

*Experiments on Amphibian Metamorphosis and Pigment Responses  
in Relation to Internal Secretions.*

By JULIAN S. HUXLEY (New College, Oxford) and LANCELOT T. HOGBEN  
(Imperial College of Science, London).

(Communicated by Prof. E. W. MacBride, F.R.S. Received July 26, 1921.)

CONTENTS.

	PAGE
1. Metamorphosis of the Axolotl by Thyroid Administration .....	36
2. Acceleration of Metamorphosis by Iodine with Triton and Salamandra Larvæ .....	42
3. Negative Effects of Administration of other Ductless Glands and Iodine on Metamorphosis in Axolotl .....	44
4. Effect of Thyroid Feeding on Necturus .....	46
5. Pigmental Reactions of Axolotls to Adrenal and Pituitary Hormones .....	48
6. The Pineal Pigment Cycle in Frog Tadpoles .....	51
Summary.....	52

The problem of metamorphosis in Amphibia attracts attention from many points of view. These organisms have, one may say, acquired interest as being indicators for the action of certain internal secretions; the underlying mechanism of metamorphosis is still imperfectly understood; and the general biological problems involved, especially with respect to the neotenus and perennibranchiate forms, are remarkably fascinating. The experiments here recorded were undertaken with a view to elucidating these issues more fully, and in the course of them data relevant to the pigmental responses of Amphibian larvæ also emerged. For various reasons it was deemed desirable to publish an account of the observations so far completed. Acknowledgment is made to Mr. D. F. Leney of New College for assisting in the care of animals, to the Royal Society and the Trustees of the Dixon Fund for grants in aid of the expenses incurred by the authors respectively, and to Prof. E. W. MacBride, F.R.S., for his kindness in reading the MS.

1. *Metamorphosis of the Axolotl by Thyroid Administration.*

(a) When a note (Huxley, 'Nature,' 1920) on the metamorphosis of medium-sized but immature Axolotls by means of thyroid feeding was published by one of the authors a year ago, and confirmed a few months later (Hogben, 'P.Z.S.,' 1920), our attention had not been called to Jensen's work (1916). Since this appears to be little known and is not readily accessible to English-speaking workers, it is proposed to give a short *résumé* of some of his most important findings.

First, however, a record of one of our own experiments will be given. Two black Axolotls, one male, the other female, and both sexually mature (though not quite full-grown), were kept in a large tank, together with two fine full-grown white Axolotls as controls. The controls were fed on worms, while the others were given pieces of ox-thyroid. In every case the feeding was controlled, the worm or piece of thyroid being kept near the animal's mouth until swallowed; if persistently refused, it was removed. The thyroid feeding began on November 30, 1919. On December 29, the thyroid-fed animals were past the critical stage of metamorphosis (see Boulenger, 1913); on January 13 no trace of gills was left; on January 19 the animals left the water, and the metamorphosis could, therefore, be considered as complete. The temperature was at first that of the room, varying from 8° to 15° C.; from January 2, when the water was warmed by an electric-light bulb, it rose to between 14° and 16° C. The length of the specimens at completion of metamorphosis was 17.4 cm. (♂) and 14.6 cm. (♀); the male had the typical sign of maturity—enlargement of the lips of the cloaca—well-marked. The controls during the same period showed no changes. It is an interesting fact that the metamorphosed animals, although tending to come more frequently to the surface about the time that the critical stage was reached, showed no inclination to leave the water until after the morphological alterations had run their course. It is only when a few days have elapsed after the completion of the visible external changes that the animals emerge from the water.

(b) *Jensen's Work.*

Curiously enough, Jensen also believed that he was the first to obtain the metamorphosis of Axolotls by thyroid, and it was only after his paper was completed that he found that he too had been anticipated—by Laufberger (1913). Jensen's experiments, however, are much fuller. He used calf-thyroid as diet. Two-week old larvæ on this diet all died within a few days, without change of form. Four immature specimens (10 to 16 cm. in length) metamorphosed in 17 to 25 days without exception. They were fed only three to eight times, with 1 to 3 grm. thyroid in all. An intra-abdominal injection of iodothyryn caused metamorphosis in the same length of time (10 specimens). The amount of iodothyryn injected had no influence upon the rate of metamorphosis within the limits used (1 to 10.5 mgrm.). One specimen *fed* upon iodothyryn started to metamorphose, but after 21 days of very slow change began to reverse the process, and became larval again. Four mature specimens, 22 to 24 cm. long, and two of them known to be over 6 years old, were also successfully metamorphosed. The time required was here greater,

varying from 27 to 32 days. Two of these animals were females who had just started to lay eggs; the very interesting fact was noticed that oviposition ceased almost immediately the thyroid diet was started. (It would thus appear that the katabolic action of thyroid will not permit of the anabolic activities necessary for the production of eggs. On the other hand, the experiment does not prove that egg laying *cannot* proceed during metamorphosis, for the amount of thyroid given was certainly very much in excess of that necessary to produce metamorphosis. It would be of great interest to attempt work along these lines with amounts of thyroid (or iodothyrim or thyroxin) close to the critical amount.) One immature animal was fed on thyroid while in deep water; in spite of this, metamorphosis proceeded at the usual rate. There were no failures to metamorphose (except the above-mentioned one on iodothyrim *diet*) among all the survivors, and the two that died under the treatment were metamorphosing regularly up to the time of their death. *Salamandra maculosa* larvæ were also metamorphosed by a thyroid diet. Eight-day larvæ came out on land in 13 to 16 days, and the same period of time was taken by 30-day larvæ. Axolotls in 1/10,000 KI solution did not metamorphose, but on the contrary developed abnormally large gills.

Experiments were also carried out by Jensen upon *Necturus* and *Proteus*. One adult *Necturus* was at first fed on thyroid, later injected with iodothyrim. The only changes noted were the resorption of one gill on one side, and a slight change in pigmentation. The animal died after some time. Four specimens of *Proteus* were also treated, some fed on thyroid, others injected with iodothyrim. The animals were 12 to 24 cm. long. In all cases, there was a distinct but slight atrophy of the tail-fin and of the gills, but the total shrinkage of the fin in height was not more than 1 to 1.5 mm., and after 6 months it regained its original size. Thus, with animals of this size, only slight effects could be obtained, indicating that in these permanently neotenic forms, the tissues are not sensitive to thyroid in the same way as in normally metamorphosing or facultatively neotenic species. Similar treatment failed to produce metamorphic effects in the *Ammocete* larva of the Lamprey.

Jensen further notes that in many, but not all, of his treated Axolotls, pathological symptoms occurred as a result of thyroid or iodothyrim. The animals refuse to eat, are usually sluggish when stimulated, rush about madly for several minutes, and exophthalmos of a very pronounced type appears. This exophthalmos, it is interesting to note, disappears with the other pathological symptoms from a few days before metamorphosis to three weeks after it has occurred.

(c) *External Agencies, Age, and Rate of Metamorphosis.*

As will be seen later, our young Axolotl larvæ fed on thyroid, all metamorphosed in from 24 to 32 days. We are thus in a position to compare the time necessary for metamorphosis induced by thyroid-feeding with that induced by air-breathing. Marie von Chauvin's classical experiments were done with great precautions, and the time taken was very large. Those of Boulenger (1913) will serve as our example, for it is not probable that the time for metamorphosis by this means can be much reduced beyond what was found by him. His results show that the time required for metamorphosis by air-breathing is a function of the temperature. Those kept at 75° to 80° F. metamorphosed in 89 to 109 days, while those kept at 55° to 60° F. took 116 to 120 days. The times required by animals fed on thyroid were always much smaller, varying from 17 to 50 days. The times recorded by Jensen are uniformly less than those found by us. This very probably is connected with a difference of temperature, since our experiments were all carried out in the depth of winter, at temperatures, be it noted, considerably below the lowest used in Boulenger's experiments. Mature animals always require a longer time than do immature. Jensen's results show that the minimum time for metamorphosis was 10 days longer in adults than in immature specimens, the maximum 7 days longer; the minimum time for adults was thus greater than the maximum for immature specimens. The difference in our own experiments was still greater. For immature specimens, it does not appear to make any difference whether they are quite young (under 3 inches in length) or half-grown; the time taken is approximately the same. This appears to indicate a real difference between the metabolism of adult and immature specimens, and that the time-difference observed is not due merely to differences of size. We may sum up by saying that thyroid-feeding causes metamorphosis much more rapidly than does enforced air-breathing; and that the change to sexual maturity in Axolotls increases the time necessary for metamorphosis by 25 to 50 per cent.

Some *Amblystoma* used in our experiments were bred in the laboratory in the spring of 1920. The eggs were laid in the second half of April. Young specimens from these eggs were used for experiment later in the same year. The first experiments were made in the first week of July, the larvæ then being not quite 3 months old, and measuring almost precisely 50 mm. in length. Four of these were fed upon thyroid, and showed all the usual metamorphic changes; the gills were reduced to mere stumps without filaments, the fin along the back was resorbed, and the eyes became

prominent. All four, however, died between the fourth and fifth weeks of the thyroid diet, when practically metamorphosed. It was not possible to say whether death was due to internal causes resulting from a too-precocious transformation, or whether it was due to the animals not being able to climb out of the water: that this latter suggestion might well be valid is shown by the fact that the animals' legs at this stage were proportionately smaller, and especially slenderer, than at later stages, and were quite possibly too weak to support the creatures on land. On the other hand, other *Amblystoma*, transformed by thyroid, usually remained several days in water after completion of all visible morphological metamorphic changes; so that death is most probably to be ascribed to internal changes, especially since it is known that in *Anura* there is a lower limit of size below which thyroid diet induces an attempt at metamorphosis, but one which is always followed by death.

A single larva was next put on thyroid diet on August 16, 1920, *i.e.*, when about 4 months old and 59 mm. in length. The animal showed metamorphic changes after 15 days, came out of the water after 26 days, and lost the last traces of gills after 29 days. Metamorphosis can thus be induced in *Amblystoma* that have not yet attained a length of  $2\frac{1}{2}$  inches; it is possible from then onwards at any size or age. Three more larvæ were started on the thyroid diet on August 9, 1920, when nearly 5 months old. The first traces of change were observed on the 17th day, the gill-filaments were all resorbed by the 23rd day, and all emerged from the water between the 30th and 32nd days. A slight diminution in size had taken place in the process, the original lengths of 68, 60, and 60 mm. being reduced to 64, 59, and 58 mm. respectively.

The following experiment was carried out to see whether immersion in dilute alcohol would accelerate or retard the metamorphosis caused by thyroid. Five dishes were prepared, one with larva in each. All contained 600 c.c. of liquid. (P) contained water, and the individual in it was fed on meat as a control. (T) also contained water, but the animal in it was fed on thyroid. (Q) contained  $\frac{1}{3}$  per cent. alcohol, (R)  $\frac{1}{6}$  per cent. alcohol, and (S)  $\frac{1}{12}$  per cent. alcohol. The animals in (Q), (R) and (S) were all fed on thyroid. The alcohol solutions were changed thrice weekly. The experiment was started on November 5, 1920. It was very noticeable that the animals in the alcohol solutions were, especially at first, more sluggish than either of those in plain water. They did not at first react to stimulation, but, after the stimulus had continued for some time, they responded by abnormally violent movements. After 2 to 3 weeks, however, they apparently became acclimatised to the alcohol, for their sluggishness was not nearly so marked.

(S) was distinctly less sluggish than the other two, between which not much difference could be noticed.

The experiment was not wholly conclusive. The differences between the rates of metamorphosis of the four thyroid-fed animals was very slight. There was, however, a slight retardation seen in the specimens kept in alcohol, and a retardation which increased with the concentration.

The experiment was therefore repeated with the following variations: (1) Control: meat-fed in water. (2)–(5) Thyroid-fed: (2) in water; (3) in  $1/3$  per cent. alcohol; (4) in  $2/3$  per cent. alcohol; (5) in 1 per cent. alcohol; two animals in each dish. The same slight retardation of metamorphosis with alcohol, increasing with concentration, was observed. Again, larger numbers are really necessary before the retardation can be regarded as proved; but, so far as it goes, these experiments appear to be significant.

All the alcohol specimens this time showed redness and congestion of the gills, increasing with concentration. The congested gills were not reduced till relatively very late, and then decreased remarkably rapidly in size. The  $2/3$  per cent. and 1 per cent. specimens all died suddenly just before they were due to metamorphose. Metamorphosis in the control took 29 days; in the  $1/3$  per cent. 29 and 31 days.

(d) *Size of Thyroid.*

From all recent work upon the metamorphosis of Amphibia, it would appear certain that metamorphosis is normally associated with thyroid activity, taking place when certain substances produced by the gland reach a definite concentration in the body. In those forms, therefore, in which a typical metamorphosis rarely or never occurs, we should *a priori* expect to find an abnormally small thyroid.

In the Axolotl, however, the thyroid is not abnormally small; further, it presents a perfectly normal histological picture. It would only be possible to give definite data after a careful examination of the relation between thyroid-weight, body-weight, and, if possible, iodine-content (or other criterion of activity) of thyroid in a number of Urodela, including normally metamorphosing forms, the Axolotl, and some Perennibranchiates. There is, however, one observation which it is of interest to mention here. That is the abnormally large size of the thyroid in Siren. This has been recorded by Wilder in his anatomical account of the animal (1891); and his account we have confirmed from the dissection of two specimens preserved in spirit in the Oxford University Museum. The thyroid is not only as large as in a normally-metamorphosing species, but much larger. Of this fact there can only be

two explanations. Either the thyroid of Siren is no longer producing the same type of substances as in other Amphibia; or, if it is continuing to do so, the body of the organism does not respond to the substances produced in the same way as in other species. That this latter is a possibility is shown by the example of *Proteus* and *Necturus*, which cannot be transformed by thyroid-feeding. In either event, the large size of the thyroid is very peculiar and demands investigation; possibly it has taken on some new function. From the particulars given by Platt (1896) it does not appear that the thyroid is under-developed in *Necturus*. In *Typhlomolge*, on the other hand, Emerson (1905) reports the complete absence of a thyroid gland. Leydig (1853) describes the thyroid of *Proteus* as being small, median, and composed of but 3 to 15 vesicles, which, however, often contained colloid.

(e) *Exophthalmos Associated with Metamorphosis.*

It is an interesting fact, which, so far as we know, has not been previously emphasised, that Amphibia before metamorphosis have their eyes flush with the surface of their head, but that in the adult state the eyes protrude considerably. In the case of the Axolotl the eyes remain flush with the surface so long as the animal remains in the aquatic form, even if it becomes sexually mature. The phenomenon appears to occur equally in *Anura* and *Urodela*. In view of the connection of the thyroid with Amphibian metamorphosis, this protrusion of the eyeballs becomes interesting when it is remembered that exophthalmos is one of the most prominent symptoms of Graves's disease. Whether the exophthalmos in Amphibia at metamorphosis has any relation to that of exophthalmic goitre cannot be definitely stated.

2. *Acceleration of Metamorphosis by Iodine with Triton and Salamandra Larvæ.*

Following Swingle's results (1919), a solution of iodine was made by shaking up an excess of iodine crystals in tap-water in a 2-litre flask, allowing to stand for 2 days, and then diluting as required with tap-water. Two larvæ of *Salamandra maculosa* were put into each of a series of dishes containing 350 c.c. of fluid each.

No. 1 was the control (tap-water only), the rest contained a saturated solution of iodine, diluted respectively 10, 50, 100, 500, and 1000 times. The experiment was performed at room temperature in January, 1920. In all except the 1/10 solution the animals fed well. In the control and in the 1/100 solution the gill-filaments were unchanged after a week. In the 1/500, the 1/100, and the 1/50 solutions the gill-filaments were somewhat reduced after 5 days. For some reason the greatest reduction occurred in the

1/100 solution. In the 1/10 solution the animals refused to feed at all. One (the smaller) died after one day. The other was more sluggish than normal animals. It showed distinct reduction of the gill-filaments after 3 days. The filaments were nearly absent after 5 days; on this day the solution was diluted to 1/12, as the animal seemed ill at ease. On the eleventh day mere stumps of gills were left, and the tail-fin had been nearly resorbed. The animal was poorly, not reacting to stimuli properly, and on the twelfth day it was dead. The saturated solution contained 0.076 mgrm. of iodine per litre.

Another series of experiments was started on January 26, 1920. Unfortunately, it had to be discontinued after 16 days, owing to the ill-health of Miss F. Peterson, who kindly helped with the work, and to whom grateful acknowledgment is made. Twelve sets of four animals were taken, there being two larvæ of *S. maculosa* and two of *Triton vulgaris* in each lot. These were distributed as follows, according to temperature and to strength of iodine solution:—

Temperature.	Strength of solution (in dilutions of a solution of iodine saturated at room-temperature).			
	1.	2.	3.	4.
A. 26° C. ....	1/20	1/50	1/125	Control.
B. 17°-18° C. ....	1/20	1/50	1/125	"
C. 2°-5° C. ....	1/20	1/50	1/125	"

In Series C no animals metamorphosed during the 16 days. In Series B five metamorphosed after an average time of 11.6 days. In Series A five metamorphosed after an average time of 6 days. This shows a very decided retarding effect of low temperature upon metamorphosis.

All animals were here kept in the same volume of water, so that only two variables affecting gill-size remained. High temperature was found to favour gill-development, while high iodine-concentration had the reverse effect. This was well brought out in the results. The gills of those kept at 26° C. could be arranged in a graded series according to size, the controls having the largest filaments, those in the 1/20 solution the smallest. Those kept at 2°-5° C. also showed a series which was identical, except for the fact that it started at a much lower level; in fact, the gills of the controls at the low temperature were slightly smaller on the average than the gills of those kept in the 1/20 solution at the high temperature. The 17°-18° C. series was intermediate, but rather nearer, as would be expected, to the high-temperature series.



Great diversity in the rate of metamorphic change was found, so much so that the original purpose of the experiment—viz., to get some quantitative data on the time-relations of metamorphosis to iodine concentration—had to be given up. It appeared that the prominence of the eyes which accompanies metamorphosis in these forms, might begin at high temperatures while the gills were still hyper-normal in size. Thus the effect of high temperature may be to mask the effect of iodine as far as the gills are concerned. When the resorption of the gills did begin at high temperatures, it went very quickly, the effect of the iodine overcoming the antagonistic effect of temperature. This is probably, according to Swingle, due to the power of all tissues of the body, but especially the thyroid, to manufacture from iodine some substance which causes the initiation of metamorphosis, when it reaches a certain critical concentration inside the body.

It, however, appears probable that the iodine may also have a direct effect on the tissues of the gills. The violent action of the 1/10th solution upon the filaments in the first experiment is very likely to be explained in this way. It is at any rate certain that the filaments are very sensitive to external agencies, as is also the tail-fin. Both of these structures, for instance, are much reduced when *Amblystoma* larvæ are kept in a very small amount of water or in damp moss, the mechanical alterations seeming to initiate the reduction (although of course the final complete metamorphosis which takes place in these circumstances must depend upon other, more deep-seated changes). We may say, therefore, that low temperature, exposure to air instead of water, and probably iodine solutions, have a directly unfavourable effect upon the gill-filaments, causing a certain amount of dedifferentiation and resorption, while the other agencies, such as thyroid diet and iodine accumulated in the body, exert an indirect effect by altering the internal environment to the point where metamorphic changes are started. Once these begin, the character of the gill-epithelium is altered, and the gills are rapidly reduced to mere blobs (fig. 4).

### 3. *Negative Effects of Administration of other Ductless Glands and of Iodine on Metamorphosis in Axolotls.*

Attempts were made to induce metamorphosis by administration of iodine in the medium and with the food to three animals 12 cm. in length. In the latter case, as in Swingle's earlier experiments (1919), a small quantity of finely powdered iodine was used, being in this case dusted on to thin slices of meat rolled into pellets. Such treatment does not produce obvious discomfort, nor is it poisonous, and with several animals was continued bi-weekly for 8 to 10 weeks without any diminution of gills and tail-fin or any evident

toxic consequences. All our endeavours confirm the conclusion of Jensen that iodine free of organic combination is not efficacious in producing metamorphic changes in the Axolotl. It is thus evident that in the neoteny of the Mexican Salamander it is not primarily the exogenous factors (available iodine supply and temperature) contributing to normal Amphibian metamorphosis that are significant.

Hence it became desirable to test the effect of administering other ductless glands. In this connection the results of D. I. Macht (1919), who has claimed to accelerate transformation in frogs by prostate-feeding, as also Bennet Allen's experiments (1920) on the part played by the pituitary gland (anterior lobe), demanded some attention. On the other hand the experience of Gudernatsch (1914) and others does not indicate the likelihood of influencing Amphibian metamorphosis by administration of pituitary gland *per os*.

Both young (4 to 6 months) and old (18 months) larvæ were fed with pituitary gland (anterior lobe). In the experiments glands of both old animals (ox) and of calves were employed and the treatment was continued for three months without producing metamorphic phenomena. As an illustration, particulars of the following experiment will serve. Fresh ox pituitaries were obtained from a slaughter-house, and the posterior and anterior lobes separated. On October 2, 1920, four vessels were prepared with four larvæ in each, which were nearly 6 months old. Those in vessel A were fed on thyroid; those in B on pituitary anterior lobe (henceforth called pituitary for brevity's sake, since posterior lobe was never employed in any of these experiments); in C on thyroid and pituitary on alternate days; and in D on raw meat, as controls.

Those fed on thyroid showed the first visible signs of transformation on the 14th day, their gill-filaments were resorbed by the 21st day, and the metamorphosis was morphologically complete on the 26th day. The controls showed no change throughout. Those fed on pituitary have showed no metamorphic changes at all; three were kept on the diet for 3 months, one for 8 months. It is obvious that the diet has no effect on their transformation. Those fed alternatively upon pituitary and thyroid metamorphosed in a perfectly normal way; the process, however, took slightly longer, the first signs of change appearing two days later than in those fed only on thyroid, the total resorption of the gill-filaments, and the morphological completion of the process, each taking place 3 days later than with the thyroid-fed ones.

The rates of growth upon the different diets are illuminating; they may best be presented in tabular form:—

	Original length.	Increase in length in 31 days.	
	Average.	Average.	Range.
	mm.	mm.	mm.
A. Thyroid diet .....	63·0	1·5	0·5-3·0
B. Pituitary diet .....	62·0	15·0	14·0-16·0
C. Alternate thyroid and pituitary diet .....	62·5	4·5	3·0-6·5
D. Meat diet .....	61·5	13·0	10·0-17·0

It will thus be seen that thyroid diet in this experiment did not cause a diminution of size, but permitted a very small increase. The controls fed on meat grew rapidly, but their growth was definitely, if slightly surpassed, by that of the pituitary-fed animals. Those fed alternately on pituitary and thyroid showed only a small amount of growth, but it was almost three times that recorded for those fed on thyroid alone, whereas the proportion of delay of metamorphosis in (C) was by no means equally great. From this, as well as from (B), it would appear that pituitary has definite growth-promoting properties for *Amblystoma* larvæ.

Half-a-dozen animals used in the pituitary-feeding experiments were taken at intervals of time and transferred to thyroid diet. The time taken for metamorphosis in three cases was slightly longer (33 to 35 days to emergence) than in controls, so that there may have been a retardation. In any case, these larvæ were clearly able to complete their metamorphosis.\*

One individual was also fed for 3 months on fresh prostate without showing any perceptible signs of transformation, though subsequent thyroid treatment induced metamorphosis.

#### 4. *Effect of Thyroid-feeding on Necturus.*

The suggestive, but inconclusive, experiments made by Jensen, with a view to elucidating more fully the significance of the Perennibranchiate condition in relation to the physiological processes underlying metamorphosis

\* One animal was continued on the pituitary diet for some time, a control in identical conditions being fed on ox muscle. After five months, the pituitary-fed specimen weighed 32·5 grm., the control 11·2 grm. Other animals of the same age fed on meat were all of about the same size as the control. It has since been suggested to me that if the control had been fed on more succulent diet, such as brain or liver, it would have rivalled the pituitary-fed specimen. Until the suggestion can be tested experimentally, I content myself with stating the facts. The growth of meat-fed specimens in the previous experiment, lasting 31 days, was about as great as that of the pituitary-fed animals. Later, the meat-fed animals' growth became relatively much slower. Another noticeable feature was that in thyroid-metamorphosed specimens previously fed on pituitary the size of the limbs was greater than in those not so treated.—J. S. H.

in other Urodeles, indicated the advisability of extending such observations. It was not possible to obtain specimens of *Proteus*; but, through the courtesy of Prof. MacBride, three live medium-sized *Necturus* were secured, and submitted to thyroid treatment in his laboratory at the Imperial College of Science.

The experiment began on November 10, 1920. One animal was kept as a control, and fed on small pieces of raw beef: the other two were given fresh thyroid (ox) gland tri-weekly, as in the case of the first experiments with *Axolotls*. Up till the time of writing, the treatment has been continued without interruption for 7 months. No pigmental changes have resulted; and there has been in neither case any appreciable reduction of the tail-fin. As regards the condition of the external gills, observation is embarrassed by the fact that the filaments are in a very marked degree erectile, their length, when fully dilated with blood, being many times greater than when the animal is not actively respiring: they can, however, be induced to extend by compelling the animal to perform muscular exercise, after which a rough estimate may be made of their maximum dimensions. Constant attention to this point showed very clearly that the filaments of the experimental individuals were relatively shorter even when fully extended; but, for an obvious reason, it is not possible to interpret this as necessarily consequent upon a reduction in the actual amount of tissue; for it is well known that thyroid administration influences the blood pressure in Vertebrates, and the behaviour of the gills in *Necturus* is evidently a vaso-motor phenomenon.

It does not seem likely, therefore, that an administration of thyroid in this Perennibranchiate form is effective in producing somatic modifications comparable to those occurring at metamorphosis in other Urodeles. In view of the morphological data given by Platt, and of the experimental evidence available, it may therefore be stated that, if the Perennibranchiate forms like *Necturus* and *Siren* are *not* primitive in the invariable retention of the larval type of Urodele organisation throughout life, their failure to develop the predominantly adult characteristics is not due primarily to thyroid deficiency—this, of course, does not apply to *Typhlomolge*, and possibly to *Proteus* also—nor to a defective supply of iodine in the environment. There remain at least four possible interpretations, then, of the Perennibranchiate state:—

(i) That these animals have never possessed genetic factors responsible for the structures of typical adult Urodela; *i.e.*, that in this respect they are actually primitive. This appears to be negatived by the purely morphological evidence available (see Gadow, 1901, pp. 65, 136).

(ii) That their thyroid mechanism is unable to make use of the available iodine supply so as to produce the requisite amount of active iodine compound required to stimulate metamorphosis.

(iii) That endogenous factors involved in the maintenance of the thyroid in a condition of functional activity are not operating effectively.

(iv) That the larval tissues concerned have collectively lost the power to respond to the thyroid activator.

The second and last possibilities are emphasised by the fact that, whereas in normal *Amphibia* Swingle has shown that iodine free of organic combination can suffice to induce metamorphosis not only in normal but in thyroid-ectomised larvæ, in the case of the mature *Axolotl*, iodine alone is not an efficient substitute for the thyroid autacoid. The importance of the third is sufficiently demonstrated by the work of Uhlenhuth (1919) on the relation of growth to metamorphosis in Salamanders, and Allen's recently published account of the inhibition of transformation in *Anura* by hypophysectomy. The state of affairs encountered in the Mexican Salamander is eminently suitable to a further analysis of what we have termed the endogenous factors in Amphibian metamorphosis; and it is hoped to obtain shortly in this connection data respecting the relation of the method of thyroid-feeding to enforced air-breathing as a means of bringing about the assumption of adult characters.

##### *5. Pigment Reactions of Axolotls to Pituitary and Adrenal Hormones.*

While the feeding of *Axolotls* with pituitary gland did not prove productive of positive data in relation to metamorphosis, it yielded results which encouraged further enquiry into the reaction of Amphibian melanophores to the internal secretions.

A few hours (6-12) after feeding *Axolotls* on pituitary (whole) gland, a marked darkening of the skin was observed; this was not noticed the first few days of pituitary diet, but became increasingly pronounced as the feeding continued. At first the darkening was very gradual, attaining its maximum intensity about 18 hours after feeding, which took place every 48 hours. Curiously enough, when this effect had quite passed off by the morning of the second day, the animals were of a ghostly pallor—considerably lighter than their normal shade, and remained thus until fed again. The rate of darkening progressively increased; after 3-4 weeks of the pituitary diet the maximum degree of expansion of the pigment cells was reached within an hour. The extreme subsequent paleness still occurred, appearing within 24 hours of feeding. Finally, after about 3 months' treatment the response diminished.

These observations were based on a dozen medium-sized Axolotls (and as many controls) of the albino variety, in which the pigment cells are sparse and confined to the upper surface of the head and mid-dorsal region. The reaction was more pronounced when the whole gland or the posterior lobe alone was administered than when the anterior lobe only was used. While these experiments were in progress Bennet Allen (1920) issued a preliminary notification of experiments on pituitary removal and transplantation in tadpoles, mentioning *inter alia* that animals after pituitary removal display a silvery-white appearance, in marked contrast to the coloration of the normal form. Thus the expansion of melanophores in reaction to the pituitary hormones would not appear to be confined to Axolotls.\*

The interest of this reaction is twofold: firstly in relation to Spaeth's thesis (1916) that melanophores represent a modified form of smooth muscle fibre; and secondly, in view of a suggestion by Fuchs (1906) that internal secretion may underlie the well-known phenomenon of "colour adaptation." Spaeth instances, as intermediate in character between typical smooth muscle and pigment cells, the sphincter pupillæ, in which, in certain cases, the cytoplasm is densely charged with melanin granules; he points out that the melanophores are mesodermal in origin; and draws attention to a remarkable parallelism between the reactions of the sphincter pupillæ fibres and melanophores in response to electrical and light stimuli and chemical stimuli such as atropine. To this it may be added that both react in the same way to the pituitary hormone, for it has been shown by Cramer (quoted by Schäfer, 1913) that the latter induces dilatation of the pupil.

The phenomenon of "colour adaptation" in reptiles, amphibia and fishes, has been provocative of much controversy and is little understood. It is widely known that these organisms can respond to the colour of their surroundings by pigmental changes. The most recent work of Laurens (1917) and others shows that, though the melanophores respond directly by expansion in bright light, in the case of Axolotls which have been blinded there is no secondary modification of the response (partial contraction) after continued illumination, and no power to respond to their background, *e.g.*, to become darker when illuminated only from above in a blackened container. The pigmental responses of these animals thus appear to be under the control of

\* Allen's (and also Smith's) results indicate that it is the intermediate lobe of the pituitary which is concerned in pigmental control in Anura. Presumably the same holds good for the effect of a diet of Mammalian pituitary on the Axolotl. The intermediate lobe will remain attached to the posterior lobe unless specially dissected apart. Swingle's recently published work (1921) confirms and extends the above-mentioned results.

stimuli received through the organs of vision. How this control is exerted is at present an unsolved problem.

Two possibilities invite consideration. Either the stimuli received by the eyes are transmitted entirely through the nervous system *viâ* the fibres innervating the pigment cells—assuming that in all cases pigmental cells are innervated from the C.N.S.; or nervous stimulation of internal secretions efficient to produce the appropriate reaction may be involved. Obviously both mechanisms may operate concurrently. In order to interpret the pigmental responses in these animals in a manner consonant with the second hypothesis, it is first necessary to demonstrate that the melanophores react in one way to one type of internal secretion and in an opposite sense to another. It has been stated that a pituitary hormone causes the melanophores to expand; and the question arises whether other internal secretions can bring about the reverse effect. In this connection two observations provide a clue. McCord and Allen (1917) have recorded that after feeding tadpoles (*Rana sylvatica*) on pineal glands for 10 days, each subsequent meal was followed by transient, and complete, contraction of melanophores, noticeable in half-an-hour, reaching its maximum in about 45 minutes, and passing off after 2 to 3 hours. Bigney (1919) again finds that by injecting adrenalin into the adult frog, the contraction of the pigment cells is produced, confirming earlier work of Lieben (1906).

To test the reaction of the pigment cells in Axolotls to pineal treatment, eight medium-sized (9 month) larvæ were placed (November, 1920) in separate containers, of which the sides had been blackened, and illuminated from above. In this way maximum expansion of the pigment cells is brought about within a few hours. Four were fed on fresh pineal glands tri-weekly, and the remaining four (controls) were kept on a normal meat diet. The experiment was continued for 2 months and proved quite fruitless. No pigmental differences either of a permanent or temporary character could be observed in Axolotls, although, as will be seen later, McCord and Allen's observations as to the effect of pineal feeding on tadpoles were afterwards confirmed. Two similar experiments with the same numbers were then repeated, with adrenal medulla instead of with pineal gland; whether administered as food or by adding fresh extract to the medium, a complete contraction of the pigment cells invariably ensued, with great vaso-dilatation of the gills.\*

*It thus appears that the pigment cells of medium-sized Axolotls react in an opposite manner to pituitary and adrenal extracts, and the fact that in Allen's experiments the removal of the pituitary was accomplished by melanophore*

\* Probably the effect is in either case due to adrenalin acting on the skin *viâ* the medium.

contraction, is indicative that internal secretion underlies the mechanism by which the regulation of pigmental reactions is effected in normal life. In any case there would appear to exist a double compensating mechanism for the control of the behaviour of the melanophores: though we cannot yet legitimately infer that this constitutes the effective apparatus through which the organs of vision influence them.

#### 6. *The Pineal Pigment Cycle in Tadpoles.*

In view of the lack of success with which efforts to induce a response on the part of the melanophores of the Axolotls were attended, it was decided to repeat McCord and F. Allen's experiments upon pineal administration to Anuran tadpoles. About 500 tadpoles of *Rana temporaria* were employed for this purpose in glass containers during the spring of 1921. At first the controls (meat-fed), and the experimental animals which were fed on fresh ox pineal glands tri-weekly, were kept respectively in single containers. Later, for purposes of observation, the tadpoles were separated in glass bowls (placed on a white background) in colonies of twenty. Contemporaneously, tadpoles raised from eggs laid on the same day were being fed on suprarenal cortex, suprarenal medulla, corpus luteum, and anterior pituitary lobe for ulterior purposes, so that it was possible to compare the phenomena consequent upon pineal treatment with the results of administration of a more varied range of tissue extracts than were employed by the authors named above.

In these experiments no change in the pigmental characteristics of the tadpoles was noticed during the first fortnight of pineal diet, which began about a fortnight after hatching. Before three weeks had elapsed, the phenomena of the pineal pigment cycle, as recorded by its discoverers, became evident. Within a quarter of an hour of feeding the tadpoles became visibly more pale, till, when the reaction reached its maximum, half an hour after the meal commenced, they assumed a quite unique appearance, by virtue of the contrast between the complete translucence of the head region and tail on the one hand, and the opacity of the visceral portion of the body on the other. This condition passes off after the lapse of five or six hours: till metamorphosis took place the same reaction followed each administration with the utmost regularity. No modification of the pigmental features occurred in the controls or in the additional cultures which were being fed synchronously with other glandular tissues. Histological preparations confirmed McCord and Allen's conclusion that the behaviour of the melanophores is the significant element in the situation. After a pineal meal the melanophores are fully contracted,



the pigment being congregated into compact masses in the centre of the cell.

The time relations observed are somewhat different in these experiments from those obtained by McCord and Allen. Thus the latent period was a little longer; the time required for the effect to manifest itself after feeding a little shorter, and the duration of the effect considerably longer. In the case of *R. sylvatica*, these investigators found that the reaction passes off in about two hours. Apart from these insignificant details, the experiment recorded entirely confirms their results. It may be added that extracts were also employed and produced rapidly (less) corresponding effects to those produced by administration *per os*.

As regards the cortex-fed cultures, no difference was seen in the pigmentation of tadpoles fed continuously from hatching till metamorphosis on supra-renal cortex as contrasted with controls. We take the opportunity of putting this observation on record, because Gudernatsch (1914) stated that cortex-fed tadpoles show progressively less pigment after five weeks' treatment, an observation which, if confirmed, would seem significant in relation to the aetiology of Addison's disease.

That the pineal gland of Amphibia does actually function in relation to pigmental responses, although it is probable, cannot be legitimately contended on the basis of evidence so far available, as mammalian pineal glands were employed in the experiments. What can be definitely stated is that pineal tissue is specifically distinguished by the possession of a physiologically active substance, a conclusion which goes far to establish its claim to be classified as an endocrine organ.

#### *Summary.*

##### *A. Metamorphosis.*

1. Salamandra and Triton larvæ may be metamorphosed by immersion in a dilute solution of iodine. Metamorphosis is retarded by low temperature. High temperature at first causes increased growth of the gills.

2. Sexually mature Axolotls can, as Laufberger and Jensen originally showed, be made to undergo metamorphosis by means of a thyroid diet.

3. Metamorphosis is accompanied by exophthalmos, apparently in all Amphibia.

4. In the case of the Axolotl, the time required for metamorphosis induced by enforced air-breathing is considerably longer than when induced by thyroid-feeding; in the latter case, it is longer for sexually mature than for young larvæ; and is in all cases accelerated by increase of temperature.

5. Administration of iodine free of organic combination, or fresh glandular

substance of the prostate and pituitary anterior lobe, is without any effect in relation to the metamorphosis of the Axolotl.

6. Thyroid-feeding continued for 7 months was not accompanied by any noteworthy somatic changes in *Necturus*.

*B. Pigmental Responses.*

7. Pituitary feeding (posterior lobe or whole gland) produces a marked temporary dilatation, followed later by excessive contraction, of the dermal melanophores in albino Axolotls.

8. Adrenal medulla extract produces temporarily complete contraction of the dermal melanophores in the Axolotl.

9. Pineal administration (as extract or as food) rapidly brings about a striking transient contraction of the dermal melanophores in frog tadpoles: McCord and F. Allen's observations in this connection are fully confirmed. It has, however, no effect upon the melanophores of the Axolotl.

LITERATURE.

- Allen, B., 'Science,' 1920.  
Bigney, 'J. Exptl. Zool.,' vol. 27 (1919).  
Boulenger, 'Proc. Zool. Soc.,' 1913.  
Von Chauvin, 'Zeit. f. Wiss. Zool.,' vol. 27 (1877), and vol. 41 (1885).  
Detwiler, 'J. Exptl. Zool.,' vol. 31 (1920), especially pp. 149-151.  
Emerson, 'Proc. Boston Soc. Nat. Hist.,' vol. 32 (1905).  
Fuchs, 'Biol. Centralbl.,' vol. 26 (1906).  
Gadow, H., "Amphibia and Reptiles," 'Cambridge Nat. Hist.,' vol. 8 (1901).  
Gudernatsch, 'Am. Journ. Anat.,' vol. 15 (1914).  
Jensen, 'Meddelelser f. d. Kgl. Veter. og. Landb.,' vol. 44 (1916).  
Laufberger (in Czech), 'Biologické Listy' (1913).  
Laurens, 'J. Exptl. Zool.,' vol. 23 (1917).  
Leydig, F., 'Anatomisch-histologische Untersuchungen über Fische und Reptilien' (Berlin, 1853).  
McCord and F. Allen, 'J. Exptl. Zool.,' vol. 23 (1917).  
Platt, 'Anat. Anzeiger' (1896).  
Schäfer, 'Lane Medical Lectures' (1913), California.  
Smith, P. E., 'Amer. Anat. Mem.,' vol. 11 (1920).  
Spaeth, 'J. Exptl. Zool.,' vol. 20 (1916).  
Swingle, 'J. Exptl. Zool.,' vol. 27 (1919).  
Swingle, 'J. Exptl. Zool.,' vol. 34 (1921).  
Uhlenhuth, 'Journ. Gen. Phys.,' vol. 1 (1919).  
Wilder, 'Zool. Jahrb. (Anat. Abt.),' vol. 4 (1891).
-