Fig. 2.**—The specimens of group 2. These specimens received two injections (9th and 16th January) of $\frac{1}{2}$ c.c. thyroid extract.

**Fig. 3.**—The specimens of group 3. These specimens received the same thyroid dosage as those of group 2 ($\frac{1}{2}$ c.c. on 9th and 16th January), and in addition received injections of $\frac{1}{4}$ c.c. fresh anterior lobe fluid three times a week from 9th January to 27th February.