There is a general agreement concerning the influence of the thyroid upon the metamorphosis of anuran amphibian larvæ. All authors who reported experiments on feeding thyroid to tadpoles, regardless of the locality in which they were carried on and on whatever species of anura they were performed, invariably found that thyroid feeding always resulted in a precocious metamorphosis. The experiments on thymus feeding, however, are characterized by just the opposite feature, the results being very inconstant. Thymus feeding sometimes resulted in retardation or entire prevention of metamorphosis; sometimes it had no effect as compared with the controls. Such different action not only was obtained in the experiments of different authors, performed on different species and at different places (Gudernatsch obtaining positive, Swingle negative results), but even when the same investigator experimented on the same species different individuals were affected differently by the thymus diet (Romeis). Nevertheless, it is certain that in some cases thymus feeding actually prevented metamorphosis and resulted in giant larvæ, while no such effect was obtained in the controls on the normal diet.

It seems necessary therefore to explain why thymus feeding sometimes does and sometimes does not prevent metamorphosis.

The writer performed a large number of experiments on the larvæ of *Ambystoma maculatum,* *Ambystoma opacum,* and *Ambystoma tigrinum,* and it was found that while the thyroid contains a specific substance enforcing metamorphosis, the preventive effect of thymus feeding is due to the absence from the thymus of a substance necessary for the formation or excretion by the thyroid of the substance causing metamorphosis.

1 For nomenclature of salamanders see Stejneger, L., and Barbour, T., A check list of North American amphibians and reptiles, Cambridge, 1917.
Inhibitory Effect of the Thymus.

Some experiments may be reported briefly, showing that thymus feeding sometimes actually prevents metamorphosis, though in the majority of the larvae it has no such effect.

In a set of larvae of Ambystoma maculatum, kept at approximately 15°C., fed on tubifex and earthworms, and consisting of about 100 specimens, most of the larvae, which had hatched in the beginning of May, 1916, metamorphosed during the months of August and September. On October 21, eight of these were still larvae. These were picked out to form a series of normal controls (c. W. 1916). In a set of eighteen larvae of the same species and of the same age and kept under the same conditions as the above series, but fed on thymus soon after hatching, nine larvae had metamorphosed up to October 21. The remaining nine were picked out to form a thymus-fed series (c. T. 1916). The worm-fed larvae of Series c. W. 1916 metamorphosed at an age of 28 weeks and 6 days on an average; the first larva to metamorphose was 25 weeks and 4 days old at the time; the last one 33 weeks and 4 days. Among four larvae of the thymus-fed Series c. T. 1916, the first animal was 27 weeks old when it metamorphosed, the last one 55 weeks and 2 days. Of these four larvae the average age at the time of metamorphosis was 35 weeks and 2 days. The other five larvae of the thymus-fed series died before metamorphosis; three at the age of 31 weeks and 2 days, one at the age of 40 weeks and 3 days, and one at the age of 62 weeks and 3 days. Thus one animal of the thymus-fed series remained larval for about 1 year and 3 months; at this time it did not show any signs of metamorphosis and it seems possible that it would have remained permanently in a larval state. The writer so far has never observed in his normal controls individuals which remained larval for so long a time. Thus there can be no doubt that in this thymus-fed series metamorphosis was retarded as compared with the controls and in one case probably was even prevented.

In a series of eight larvae of Ambystoma opacum, which were kept at about 25°C. and fed moderately on earthworms, the average time of metamorphosis was 26 weeks; in a series of eight larvae of the same species of the same age and from eggs of the same female as the
larvae of the foregoing series, the larvae were fed on thymus and with the largest possible amount of this diet; otherwise they were kept like the worm-fed controls. The average time of metamorphosis in this series was 17 weeks and 5 days; i.e., less than in the worm-fed series. In this number, however, two larvae are not included, both of which died before metamorphosis; one of them reached the age of 31 weeks and 2 days, the other 34 weeks and 1 day without having metamorphosed. Thus in this series again a considerable delay (if not an inhibition) of metamorphosis was produced by the thymus diet in two animals.

A third species, *Ambystoma tigrinum*, was experimented on. This species, as mentioned in a recent publication\(^2\) shows the least effects when fed on thymus. Among six animals kept at approximately 25°C. and fed on thymus, the larvae metamorphosed simultaneously with the controls. Among six other animals kept at approximately 15°C. and fed on thymus, only five larvae metamorphosed simultaneously with the controls (between 22 and 26 weeks after hatching), while one individual though now over 74 weeks old is still in larval condition. Thus also in this species thymus feeding apparently resulted in a considerable retardation of metamorphosis though only in one individual.

From these experiments on urodelan larvae it is again evident that the effect of the thymus is extremely variable. But it is also a fact that in some of the larvae metamorphosis was retarded by the thymus diet and in two probably completely prevented. Further experiments, therefore, were carried out to determine this point.

**Inhibitive Effect of Thymus upon Amphibian Metamorphosis Is a Deficiency Phenomenon.**

It is clear that the considerable variability of the action of the thymus cannot be explained on the assumption that the inhibitive effect of that gland is due to the presence of a specific inhibiting substance in the thymus. But it can be explained if this effect is due to the absence from certain parts of the thymus of a substance necessary to produce metamorphosis and which is contained, in minute quan-

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\(^2\) Uhlenhuth, E., *J. Gen. Physiol.*, 1918, i, 23.
tities, in other parts of the thymus, in normal food, and perhaps in the water of certain localities where unsuccessful experiments on thymus feeding have been carried out.

If the preventive influence, which the thymus exhibits in some of the larvae, is due to the presence of some specific metamorphosis-inhibiting substance, metamorphosis evidently should be prevented by the thymus even if normal food is added to the thymus diet. This is the case, for instance, with the metamorphosis-producing substance of the thyroid gland. Lenhart\(^3\) has shown that if a certain amount of the active substance of the thyroid, able to produce accelerated differentiation and not large enough to result in death from emaciation before differentiation can take place, is introduced into the organism, differentiation will be accelerated and at a definite rate, whether the tadpoles are fed only on thyroid or whether some other food (liver) is added to the glandular diet. In fact, it seems, from all experiments so far performed with thyroid, that it is of no importance what food the larvae receive; the addition of only a minute quantity of thyroid substance causes metamorphosis at an accelerated rate. We found the same to be true for the larvae of salamanders. Young (5 weeks old) larvae of *Ambystoma opacum* which were fed on earthworms, were placed in a 0.02 per cent solution of iodothyrin; in spite of the earthworm diet and of the small quantity of thyroid substance used (Bayer's iodothyrin), all larvae metamorphosed from 8 to 9 days after the commencement of the thyroid treatment, while the controls needed 7 to 8 weeks more to metamorphose. The thymus itself contains a specific substance which is highly toxic and produces tetany in the larvae of *Ambystoma maculatum* and *opacum* as described in a former article.\(^3\) The action of this substance as regards its constancy is quite similar to the action of the metamorphosis-producing substance of the thyroid and very unlike the metamorphosis-inhibiting action of the thymus. No matter what food is added to the thymus diet larvae fed on thymus always had tetany. And like the thyroid substance, the tetany toxin of the thymus is also characterized by the constancy of its action; it produces tetany in each individual.

It is quite different with the prevention of metamorphosis by the thymus. If normal food is added to the thymus diet, metamorphosis will take place in each individual at the same time as in the controls. This is shown in the following experiments on larvae of *Ambystoma maculatum* of the same age, from eggs of the same female, and all kept at approximately 25°C. One series of 30 larvae was fed only on worms, one series of 15 larvae only on thymus, and one series on thymus and worms alternately. Fig. 1 shows the result. The larvae of the mixed food series metamorphosed simultaneously with those of the control series, which were fed on worms only; while in the series fed on thymus, metamorphosis started when all animals of the series fed on worms and on worms and thymus alternately had already metamorphosed, and the last larval animal died about 12 weeks later without having metamorphosed at all. In other words, in the series fed on mixed diet the food added to the thymus diet contained the substance which is necessary for metamorphosis and which is lacking in the thymus; and thus this substance was introduced into
the larval organism in such a large amount that metamorphosis occurred in this series simultaneously with the controls. The ability of the normal food to counteract the inhibitive effect of the thymus upon metamorphosis was the more conspicuous as the same food was quite ineffective in preventing the tetanic convulsions produced by the tetany toxin present in the thymus; this may be seen from the curves which indicate the percentage of tetanic animals found at the time of observation among the thymus-fed and mixed food series.\(^4\)

That the action of the thymus is merely due to the absence of the substance necessary to produce metamorphosis, is also demonstrated in experiments in which thymus-fed larvae are placed in a solution of thyroid substance. If the thymus contained a specific metamorphosis-preventing substance, one would expect an antagonistic neutralization of the thyroid substance by the thymus substance. But instead the thyroid substance even when present in minute quantities induces prompt metamorphosis in the thymus-fed animals. For the sake of illustration one experiment may be reported here. A set of six thymus-fed larvae of *Ambystoma opacum* was fed on thymus. At an age of 6 weeks the larvae were placed in a 0.02 per cent solution of iodothyrin which after about 20 hours was replaced by a 0.006 per cent solution. 8 days after the beginning of the thyroid treatment all the larvae were metamorphosed although fed on thymus, while the controls not treated with iodothyrin needed from 6 to 7 weeks more to metamorphose. In this series again we observe that the effects of the thymus, which actually are due to the presence of a special\(^5\) substance in the thymus, are not stopped by the thyroid treatment. The larvae exhibited severe tetanic convulsions caused by the tetany toxin of the thymus; these convulsions occurred in spite of the thyroid treatment with undiminished strength.

\(^4\) For detailed discussion see Uhlenhuth, *J. Gen. Physiol.*, 1918, i, 33.

\(^5\) The term specific in connection with the active principle of the thyroid gland has been avoided here, for it does not seem to be proved definitely that the effects exerted by the iodothyrin cannot be brought about by any other substance or any other factor. Since the term specific refers not only to the origin of the inner secretory substances but also to their effects, it is misleading in connection with the thyroid substance.
DISCUSSION.

From the above experiments it is evident that an exclusive thymus diet sometimes can retard or even prevent metamorphosis. But while the ability of the thyroid to enforce metamorphosis is due to the presence in the thyroid of a special substance, the inhibitory action of the thymus is due to the absence of a substance without which metamorphosis is impossible.

In order to appreciate this fact fully we must remember the experiments performed by Allen⁶ and Hoskins,⁷ which have demonstrated that tadpoles whose thyroid glands have been extirpated are unable to metamorphose. This means that under normal conditions at the time of metamorphosis the thyroid begins to excrete the metamorphosis-producing substance which under experimental conditions is introduced into the organism by feeding thyroid to the larvae or keeping them in a solution of thyroid substance. Since under normal conditions no thyroid is fed to the animals and since it is the thyroid of the animal itself which excretes the substance in question, we must assume that the normal food of the larvae contains a substance which is necessary to develop the thyroid of the larva to a state in which it can excrete the metamorphosis-producing substance. Whether or not the substance necessary to develop the thyroid and furnished in normal conditions by the normal food of the larvae is identical with the metamorphosis-producing substance excreted later on by the thyroid, cannot be decided at present; but in this respect the attempts made by Allen⁶ to enforce metamorphosis of thyroidless larvae by feeding them on thyroid are important. If it is possible to enforce metamorphosis in thyroidless larvae by feeding them on thyroid, but impossible by feeding them normal food, the substances contained in the normal food are able to develop the thyroid to the excreting stage, but they are unable to evoke metamorphosis in the absence of the thyroid, and, therefore, they are not identical with the thyroid substance. The results so far obtained by Allen point in the latter direction. It is this substance, necessary to develop the secretory stage in the thyroid, which is missing in the thymus.

The question arises now in which way do some of the thymus-fed larvae procure enough of the substance required for the development of the thyroid, while other thymus-fed larvae are unable to obtain enough of it though all of these larvae apparently are fed on an equally exclusive thymus diet? Although at present an exact statement on this matter is impossible we must seek its explanation in the fact that evidently the amount required is so small that it was difficult in our experiments so far performed to control the sources which sometimes supplied this substance against our will. As to the actual source of the substance it is possible that the connective tissue constituting the septa between the lobules of the thymus may contain some of it; in fact, this is probable since the septa are in no way a specific tissue like the rest of the thymus. The possibility of some of the larvae having obtained, by chance, more of the septa than others must be admitted. In this way the great variability of the results of the same author could be explained. There is also a possibility that the water may contain some of that substance; in favor of this would be the fact that some authors, like Swingle, did not obtain any retarding effects at all in their thymus-fed series. Of course, the difference in the reaction of different individuals of the same series of one experimenter can be explained less readily on that possibility.

Our experiments suggest the possible character of the influence of environmental factors on metamorphosis. The problem of amphibian metamorphosis, as well as the problem of internal secretion, assumes a new shape in the light of that fact. On the one hand, it has become clear from the experiments of Allen and his followers that metamorphosis is directly dependent on the action of a certain inner secretory gland of the amphibian larva; on the other hand, it is evident that the development of the secretory stage of that inner secretory gland depends ultimately on certain purely environmental, non-glandular factors. And it is now time that we should recall such attempts as those made by Duméril and von Chauvin to enforce or prevent metamorphosis by purely external non-glandular factors.

factors; these attempts in the case of von Chauvin doubtless were successful, though the actual relation between the factors employed and the result obtained cannot well be understood at present.

SUMMARY.

1. Though thymus-fed salamander larvae often metamorphose normally, thymus feeding sometimes retards and in rare cases inhibits metamorphosis completely.

2. The addition of normal food to a thymus diet abolishes the inhibitory effect of the thymus.

3. Addition of a small quantity of iodothyrin leads rapidly to precocious metamorphosis of thymus-fed larvae.

4. The inhibitory effect of the thymus is not due to a specific inhibiting substance in the thymus, but to the absence from the thymus of a substance required to develop the thyroid to the secretory state.