RELATION BETWEEN THYROID GLAND, METAMORPHOSIS, AND GROWTH.

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It has been demonstrated by a number of workers that the active principle of the thyroid gland in the causation of amphibian metamorphosis is iodine. Recently Swingle has obtained definite proof that inorganic iodine when fed to larvae of Salientia induces metamorphosis in a short time after the beginning of the feeding. On the other hand, it has been shown by the writer that the retarding influence of the feeding of thymus upon amphibian metamorphosis is due to the absence from the thymus gland of a substance required for metamorphosis, and it is possible that this lacking substance is iodine. Although minute amounts of iodothyron have been found in some thymus glands, it is likely that the amounts found are insufficient to produce metamorphosis and that some thymus glands may not contain the iodine at all. This would account for the variability of the results obtained with thymus feeding.

Swingle also subjected tadpoles deprived of their thyroid to an iodine diet; although such larvae when kept on a normal diet never metamorphose, they very soon metamorphosed when fed on iodine crystals.

Hence it is manifest that iodine is one of the substances involved in the causation of amphibian metamorphosis. The quantities contained in normal food are, however, so small as to have no immediate effect upon the organism. If the larvae have no thyroid glands, the small quantities of iodine taken up with the food apparently cannot be retained by the organism and the iodine leaves the body without

2 Uhlenhuth, E., J. Gen. Physiol., 1918-19, i, 305.
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brining about metamorphosis. If, however, a thyroid gland is present, all or most iodine taken up by the organism is retained and stored up in the thyroid. At a certain time during the life of the larva the thyroid suddenly begins to excrete the iodine stored up during the larval period, and metamorphosis results.

From this it is evident that under normal conditions the iodine is not the only substance needed in metamorphosis; there must be still another substance which, when present in a certain quantity, causes the thyroid to excrete the thyroid hormone. The experiments to be reported in this article not only furnish evidence of the actual existence of such an excretor substance, but they also demonstrate that it is evolved during the processes which lead to the growth of the organism.

Metamorphosis and Rate of Growth in Worm-Fed Larvae.

In eleven series of larvae of the species Ambystoma opacum the rate of growth during the larval period of active growth has been determined in the following way.

In each series the time of metamorphosis was recorded for each single individual; this can be done very accurately in this species if the first shedding of the skin and the reduction of the gills to mere stumps without fringes are taken as the indication of metamorphosis, both phenomena occurring within a day. That these two processes are actually controlled by the influence of the thyroid, in contradistinction to many other processes, has been indicated already in former articles and will be shown in detail in a later publication. From the values obtained in this way the average length of time of the larval period was calculated for each series (Table I).

Each individual was measured once a week and the average sizes obtained from those values for each series were plotted in curves. At the time of metamorphosis a sharp drop of the curve takes place, due to a discontinuation of growth, and even a diminution of the size of the animals which may last for one or several weeks before growth is resumed. In Series A 1916, the curve of which is shown in Fig. 1,
this drop occurred in the 24th week, though less distinctly than in most of the other series. From the average sizes reached in each series when the drop occurred and from the time when the drop took place, the rate of growth was calculated in day-millimeters, as indicated in Column 3 of Table I.

In comparing the length of the larval period for each series with the corresponding rate of growth it becomes evident that the smaller the rate of growth observed, the later metamorphosis took place. Furthermore, the rates of growth appear to be proportional to the

TABLE I.
Rate of Growth (R) in Worm-Fed Ambystoma opacum during Larval Period of Active Growth (Calculated from Averages).

<table>
<thead>
<tr>
<th>Series</th>
<th>Age at metamorphosis (days)</th>
<th>R</th>
<th>R × A</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 1916</td>
<td>245</td>
<td>0.27</td>
<td>66</td>
</tr>
<tr>
<td>A 1916</td>
<td>182</td>
<td>0.31</td>
<td>56</td>
</tr>
<tr>
<td>E 1917</td>
<td>161</td>
<td>0.36</td>
<td>58</td>
</tr>
<tr>
<td>D 1917</td>
<td>127</td>
<td>0.48</td>
<td>61</td>
</tr>
<tr>
<td>Wk 1917</td>
<td>106</td>
<td>0.58</td>
<td>62</td>
</tr>
<tr>
<td>WNa 1917</td>
<td>100</td>
<td>0.58</td>
<td>58</td>
</tr>
<tr>
<td>WMe 1917</td>
<td>100</td>
<td>0.58</td>
<td>58</td>
</tr>
<tr>
<td>W 1917</td>
<td>97</td>
<td>0.62</td>
<td>60</td>
</tr>
<tr>
<td>Wca 1917</td>
<td>96</td>
<td>0.62</td>
<td>60</td>
</tr>
<tr>
<td>C 1917</td>
<td>80</td>
<td>0.79</td>
<td>63</td>
</tr>
<tr>
<td>XIV 1918</td>
<td>70</td>
<td>0.83</td>
<td>58</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>60</td>
</tr>
</tbody>
</table>

This means that during the process of growth a substance is evolved which when present in a definite amount induces metamorphosis, provided that the larvae have been fed on normal food which apparently contains enough iodine to furnish the other substance (iodine) required for metamorphosis in a sufficient quantity; the greater the rate of growth the quicker that quantity of the first substance is formed which is required to induce the secretory action of
the thyroid gland. Hence besides iodine still another substance is needed in the amphibian metamorphosis; namely, the excretor substance which causes the thyroid to excrete the stored up iodine.

It may be mentioned here that the sudden drop of the growth curve at the time of metamorphosis may be explained if we assume that the thyroid, when it is stimulated by the excretor substance, excretes at first an overdose of iodine, while later the excretion becomes less energetic. Janney⁴ has shown that while an overdose of thyroid hormone leads to a negative nitrogen balance and a loss in weight and size, a certain minimum dose results in a positive nitrogen balance and a gain in weight and size.

Metamorphosis and Rate of Growth in Thymus-Fed Larvae.

In a recent publication Janney has shown that while a certain minimum amount of the thyroid hormone results in an increased protein breakdown, it finally leads to a positive nitrogen balance and an increase in weight and size, since it not only accelerates the protein breakdown but at the same time facilitates the assimilation of the nitrogen into the proteins of the body tissues. This is shown also in the metamorphosis of the amphibians where the initial breakdown of the tissues, as demonstrated, for instance, by Morse on tadpoles, and the decrease in size, resulting probably, as suggested above, from the excretion of an overdose in the beginning of the functional period of the thyroid, are followed soon by an increase in size and weight.

On the other hand, Janney's experiments have shown that in certain diseases, such as exophthalmic goiter, the normal synthesis by the thyroid of the thyroid hormone from iodine and certain organic substances is disturbed, and on the basis of Swingle's experiments we may assume that in these diseases the thyroid is unable to retain the iodine consumed by the organism with the food. This assumption is supported by the fact, as mentioned by Janney, that in exophthalmic goiter the thyroid frequently is found very poor in iodine. But since in this case the excretory function of the thyroid remains undisturbed, the thyroid is excreting, instead of the hormone, certain substances, probably the indole-containing amino-acid tryptophane which normally is used to build up the hormone, but which in itself is toxic causing a permanent protein breakdown without facilitating assimilation of the food nitrogen; consequently a negative nitrogen balance is brought about and a permanent loss of weight.

If we apply this hypothesis to amphibian metamorphosis, we should expect that in such larvae, which have not been able to take up an amount of iodine sufficient for metamorphosis and whose thyroid consequently was unable to develop the normal hormone, the action of the excretor substance would lead to a prolonged and increased loss in size of the larvae, without resulting metamorphosis, since in these animals excretion by the thyroid would commence as soon as the excretor substance reached the amount required, but the excreted

5 Morse, W., Biol. Bull., 1918, xxxiv, 149.
substance would be the toxic substance tryptophane instead of the normal hormone.

This expectation is actually fulfilled in the thymus-fed larvae of *Ambystoma opacum*, as is shown by Curve B, Fig. 1. In this curve is represented the growth of a series of larvae of the same age and from the same mother as the larvae used for the experiment plotted in Curve A; both series were kept under the same conditions, but while Series A was fed on earthworms, Series B was fed on thymus. The thymus-fed larvae grew normally in the beginning—and in this particular series even more quickly than the controls, a fact explained by the writer in a previous paper.\(^6\) At the 13th week we observe a sharp drop in Curve B and hereafter growth was stopped and never resumed again. Exactly the same results were obtained for the thymus-fed larvae of *Ambystoma maculatum* and *Ambystoma tigrinum*.

The time when the drop of the curve takes place in the thymus-fed animals is that at which metamorphosis should have occurred if they had received normal food. This was not only suggested by the general appearance of these larvae and by the fact that a few larvae actually did metamorphose (in Series B 1916 only one larva metamorphosed at this time), but it can be proved if we calculate the time of metamorphosis from the value of the product \(R \times A\) for the worm-fed larvae. This has been done in Table II for eight thymus-fed series of *Ambystoma opacum*.

Again the product \(R \times A\) was calculated from the duration of the larval period and the rate of growth, the latter value being obtained as in the worm-fed larvae from the growth curve up to the point where the drop occurred. First we notice that the product \(R \times A\) (Table II) in the first three series is far above the average value of \(R \times A\) as obtained for the worm-fed series. This is due to the fact that the larvae of these series did not metamorphose at a time proportional to their rate of growth but much later, due to the absence of iodine at this time. The time at which they should have metamorphosed if iodine had been present in the food in a normal amount, can be calculated, however, from the product \(R \times A\) in the worm-fed series, which is 60, and the rate of

growth in each particular thymus-fed series. We find, then, that in the first three series metamorphosis was greatly retarded (Column 5, Table II). In Series D 1916 metamorphosis should have taken place at 162 days instead of at 207 days, in Series B 1916 at 109 days instead of at 133 days, and in Series TCa 1917 at 113 days instead of at 122 days. In the other series the retardation of metamorphosis, if there was any at all, was only slight; how far this was due to the special treatment these thymus-fed animals received will not be discussed here. It may be mentioned, however, that in the last series (T 1917) the retardation of metamorphosis was prevented probably

### TABLE II.

*Rate of Growth (R) in Thymus-Fed Ambystoma opacum during Larval Period of Active Growth (Calculated from Averages).*

<table>
<thead>
<tr>
<th>Series</th>
<th>Age at metamorphosis</th>
<th>R</th>
<th>R × A</th>
<th>Age at which animal should metamorphose</th>
</tr>
</thead>
<tbody>
<tr>
<td>D 1916</td>
<td>207</td>
<td>0.37</td>
<td>77</td>
<td>162</td>
</tr>
<tr>
<td>B 1916</td>
<td>133</td>
<td>0.55</td>
<td>73</td>
<td>109</td>
</tr>
<tr>
<td>TCa 1917</td>
<td>122</td>
<td>0.53</td>
<td>65</td>
<td>113</td>
</tr>
<tr>
<td>B 1917</td>
<td>122</td>
<td>0.50</td>
<td>61</td>
<td>120</td>
</tr>
<tr>
<td>TTx 1917</td>
<td>122</td>
<td>0.50</td>
<td>61</td>
<td>120</td>
</tr>
<tr>
<td>TMs 1917</td>
<td>109</td>
<td>0.58</td>
<td>63</td>
<td>103</td>
</tr>
<tr>
<td>Tkj 1917</td>
<td>104</td>
<td>0.57</td>
<td>59</td>
<td>105</td>
</tr>
<tr>
<td>T 1917</td>
<td>101</td>
<td>0.60</td>
<td>61</td>
<td>100</td>
</tr>
</tbody>
</table>

Average: 65 days

by adding parathyroid to the thymus diet; since the parathyroids are known to contain small amounts of iodine, it is probable that the thyroid of these larvae was apparently able to store up enough iodine to permit normal thyroid excretion and metamorphosis when the excretor substance began to act.

The number of days at which the second thymus-fed series (B 1916) should have metamorphosed, i.e. at which the excretor substance began to act, is 109. This approaches very closely the time when the drop of the curve occurred (95 days), if we consider that the value 109 was calculated only from an average value of $R \times A$. 
If prevention of metamorphosis by the absence of iodine, but in the presence of a thyroid gland, is accompanied by a check in growth (for the reasons given above), we should expect that prevention of metamorphosis in the absence of a thyroid gland would not be accompanied by an abnormal check of growth. Hence amphibian larvae deprived of their thyroids should be able to complete the normal growth of the species without any disturbances. That this is actually the case is shown in Allen's tadpoles which were deprived artificially of their thyroid glands and which reached frequently enormous sizes, becoming real giant larvae. A similar phenomenon is found in such forms as *Typhlomolge rathbuni*, which for some reason do not develop a thyroid gland—as discovered by Emerson—and hence remain permanently in a larval condition, but which seem able to grow for years without disturbances.

Thus the experiments reported in this article seem to prove that metamorphosis of the normal larvae of *Ambystoma opacum* which possess a thyroid depends not only upon the presence of a sufficient amount of iodine in this gland but also on the presence of the action of a second substance inducing the excretion of the iodine by the thyroid gland. By means of this hypothesis we are able to explain why thymus-fed larvae suddenly stop growing at the time when metamorphosis should occur, without, however, metamorphosing, and why species not possessing thyroid glands, such as the *Typhlomolge*, can complete their growth without disturbance.

*Metamorphosis and Temperature.*

There is still another phenomenon which lends itself readily to explanation on the basis of the assumption of an excretor substance. It is a well known fact that growth is retarded at low temperature, and since we have seen that the excretor substance is evolved during growth, it is not surprising that metamorphosis also should be retarded at low temperature. This has long been observed by many students of amphibian metamorphosis. But what remains unexplained is the fact that amphibian larvae when kept in low temperature are always much larger at the time of metamorphosis than

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they would be if kept at high temperature. We have observed this phenomenon frequently and in looking for an explanation have tried to find a relation between the size of the larve and the age at the time of metamorphosis, similar to that existing between the latter quantity and the rate of growth. Such a relation, however, does not exist.

But if we assume the action of an excretor substance in metamorphosis, the phenomenon in question can be readily explained. Comparing \( R \times A \) for the first worm-fed series (C 1916) in Table I, which was kept at a temperature of 10\( ^\circ \)C. below that of the other series, with the rest of the series of Table I, we observe that it is very high (10 per cent) above the average, which would indicate that metamorphosis in this series was more retarded than the corresponding rate of growth would demand. Since in this series a drop of the growth curve similar to the drop of the growth curve of the thymus-fed animals did not occur, this case of undue retardation of metamorphosis cannot be explained in the same way as in the thymus-fed larve, i.e. by assuming that from a lack of iodine in the thyroid gland a destructive compound was excreted by the thyroid. The only way to explain this case of retardation is to assume that at low temperature less excretor substance is evolved than at high temperature during an equal rate of growth processes. That this should be possible is not at all surprising, but was to be expected since it is well known that the temperature coefficients for different physiological processes may differ greatly. Loeb, for instance, pointed out that not only the temperature coefficients but also their variations at the lower and upper temperature scale differ considerably in different physiological processes.\(^8\)

But if the amount of excretor substance produced by an equal rate of growth is less at low temperature than at high temperature, the animals kept at low temperature must grow longer at an equal rate than those kept at high temperature before that amount of excretor substance is produced which is required to bring about thyroid excretion. Consequently the low temperature larve must reach a larger size than the high temperature larve, before they can metamorphose.

\(^8\) Loeb, J., Mechanistic conception of life, Chicago, 1912, 212.
The same phenomenon was observed also in the thymus-fed larvae (Series D 1916, Table II) and since here the lack of iodine and that of the excretor substance are combined, it is not surprising that the value for $R \times A$ is still higher (28 per cent) above the average than in the low temperature series of the worm-fed larvae (Series C 1916, Table I).

Thus with the hypothesis of an excretor substance we can explain a phenomenon which for a long time was confusing to experimental biologists as well as to systematists.

SUMMARY.

1. Two substances are involved in amphibian metamorphosis as studied in Ambystoma opacum: first, iodine, which is taken up by the food, and second, an excretor substance, which is evolved during the processes of growth and serves to induce the excretory function of the thyroid gland.

2. This explains why in larvae, whose metamorphosis is inhibited by lack of iodine, growth is checked at the time when metamorphosis should occur; for at this time the excretor substance commences to act and this results, if iodine is absent, in the excretion by the thyroid of toxic substances which cause the breakdown of proteins and consequently a decrease in size of the larvae.

3. Larvae whose metamorphosis is inhibited by extirpation of the thyroid or by the hereditary lack of a thyroid (as is the case in Typhlonolge) can grow normally, since in them the action of the excretor substance cannot result in the excretion by the thyroid of a toxic growth-inhibiting substance.

4. At low temperature less excretor substance is produced than at high temperature during an equal rate of growth; therefore larvae kept at low temperature reach a larger size than larvae kept at high temperature, before they metamorphose.

The writer wishes to express his thanks to the Library of the Brooklyn Museum, and especially to Miss S. H. Hutchinson, for courtesies extended to him.