ACCELERATED METAMORPHOSIS OF FROG TADPOLES BY INJECTIONS OF EXTRACT OF ANTERIOR LOBE PITUITARY GLAND AND THE ADMINISTRATION OF IODINE.*

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With Two Plates.

The accelerated transformation of frog tadpoles to adults by thyroid feeding and also iodine, demonstrated by many workers since Gudernatsch's original experiments, and further, the inability of thyroidless tadpoles to change unless given either thyroid or iodine (Swingle), has established the importance of both thyroid and iodine in amphibian metamorphosis.

Various glands of internal secretion, including the pituitary, have been fed to tadpoles and axolotls without noticeable effects, but the extirpation of the pituitary of tadpoles (Smith, 1916; Allen, 1917) was found to prevent metamorphosis, and on the other hand, grafting the anterior lobe into normal tadpoles caused a considerable acceleration in the assumption of adult characters, so that the possibility of pituitary influence has been investigated more thoroughly. As a result Hogben succeeded in inducing metamorphosis in axolotls when injected with commercial extracts of the anterior lobe. In the experiments recorded here an increased rate of change was obtained when tadpoles were injected with such extract, thus confirming the results of implantation work and the functional activity of the anterior lobe secretion during metamorphosis.

Allen has shown that the administration of iodine to tadpoles deprived of the pituitary or the thyroid or both induces them to undergo metamorphosis, suggesting independent action of the iodine. Evidence of this effect is obtained from a further series of experiments detailed in this paper, in which

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greater metamorphic acceleration was produced in injected tadpoles kept in iodine solution of suitable concentration.

The supply of tadpoles for this work was drawn from two sources: (a) animals reared in the laboratory, (b) animals collected outside.

I. Injections of Extracts of Anterior Lobe.

Throughout all the experiments conditions were kept uniform. The temperature was constant (17°), the injections made tri-weekly, each injection consisting of 0.1 c.c. of commercial extract of the anterior lobe pituitary made up with Ringer's solution, so that 0.15 gr. of fresh gland was injected on each occasion.

For experimental purposes the tadpoles were placed in small shallow dishes on a white background. The dishes were arranged in pairs, one for controls, and the other for the specimens undergoing treatment. They were fed twice a week on raw meat, the meat being left in the dish for a few hours and then removed and the water changed. In the case of iodine a fresh solution of iodine of the same concentration was given after each meal.

The injection was made in the dorsal region to the side of the kidney. The difficulties attending the operation were many and resulted at first in several deaths, but an improved technique and careful manipulation enabled them to be successfully overcome and the fatalities considerably reduced. Other factors, however, both internal and external, which could not be controlled were mainly responsible in these cases. The method adopted consisted in covering the animal to be operated on with just sufficient water, and placing over it a flat ring with a piece of gauze stretched across it. The individual was thus securely held and could be slowly injected through the gauze. The wound caused by the puncture healed very quickly, and the animal showed no ill-effects behaving quite normally.

The extract was the same preparation used by the author in previous work on axolotls, but in order to test its strength and activity an axolotl was injected as a control. It proved to be of the same standard as metamorphosis occurred at the expected time and the animal's behaviour throughout agreed with former experimental observations.
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The first series of individuals to be used were reared in the laboratory and 5 weeks old. Five were placed in each dish, but after three injections all had succumbed. Nevertheless there were distinct and notable changes. There was an obvious diminution in size after the first injection, which enhanced the difficulties of injection, and also a peculiar transparency of the skin which persisted, the internal organs appearing as a black mass and the eyes as black spots. Allen records a similar condition after the removal of the hypophysis of tadpoles, and Hogben notes a paling of axolotls after hypophysectomy.

Other changes in the injected specimens were their sluggish movements, but when disturbed a sudden display of great activity was noted. The controls were lively, appearing normal in every way with no alteration of pigment or size.

Batches 6, 7, and 8 weeks old were started in the same manner with similar results, and although many survived several injections only one remained alive after eighteen days from the first injection. This was a member of the 8th week batch, and it was then too small to inject again. After twenty-four days limb buds appeared, and with the exercise of great care as regards diet and change of water it was kept alive a few more days. Its tail developed a peculiar twist, resembling that described by Swingle in hyperthyroidism in tadpoles, and the head and body were becoming more adult-like in shape. The controls meantime showed no signs of such changes.

This series of changes agrees with that found by Gudernatsch in his thyroid fed tadpoles, and by Morse and Swingle in tadpoles kept on thyroid and iodine diets during their metamorphosis. The mortality was particularly high in some of these experiments, and Morse points out that it is impossible to bring about complete metamorphosis in extremely young larvae by feeding large quantities of thyroid or iodine, for although marked changes do appear death supervenes before complete transformation. This applies equally well to tadpoles injected with anterior lobe extract as results of this work show.

It seems possible, particularly when the rôle of the anterior lobe extract is considered in relation to the metamorphosis of the axolotl, that here it is producing heightened metabolism.
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which induces certain somatic changes similar to those normally occurring in metamorphosis, the latter process being thereby accelerated. The effect, as in thyroid feeding, varies with age, and it is apparent that the rapid rate is responsible for death in the young larvae, and only in the older tadpoles is there any chance of complete transformation.

To demonstrate these deductions more conclusively another series of experiments on exactly the same lines were conducted under identical conditions, with the large tadpoles obtained from outside sources. They were placed in dishes as before, but on account of their size only three were put in each. As their exact age was unknown and a few already showed signs of limb buds, a careful selection was made to obtain approximately the same stage of development in those to be experimented with, and further controls showing limb buds were kept under observation under the same conditions as an additional check. It was found in the first series of experiments that the meat diet had a retarding influence, for tadpoles in the storage tank, feeding on algae, showed slightly greater development than the controls, so that in this group another control of alga-fed specimens with and without limb buds was used.

The experimental group was divided into two, the first for ordinary injections as before, and the second for work in iodine solutions to be described later. All were fed on raw meat as before. Injections were made with far more success, the fatalities due to injection being very small, since the greater size of the tadpoles made the operation comparatively easy and the risk of internal injury much less.

The same sequence of changes was again noted, although the pigmentation effect and diminution in size were not so marked. The body lost its rounded shape so characteristic of the well-fed larval stage, becoming slender, and at the eighth day the hind limbs were prominent. The latter rapidly increased, the eyeballs became prominent, the mouth larger, and at the sixteenth day there were signs of tail absorption and fore limbs. They were even more advanced than those controls having hind limb buds at the commencement of the experiment irrespective of diet, whilst the other controls were unchanged only one or two just showing hind
Fig. 1. Ventral view of tadpoles after seven days; (1) control, (2) anterior pituitary injected.

Fig. 2. Ventral view of tadpoles after eight days; (1) control, (2) anterior pituitary injected, (3) anterior pituitary injected and kept in 1 in 250 iodine solution.

Fig. 3. Side of view of tadpoles after eight days; (1) control, (2) anterior pituitary injected, (3) anterior pituitary injected and kept in 1 in 250 iodine solution.

Fig. 4. After sixteen days; (1) control, (2) anterior pituitary injected, (3) anterior pituitary injected and kept in 1 in 250 iodine solution.
Fig. 5. Tadpoles kept in iodine solution twenty-four days; (1) 1 in 100, (2) 1 in 500, (3) 1 in 1000.

Fig. 6. Tadpoles kept in iodine solution twenty-four days; (1) control, (2) 1 in 300, (3) 1 in 100.

Fig. 7. After fifteen days; (1) control, (2) anterior pituitary injected and kept in 1 in 250 iodine solution, (3) anterior pituitary injected and kept in 1 in 150 iodine solution.

Fig. 8. After fifteen days; (1) anterior pituitary injected, (2) anterior pituitary injected of half strength and kept in 1 in 250 iodine solution.
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limb buds. Hence in spite of diet and backward development, the injected specimens showed the most advanced state of metamorphosis at the end of this period. Although some died the mortality was not nearly so high as previously, and a few had practically completed metamorphosis in a little more than three weeks when the experiments were stopped. However, sufficient evidence had been obtained to substantiate observations and deductions from the first series of experiments.

2. Administration of Iodine.

Experiments have shown the significance of iodine in the metamorphosis of tadpoles; but the actual relationship between iodine and the pituitary during these changes is not clearly understood, hence in an endeavour to throw more light on this question, the second batch of large tadpoles already referred to were placed in iodine solutions of various concentrations, and whilst one group acted as controls, others in similar strength solutions were injected. A saturated solution of iodine was made up at room temperature and used in the following concentrations: 1 in 10 c.c., 1 in 50 c.c., 1 in 100 c.c., 1 in 150 c.c., 1 in 200 c.c., 1 in 250 c.c., 1 in 300 c.c., 1 in 400 c.c., 1 in 500 c.c., and 1 in 1000 c.c. of water. Three specimens were placed in 75 c.c. of each of these solutions, and two complete series made. There were controls in tap water for each set. Environment, diet, temperature, and other conditions were the same as before, and also there were tri-weekly injections of the same dosage in the injected series.

In the iodine control series the mortality was higher in higher concentrations; those in 1 in 10 and 1 in 50 died within a few hours owing to toxic effect, whilst only one managed to survive any length of time in 1 in 100. It became very emaciated, somewhat pale and sluggish, remaining motionless on the bottom of the dish. A similar condition was observed in the injected specimens of the previous experiments, except that there was no evident transparency in this case. Hind limbs appeared within fourteen days and later fore limbs, whilst the tail shortened, and at the time of death metamorphosis was well advanced. During this period those
kept in 1 in 1000 showed no signs of progress beyond the controls, but hind limbs had appeared in those in 1 in 500. Hence between 1 in 100 and 1 in 500 there is a definite acceleration. Between these limits there is an upward trend in the mortality, diminution in size, and rate of metamorphosis from the lower to the higher concentrations. After fifteen days only one survived in 1 in 150, but the numbers slowly increased with reduction in concentration and all remained alive in 1 in 500. The greatest diminution in size occurred in 1 in 150, whilst scarcely any change was noted at the lower limit on comparison with controls. Fore limbs, prominent eyes, altered shape of body, and size of mouth were features at this stage of those in the highest strength solution, but progress was slower going down the series and only hind limbs were noted at the lower limit.

Observations on the injected specimens kept in iodine solutions revealed a much greater though similar upward trend from the lower to the higher limits; in fact, although one kept in 1 in 150 was successfully reared it was very sluggish and not normal, and 1 in 250 was the highest concentration at which the transformation could be best completed under the double stimulus, the rate of metabolism being too great above this point, death resulting after a few days. An injected specimen at this concentration showed fore and hind limbs and rapid absorption of the tail after fourteen days. Those in 1 in 500 progressed more rapidly than those kept in 1 in 150 without injections. The usual sequence of changes was noted in all these transformations, the only variations compared with the iodine controls being pigmentation, in which some were dark and some light. Those injected were mainly lighter in colour, but from the indifferent results from the iodine controls, it seems obvious that iodine has no pigmentary effect. This agrees with Swingle's conclusions, who observed similar inconsistencies when feeding iodine to toad tadpoles and keeping them in suitable iodine solutions. The outstanding feature, however, of these results is the actual acceleration in metamorphosis produced. It was greater than that produced by either acting alone and yet increased as the amount of iodine increased. It seems highly probable, therefore, that the
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Acceleration noted represents the combined effect of the influences of the iodine and the anterior lobe extract in stimulating metamorphic processes, each acting independently and producing its effect irrespective of the presence of the other. Quantitative evidence in favour of this could not be obtained, as inconsistencies in the rate of metamorphosis and other factors made accurate determination impossible. Also the possibility of estimating relationship between the rate of change and iodine concentration proved out of question for similar reasons. The rate of change in different concentrations in both series, particularly the injected, certainly showed a gradual increase with concentration, but there was no consistency, and the variations prevented reliable estimations being made. Similar variations were noted in the diminution in size, and it is not surprising when all the factors capable of influencing results and the difficulties of maintaining uniform conditions are considered. However, it is apparent that the limits of concentration of iodine within which acceleration and completion of metamorphosis can successfully take place are 1 c.c. of saturated iodine solution in 100 c.c. of water to 1 in 500, or 0.0025 gm. per litre to 0.0005 gm. per litre, whilst 0.001 gm. per litre or 1 in 250 is the lowest limit at which injections can be made with resultant successful transformation. These results are only approximate and apply only to this series of experiments, and are not put forward as applicable to any such series of experiments, since the conditions of environment, diet, age, temperature, extract, etc., all influence the results in some way. Swingle and other workers have endeavoured to ascertain a definite relationship between thyroid and iodine, but found their efforts limited by such factors and the possibility of reliable standard conditions remote. One interesting quantitative experiment was carried out in conjunction with the last series. A batch of tadpoles was kept in water and injected with 0.1 c.c. of the extract as described for the first series. Another batch was taken at the same stage of development, kept under identical conditions, but placed in a solution of 1 c.c. of saturated iodine solution in 250 c.c. of water, and injected with 0.1 c.c. of the extract diluted with Ringer's solution to half its original strength. Accelerated
metamorphosis occurred in each case, but the rate of differentia-
tion was practically the same throughout the time of the
experiment. After fifteen days hind limbs were well developed,
the tail diminishing, and fore limbs just appearing. It seems
as if the action of the iodine was equivalent in its effect to that
of the other half of the anterior lobe extract. Probably the
iodine acts independently and may function as a hormone
within the tadpole itself without the intermediation of the
gland, as Swingle suggested after his work on the metamorphic
action of iodine on thyroid and thyroidless tadpoles. Allen's
successful initiation of metamorphic changes by iodine adminis-
tration in pituitaryless, thyroidless tadpoles, and also in
tadpoles from which both these glands had been removed, adds
still further support to this independent activity. Nevertheless,
iodine is without action on normal and thyroidless axolotls as
shown by Hogben, so that it is not characteristic of all such
changes in amphibians. Undoubtedly it does play some part
in metamorphosis, but it is quite possible that the eventual
solution of the problem will reveal the needed presence and
action of other factors. Meantime the thyroid, pituitary, and
iodine all react to metamorphic changes in the Anurans, and
increased stimulation of any one member accelerates the normal
rate whilst reduction of the activity retards.

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1. Acceleration of the metamorphosis of frog tadpoles is
induced by injections of commercial extracts of the anterior
lobe pituitary gland extract.

2. The acceleration of metamorphosis produced by iodine
in solution in suitable concentrations is not so great as that
produced by injections of the anterior pituitary extract.

3. Further increase in the acceleration occurs when the
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injected specimens are kept in suitable concentrations of iodine solution.

4. The sequence of changes is similar to that observed in the accelerated transformation of tadpoles produced by thyroid or iodine diet.

5. The highest mortality, greatest reduction in size, and most rapid transformation occurs in the highest concentration of iodine, and this is still greater in injected animals kept in iodine solutions.

6. The younger the tadpole the less chance is there of completing metamorphosis under the accelerating stimulus of injected anterior lobe or iodine.

7. There is a paling effect after initial injection less marked in older specimens which is possibly due to inhibiting influence on the posterior lobe of excess of anterior lobe secretion. Iodine has no definite pigment action.

8. Iodine appears to act independently of the anterior lobe injection in the tadpole, the acceleration being apparently the sum of their separate actions.

4. References.

Adler, L. (1914), Arch. f. Ent. Mech., 89, 21, and 40, 18.