IS THE INFLUENCE OF THYMUS FEEDING UPON DEVELOPMENT, METAMORPHOSIS AND GROWTH DUE TO A SPECIFIC ACTION OF THAT GLAND?

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The experiments on thymus feeding thus far reported in the literature have given results sufficiently different to prevent the formation of a definite idea as to the rôle of the organ in these experiments. This is even true if one has in mind only one of the various groups of animals which have been studied in such experiments. Concerning the Amphibia among which only the larvae of Anura have been studied carefully regarding their reaction to thymus feeding, it seems that most of the experiments showed a retarding influence of the thymus upon development and metamorphosis although some exceptions are reported. With respect to growth however, the results are so lacking in uniformity, that Gudernatsch as well as Romeis who studied the effect of thymus feeding in tadpoles doubted whether the effect produced by this organ was due to a specific action or only to quantitative conditions. Gudernatsch in his experiments on tadpoles noted accelerated growth leading to enormous size; but Romeis obtained completely normal growth in various series of thymus-fed tadpoles and pointed out that the thymus feeding never produces abnormally large animals.

The following experiments which will be reported elsewhere in detail, seem to indicate that the accelerated growth of thymus fed Amphibian larvae is merely the effect of quantitative conditions and not the result of a specific quality of the organ such as a specific growth-stimulating agent. Furthermore, they yielded some very interesting results concerning development and metamorphosis although these are still difficult to explain. Finally they showed that in each thymus-fed larva severe tetany

is produced. The last mentioned phenomenon will be discussed in another article; the effects of thymus upon development, metamorphosis and growth will be outlined briefly in the following pages.

In the experiments to be reported, only larvae of Urodela were studied (*Amblystoma punctatum* and *A. opacum*). The advantage of using Salamander larvae is that the quantity of food given to them can be controlled and measured with exactness, up to a certain degree, which cannot be done if tadpoles are used; and it should be emphasized that in order to avoid errors the possibility of measuring the quantity of food is very desirable and even should be demanded in experiments in which it is suspected that qualitative relations are involved.

1. DEVELOPMENT AND METAMORPHOSIS

Gudernatsch found that the development of tadpoles was delayed if the animals were fed on thymus. Similar results were obtained by Romeis in his first experiments. This led to the inference that thymus contains a substance whose specific property is a retarding effect upon development. Only recently Gudernatsch again published a paper based on this hypothesis.

However, in his work published in 1915 relative to the influence of the glands of internal secretion on Anura larvae, Romeis reports upon two series of experiments, which cannot be explained from the above-mentioned standpoint, and which caused the author himself to doubt whether inhibition of development were indeed a specific function of the thymus. The first series consisted of fairly old larvae of the species *Rana esculenta*, the individuals of which developed in a perfectly normal manner, in spite of being fed with thymus; but in this case it might have been supposed that the thymus feeding had been started too late. In the second series, however, in which larvae of *Rana temporaria* were employed, the thymus feeding was commenced at a very early stage, in spite of which fact the development of the larvae was not delayed. On the contrary, they underwent metamorphosis at an earlier stage than did the larvae fed with

muscle tissue, and before the latter had developed front limbs. This last series of experiments suggests the assumption that the influence exerted by the thymus on development must be dependent on factors not specific for the thymus. The experiments on Salamander larvae now to be described led to similar conclusions.

Both Gudernatsch and Romeis took as index of the rate of development, the growth of the hind and front limbs, the absorption of the tail and the abandonment of the water. The latter phenomenon however, which we will refer to as metamorphosis seems in Salamanders, to be dependent on a mechanism different in many respects from that which controls development, such as the growth of limbs, etc.; for in the first place even under conditions of normal feeding, different individuals show a different stage of development when they leave the water, and secondly the effect of thymus upon development and upon metamorphosis does not seem to be the same in the Salamander larvae examined. Therefore we shall distinguish between development and metamorphosis; growth and differentiation of the limbs, certain changes of the fin and gills not being in direct relation to the abandonment of the water, and the changes of the color pattern of the skin which finally lead to the definite coloration of the skin, will be referred to as development; while the abandonment of the water together with the sudden reduction of the gills to mere stumps and the complete absorption of the fin will be called metamorphosis.

In a group of eight series, O 1916, in which larvae of *Amblystoma opacum* were used, four series were fed with small fragments of earthworms and four series with equal sized pieces of thymus. As will be explained later on, these experiments were conducted with the intention of feeding the respective animals with equal quantities of worms and thymus. So far as development and metamorphosis is concerned, it would seem at least possible, that besides the quality of food, the amount of food may also have some influence upon these two phenomena; but at any rate only if we make the quantities of food alike in the experimental series and the controls, can we be sure that the differences

obtained in both series are the expression of the quality of the food.

The development of the legs and toes was carefully noted, and the development of these organs was seen to occupy a period of from 6 to 9 weeks. As the thymus feeding began as early as the second week, the development of the legs took place under the influence of thymus feeding during 4 to 7 weeks. According to Gudernatsch and Romeis this length of time sufficed in the case of tadpoles to produce the retarding effect upon development of the thymus; but in the case of the Salamander larvae absolutely no retardation could be noted as a consequence of the thymus feeding. Indeed, in those series which as a result of the simultaneous effect of a lowered temperature necessitated a longer period of time in order to attain complete development, a change in the contrary direction could even be noted in the latest stages, the thymus animals attaining complete development of their limbs more quickly than the worm-fed animals.

Thus it can hardly be argued in this connection that feeding had not lasted long enough to produce a result, in view of the fact that in the latest stages of development of the legs when the feeding had lasted a longer period than in the first stage, the opposite result was obtained. This result then indicates that a distinct difference exists between the Anura and Urodela with respect to the effect of thymus-feeding upon development.

The development of the legs in such animals to which instead of equal quantities of food as much food was given as each animal was able to take, was not studied in sufficient detail. In one group of experiments (P 1719) which consisted of *A. punctatum* larvae, development of the legs was recorded during 14 days after the beginning of the feeding; in this group the relation between the thymus-fed animals and the controls was the same as in the above experiments on *A. opacum*.

The differences between the Anura and Urodela become even more accentuated as development proceeds. But a careful distinction must be made between animals fed on equal quantities and those which obtain as much food as they will take.

In the above mentioned group (O. 1916) which consisted of larvae of *Amblystoma opacum* fed with equal quantities, the development of further advanced characteristics of the legs, of the shape of the head, of the gills and of the color of the skin proceeded much more rapidly in the thymus-fed animals than in those fed on worms.

With respect to the development of the gills, the following should be remarked: In larvae of *Amblystoma opacum* fed with worms and kept at an average temperature of 22.6°C. as late as one or more days before metamorphosis the gills attain a stage not only of considerable size, but one in which they are characterized by considerable redness and above all by the fact that they are bent upwards in a crescent shape. The long well developed branches are widely extended and the points of the stem inclined forwards so as to bend over. In worm-fed animals kept at a high temperature (22.6°C.) this condition of the gills was only attained in the 23rd week, but in thymus-fed animals kept at the same temperature as early as the 11th week; in worm-fed animals kept at a low temperature (in average 14.8°C.) only in the 29th week; in thymus-fed larvae kept at the same temperature as early as the 11th week (although in the latter case the gills were less developed than in the case of the high temperature thymus-fed animals).

A similar relationship is observed with respect to the color of the skin. In the case of the warm worm-fed animals the melanophore spots only began to develop in the 13th week, at which time they had already attained very considerable development in the case of the warm thymus-fed animals; in the warm worm-fed individuals the blue-grey pigment did not appear until the 24th week, but in that of the warm thymus-fed animals as early as the 12th week. In the cold worm-fed animals the fusion of the melanophore spots into a uniform black-brown coat only began in the 30th week, and occurred as early as the 13th week in the case of the cold thymus animals; but in the cold worm-fed animals no trace of a silver-grey pigment can be detected after 32 weeks, although this appeared in the cold thymus-fed individuals as early as the 13th week. These differences in the

rate of development are doubtless sufficiently great to indicate distinctly the differences existing between the Anura and Urodela. Under conditions of quantitatively equal feeding (which alone can be taken into consideration in a study of qualitative effects) the feeding of thymus to larvae of *Amblystoma opacum* causes accelerated development.

Nevertheless, the above mentioned experiments become even more clear if the results obtained by them are compared with the result in experiments made by a different method; for the factor to be emphasized is not the time elapsed since the hatching but the size of the animals. The thymus-fed individuals attain the stated conditions of development while much smaller in size than the worm-fed animals. The latter must attain much greater size than the thymus-fed animals, in order to acquire the same degree of development.

In a group of *A. punctatum* (P. 1916) kept at a high temperature, one series was fed with pieces of thymus and another with *Tubifex*. In both series the animals were allowed to eat according to their inclination as a result of which the worm-fed animals consumed a considerably larger quantity of food than did the thymus-fed animals, and consequently grew much more rapidly. The development of the skin pigmentation also proceeded more quickly than in the case of the thymus animals, although the latter attained these various stages while much smaller in size. At the time no exact drawings were made to show the relationship between the size and stage of development. This will be taken up in a recently initiated experimental series of *A. punctatum* (P. 1917) as yet incomplete, in which the same system of feeding is being maintained as in Group P. 1916. Meanwhile it can already be noted that the worm-fed animals do not develop the yellow network until they have attained the average size of 62.91 mm., the minimum length being 59 mm. The thymus animals, which have only attained an average length of 32.22 mm., with a maximum length of 36 mm., have not yet shown signs of this network. In group P. 1916 of *A. punctatum* in which the worm-fed animals behaved like the worm-fed animals in Group P 1917 regarding the relation between size and de-

velopment of network, the first thymus animal attained the network stage when only 41.3 mm. in length.

We thus see that the time at which the various phases of development are attained varies according to the quantity of food, with the result that sometimes the thymus animals, at other times the worm-fed animals appear to lead. But the constant factor is the size at which the various stages are attained; that is, constant to the extent that the thymus animals always develop more quickly than do the worm animals, if the various stages are referred to the size of the animals. This relationship is directly opposed to that of the *Anura* larvae, for in these animals the thymus-fed individuals must usually attain a considerably greater size than the worm-fed animals in order to arrive at the same degree of development.

Identical relationships as occur in the development are also found in the metamorphosis; but in this case one or more additional factors seem to play a rôle to complicate considerably the phenomena, as we shall see.

Here again we must differentiate between the experiments in which the food was quantitatively equal and those in which each animal was allowed to eat to the point of satiety. But it should be emphasized that only the first method permits of a correct comparison. For when the worm animals feed at will they eat approximately 10 to 20 times the quantity of food that is consumed by the thymus animals when the latter begin to suffer from tetany; as the worm animals also grow much more rapidly as a result, it would not be surprising that they also metamorphose earlier, since we might expect that if a definite size of the animal is indispensable to metamorphosis, metamorphosis will be accelerated if we accelerate growth by some external conditions.

We will now turn our attention again to the group O 1916 of *A. opacum* in which each series was given approximately the same quantity of food. In this group the warm thymus animals were the first to undergo metamorphosis; thus in the warm thymus series (22.6°C.) the first animal underwent metamorphosis in the 13th week, in the warm worm series only in the 24th week; in the cold thymus series (14.8°C.) the first animal left the water

in the 24th week; whereas in the cold worm series no animal has yet undergone metamorphosis (in the 32nd week). Thus, thymus-fed animals are seen to metamorphose earlier than worm-fed animals; that is, provided they receive equal quantities of food.

The relationship of time however becomes inverted if the worm and thymus animals, instead of receiving equal quantities of food are allowed to eat at will. In a group of *A. punctatum* (P 1916) consisting of two series, which had been kept at a high temperature and in which the last-mentioned mode of feeding was adopted, the first animal of the thymus series underwent metamorphosis after five months, whereas the first of the worm series did so after only $3\frac{1}{2}$ month

As in development, so also in metamorphosis, the relationship of time is seen to be inconstant and depends on the amount of food given to the animals. But a constant factor exists in the relationship between size of the animal and metamorphosis. Whatever method of feeding may be adopted, the thymus-fed individuals are always much smaller when they undergo metamorphosis than are the worm-fed ones. In the *Opacum* group (O 1916) consisting of equally fed animals, the warm thymus animals averaged only 47.8 mm. in length at the time that the first individual underwent metamorphosis, whereas in the worm series at the beginning of metamorphosis the average size was 53.5 mm. The same relationship can be observed at a low temperature; the average size of the thymus animals being only 57.5 mm. at the beginning of metamorphosis, whereas the worm animals had not yet begun to metamorphose when their average length was 65.1 mm. The same conditions apply in the above-mentioned *Punctatum* series (P. 1916); the thymus animals begin to metamorphose when their average size is 41.9 mm., but the worm animals only at an average size of 50.0 mm.

As in the case of development, so in metamorphosis the relationships obtaining in *A. opacum* and *punctatum* are exactly the reverse of those found in the *Anura* larvae, for in the former the worm-fed animals must attain a much greater size than the thymus-fed individuals before they can undergo metamorphosis

whereas in the case of the *Anura* larvae the thymus animals must be larger than the worm animals before metamorphosis can occur.

However, in addition to the facts mentioned above, still another phenomenon must be described which seems to aid greatly our understanding of the relation between development and metamorphosis. If we refer metamorphosis neither to the time which has passed since hatching nor to the size of the animals but to the stage of development of certain structures, metamorphosis does not appear to be accelerated in the thymus animals but rather retarded.

For example, when comparing the warm worm animals with the warm thymus animals of the *Opacum* group (O 1916) we see that as early as the 11th week the warm thymus animals attained the same stage of development at which the warm worm animals commenced to metamorphose. At this stage, however, a remarkable phenomenon is noted; the warm thymus animals fail to metamorphose while some of their organs continue to develop; the structures of their skin, which are responsible for the development of the color of the skin attain, while the animal is still larval a phase of development reached by the worm animals only some time after metamorphosis has been accomplished. After the warm thymus animals have entered upon the stage characterized by the crescent-shaped gills and the fusion of the melanophore spots, they should, if compared with controls, undergo metamorphosis, but instead they develop the silver-grey pigment and undergo reduction of the size of the fin. Simultaneously (a point to be specially emphasized) they stop growing and become reduced in length, a condition which also occurs in the case of worm animals before metamorphosis. They assume an aspect which on the whole resembles that of a worm-fed animal which had undergone metamorphosis about two weeks previously. As can already be seen, these relationships can be noted much more distinctly in the cold *Opacum* series; but as the animals of these series have not yet all undergone metamorphosis and the worm animals have not yet begun to metamorphose, we will not describe the phenomena already noted.

Exactly the same phenomenon can be seen in a group of *Punctatum* (Group P 1916) maintained at a high temperature, such as the development of definite characteristics of a metamorphosed animal during the larval stage. In this case the yellow network was separated into yellow spots during the larval stage—a phenomenon which does not occur in the case of the worm animals before they have left the water.

From what has been stated above we can see that even in those animals which metamorphosed first and, in the series of *Opacum* larvae (O 1916) fed with equal quantities, metamorphosed 11 weeks earlier than the worm animals, the process of metamorphosis was disturbed. This becomes much more apparent if for the date at which the first animal underwent metamorphosis we substitute that of the last animal metamorphosed. In that case we obtain the following relationship: In the series of *Opacum* larvae (O 1916) after 32 weeks have passed, 12 per cent of the thymus-fed animals are yet in a larval stage, whereas the worm-fed individuals all had metamorphosed as early as the 29th week. Thus, the period of metamorphosis in the worm series extended only over 5 weeks, whereas in the case of the thymus animals it has already lasted 19 weeks. In the repeatedly mentioned group of *A. punctatum* larvae (P 1916) kept at a high temperature, the last thymus fed animal had not left the larval stage even after 8 months, whereas the last worm-fed animal had metamorphosed after only $5\frac{3}{4}$ months; thus in the worm-fed animals, metamorphosis covered a period of only $2\frac{1}{3}$ months, whereas in the thymus animals it lasted 5 months. In a group of *A. punctatum* (P 1916 C) kept at a low temperature, a worm-fed series comprising individuals which out of a number of 300 larvae had not yet undergone metamorphosis was added to a thymus-fed series which had been under observation for about 5 months. In other words, this worm-fed series consisted of larvae which were abnormally late in undergoing metamorphosis. The first of these worm-fed animals left the water $5\frac{2}{3}$ months after hatching, the last $7\frac{2}{3}$ months after hatching, the period of metamorphosis extending in this series over 2 months. Of the thymus-fed animals the first metamorphosed after $4\frac{1}{3}$ months,

the last (leaving two animals out of consideration) after $6\frac{1}{3}$ months. In the case of these thymus-fed animals the period of metamorphosis lasted 2 months, for instance, not longer than in the case of the worm animals; but 2 of these thymus animals not yet mentioned, behaved very differently from all the other animals. They both remained at a low stage of development, so far as coloring was concerned, and their tails underwent but slight reduction in size. On the other hand, the gills were reduced to short stumps. Although neither of these 2 animals was shedding its skin (which should take place before metamorphosis) at the time of the reduction of the gills, they were taken out of the water and placed in a vessel, the bottom of which was covered with filter paper and just enough water to keep the vessel wet. But neither of the animals showed any further change, until finally $12\frac{2}{3}$ months after hatching one of them shed its skin and its gills became completely atrophied, while at the same time the skin became darker in color although the yellow network failed to develop. The other animal is still in the larval stage, $13\frac{2}{3}$ months after hatching.

We must not fail however to mention that it still appears very doubtful whether this is a direct effect of thymus, for a similar phenomenon was also noted in the case of worm-fed animals, although not to so extreme a degree. Out of approximately 300 worm-fed animals, only 1 individual showed such a condition; after more than 8 months it was still in a larval condition and had not developed a trace of the yellow network. The fin of its tail was but slightly reduced; its gills were more reduced and the animal was still undergoing growth and taking food spontaneously. It was used for the purpose of an operation, in the course of which it died. However, as has been said, at this stage it showed no trace of approaching metamorphosis. From this it seems very doubtful that the delay of metamorphosis in the two last mentioned thymus animals was actually due to the action of thymus and we must exclude them from discussion until the same phenomenon is obtained in a greater number of cases.

2. GROWTH

In one series of experiments (P 1917) for which purpose larvae of *A. punctatum* which had hatched on the same day and were the offspring of the same mother were employed, it was assumed that where there is an unlimited supply of food, the amount spontaneously taken up by each animal is a function of growth, and that growth is not a function of the food quantity. For that reason in these experiments which were carried out at an average temperature of about 22°C., the animals were allowed as much food as they felt inclined to take.

The group consisted of three series. The animals of the first series were given small equal-sized fragments of thymus with a pair of forceps, until each animal was satisfied. They took the pieces easily and owing to the softness of the material had no difficulty in swallowing them. The second series received fragments of earth-worms. Owing to the hardness of this food, however, the animals found great difficulty in swallowing it, and it took several minutes, or even hours for each piece to be swallowed. As they were fed only once a day, these worm animals remained hungry and consequently were soon backward in growth, as compared with the thymus-fed animals. The latter finding coincided with the observations made in the case of the *Anura*; i.e., that the thymus stimulates growth; but it failed to prove a specific influence of thymus, for the reason that the animals which were fed in a normal manner were found to be starving. In a third series the animals were fed with small worms (*Enchytraeus*), which were at first given in small pieces; these worms were thrown into the containers in such large quantities that the animals never lacked food. Besides this, each animal was fed on pieces of earthworms which the fast-growing animals soon took readily and in large quantities. The individuals of this series grew faster from the very outset than did the thymus animals. As the latter did not develop tetany until the 5th week and were in a completely normal condition until the end of the 4th week, we may look upon the result attained up to that time as the pure effect of nutrition. The salamander

larvae failed to show that the thymus had exerted any growth-accelerating influence. On the other hand, the quantity of food given plays an important part in this connection, for the animals react in a highly sensitive manner to relatively slight differences in food quantities. From these experiments it would seem that in the experiments on tadpoles conducted by Gudernatsch and Romeis the factor revealed is not a specifically growth-promoting influence, but that the accelerated growth of the thymus animals should be attributed to the fact that the jaws of the tadpoles, although adequate to supply the body with a quantity of the soft thymus material corresponding to the needs of the organism, were nevertheless not the most appropriate instrument for preparing from the hard beef muscle sufficient nutriment for the purpose of keeping up normal growth. The very fluctuating results which Romeis obtained in his experiments indicate pronounced sensitiveness on the part of the tadpoles to small quantitative differences of food which often completely escape control, rather than the presence in the thymus of a specific growth-promoting influence. It should also be remarked that in the above-mentioned experimental group it was also noted that the animals fed with worms must consume a much greater quantity of earth-worms than the thymus-fed animals consume of thymus in order to grow equally quickly; the supply of earth-worm fragments which the second series consumed was only slightly smaller than that of the first series fed with fragments of thymus. This fact speaks in favor of relatively high nutritive value in the thymus. It should also be taken into consideration that in the fragments of earth-worms a not inconsiderable part of the volume consumed consists of indigestible substance (chitin, earth) which are later eliminated in the feces.

In the preceding order of experimentation it is seen that at the moment that the tetany period begins in the thymus-fed animals we are confronted by an obstacle which prevents any quantitative judgment from being formed; for from this time on the thymus animals are seen to be abnormally placed and the amount of food taken in by them becomes abnormally low. This is all the more disturbing for the reason that it is uncertain whether

under these conditions the quantity of food spontaneously taken is really a function of growth. On the contrary it appears very probable that the reduced amount of food taken must be attributed to disturbances caused in the swallowing apparatus by the convulsions. In such a case the animals would be in a condition of starvation and in contradiction to the idea of the experiment the rate of growth would be the function of the food quantities introduced into the organism. I thought to be able to overcome this obstacle in another group of experiments, in which I proceeded from the fact that when food is present in sufficient quantities equal amounts of food produce an equal rate of growth.

In a group consisting of 4 series (O 1916) for which larvae of *A. opacum* were used, the food was given in small fragments at the point of the forceps in all the series; an attempt was made to make all the pieces of approximately the same size on the same day of feeding. The number of pieces given to each individual animal was noted, and on each feeding day approximately (for the week) the same number of pieces was given, so that all the animals of these 4 series received approximately the same number of pieces, the series comprising one thymus and one worm group at an average temperature of 22.6°C., and one thymus and one worm group at about 14.8°C. An effort was hereby made to distribute a quantitatively equal amount of food among the 4 series; but it must be remarked that this can only be roughly attempted and cannot be exactly carried out. As it can never be known beforehand how much food the animals may need on a given day in order to be satisfied, it would also be quite impossible to weigh the food. But even if this were possible, the distribution of equal quantities according to weight would not lead to the distribution of equal nutritive quantities as a given volume of thymus contains a larger quantity of substances available for metabolism than does the same quantity of fragments of earth-worms, as has been shown in the first experimental group. Although this method is not exact, it has at least furnished an approximate idea as to how important it is to control the quantity of food in such experiments.

Of course the quantity of food to be given each day was always standardized from the series which desired the smallest amount to eat. At the beginning these were the worm series, and of these the cold worm series showed less avidity for food than did the warm worm series. As a result the thymus series at first received less than they would have liked to eat. The reasons for this comparatively small appetite in the worm animals have been specified above when discussing the first experimental group. From the time that the series of warm thymus animals began to undergo metamorphosis, the animals of this series showed the least desire to eat; after that it was the worm animals in general, and the warm worm series in particular which received less than they could have consumed.

It may be emphasized at this point that when this method of distributing quantitatively equal amounts of food is followed, tetany exerts a very slight influence on growth. Sometimes the rate of growth is reduced at such points where the tetany curve reaches its apex, but in other cases, on the contrary it increases or reaches even a maximum when the tetany curve does.

The condition which exerts an influence on growth in comparison with which all other influences are reduced to insignificance, is metamorphosis, as will be apparent from the following description.

During the first few weeks the warm thymus animals are seen to lead in size; next in order come the cold thymus animals, then the warm worm series, and finally the cold worm series. Nevertheless no special importance must be attributed to this relationship, for as has already been stated, given an equal volume of food, the thymus animals probably obtain more nourishment from their pieces of thymus than do the worm animals from an equal quantity of worm fragments. The relationship of size which has just been mentioned lasts until the 10th week, and the acute tetany which has meanwhile set in among the warm thymus animals and reached its climax has failed to influence this relation at all. In the 11th week a pronounced change sets in; at this stage the warm thymus animals are all ready for metamorphosis, the first individuals being 14 days removed from

this step. During this week the curve of the body size of the cold thymus animals, which up to that time occupied the second position, can be seen to cross that of the warm thymus animals. From the time that the first animal of the warm thymus series entered upon metamorphosis, the warm thymus animals completely stopped growing. Their curve, which of course does not include the metamorphosed animals, is soon after crossed by those of the two series of worm animals, and the warm thymus animals remain smallest in size for the rest of the experiment.

The cold thymus series, the first individuals of which underwent metamorphosis in the 24th week, also increase in size only a little from the time of metamorphosis on; but as the first animals of the warm worm series which is most proximate to the cold thymus curve similarly undergo metamorphosis in the 24th week, and also because the curve of the cold thymus animals is higher above that of the warm worm animals than the curve of the warm thymus animals is above the cold thymus animals, the curve of the latter remains the first at the beginning; it is not crossed by that of the warm worm animals until the latter have all metamorphosed. Finally, in the 29th week, together with the curve of the warm worm-fed animals, it is crossed by the curve of the cold worm series, which now occupies the first position. As early as the end of the 29th week the largest animals of the cold worm series have attained a size greater than that of each non-metamorphosed (and of course of each metamorphosed) individual of the three remaining series. As for the present (after the 32nd week) the animals give no sign of impending metamorphosis and continue to grow.

The above-reported circumstance appears to us to be the most instructive with reference to the statement that thymus-fed anuran larvae attain a size by many denoted as abnormally large but stated by Romeis never to exceed normal limits, although sometimes exceeding the size of the muscle-fed animals. If we begin by comparing each of the two thymus series with the corresponding worm series, we see that the same relation exists between them as between muscle and thymus-fed tadpoles, inasmuch as the animals which metamorphose later attain greater

dimensions than do those which first underwent metamorphosis. It can be seen that this is not connected with a specific influence of thymus feeding, from the fact that exactly the same relation exists between the warm and the cold worm series, the warm worm-fed series which first underwent metamorphosis metamorphosing while smaller in size than the cold worm series, and the latter continuing to grow after the former has metamorphosed. While yet in the larval stage the cold worm-fed animals attain a size which when the largest animals of both series are used for comparison, already exceeds that of the largest warm worm-fed larva by 12.5 mm.¹ From another point of view the salamander larvae of those species so far examined show the very opposite characteristics from those possessed by the anuran larvae; for it is not the worm-fed salamander larvae which first undergo metamorphosis but the thymus-fed individuals. Thus, the point to be primarily emphasized is not the greater size ultimately attained by the worm-fed salamanders and thymus-fed tadpoles, for we have seen that this does not depend upon the specific qualities of the thymus, but that it is a general phenomenon peculiar to amphibia and one dependent upon the time at which the animals undergo metamorphosis. The point of importance in both cases—the larvae of *Anura* as well as of *A. opacum* and *A. punctatum* is the circumstance that thymus-feeding produces metamorphosis in the *Anura* only when considerable size has been attained, whereas in the *Urodela*, on the other hand, this occurs while the animal is but small in size.

To summarize, we may make the following statement: The differences in the rate of growth to be noted before metamorphosis are not the result of a specific growth-promoting influence of the thymus; they are based on the circumstance that animals which are better fed grow more quickly. In the experimental group of *A. punctatum* (P 1917) discussed in the preceding section, these

¹Although the cold worm larvae are at the time of writing larger than the largest metamorphosed warm worm animals, we do not here intend to take up the question of this relation; moreover, a comparison of the experiments hitherto conducted in connection with the *Anura* shows this not to be possible, as the respective authors never observed their experimental animals beyond the period of metamorphosis.

individuals are obviously the worm-fed animals of the third series, which are allowed to have as much food as they wish; in the experimental group with *A. opacum* (O 1916) it is the thymus animals which take in a greater quantity of nutritive material through eating thymus. The experiments furthermore show that qualitative influences exerted on the rate of growth would have to be very considerable in order that they can be experimentally tested in the case of amphibia, for in these animals the slightest quantitative differences, such as can hardly be controlled, would bring about very misleading differences in growth.

With respect to the ultimate size attained by the animals, Salamander larvae resemble tadpoles in the fact that under certain conditions the later they metamorphose the greater is their final size; this is not only true for thymus-fed animals in comparison to worm-fed animals, but also for worm-fed animals kept in high temperature in comparison to worm-fed animals kept in low temperature.

The action of thymus on development and metamorphosis may be summarized in the following way:

In animals fed on thymus the development presumably of the organism as a whole but certainly of the legs, gills, shape of the head and color of the skin, is greatly accelerated during the larval period. The thymus-fed animals, therefore, reach the stage at which worm-fed animals are ready for metamorphosis, much more quickly than worm-fed animals. As development at least to some degree may be dependent on growth, on the rate of growth and on size, it is impossible to examine the specific influence upon development of any substance without keeping alike the conditions of growth in both the experimental and control series; such was attempted by admitting an equal amount of food to both series.

When the thymus animals have reached the stage at which worm-fed animals go into metamorphosis, the development of most organs seems to stop, while certain characteristics of the skin continue to develop; the skin of such animals then behaves very similarly to the sex-organs of neotenic larvae, since the skin at least with regard to the structures determining pigmentation,

develops characteristics of a metamorphosed animal, while the animal as a whole still is in a larval stage. At the time when metamorphosis should occur disturbances in the course of development begin to appear evidently due to the suppression of the development of some factor, without which further development is impossible. In most of the animals of a thymus-fed series this factor still develops much earlier than in the controls; but even in these individuals metamorphosis becomes a grave danger to the animals' life. In high temperature some animals die during metamorphosis and those which survive metamorphosis die a relatively short time after metamorphosis. In some individuals the development of the factor necessary for metamorphosis is still more disturbed and becomes delayed in comparison with the controls; at high temperature all individuals in which this is the case die on the day when the gills and the rest of the fin undergo the sudden reduction in size, characteristic of the entrance into metamorphosis. In low temperature they may survive metamorphosis. In low temperature a very small percentage of the thymus-fed animals may remain at a low stage of development and not metamorphose for more than a year; but whether this is due to the action of the thymus diet is not yet certain, as a similar phenomenon was observed in one worm-fed animal of the stock.

It seems that we cannot understand the results reported in thymus feeding experiments if we assume that they are the pure expression of the influence of the thymus substance. The rather great fluctuations reported in individuals of the same species as well as the surprising differences between larvae of Anura and Urodela when fed on thymus, indicate that quite a number of factors are involved in metamorphosis, some of which were not controlled in the experiments. It is of course clear, that differentiation of the organism is one of these factors; that a certain degree of differentiation is indispensable for metamorphosis, or at least to facilitate it, was shown by Gudernatsch in some recent experiments on the influence of thyroid. That some of the individuals among a thymus-fed series of Salamander larvae metamorphose earlier than the controls may be due in some degree

to the fact that in the thymus-fed Salamander larvae development and differentiation and consequently metamorphosis also depend on the general conditions of growth; the experiments on Salamander larvae reported suggest that rate of growth and size play an important rôle in metamorphosis. The difference noted between Anura and Urodela when fed on thymus can be explained only by assuming a fundamental difference between the organization of these two groups of animals. It will be pointed out in another article that such a difference, namely the absence in the Salamander larvae and the presence in the anuran larvae of the parathyroids, seems to explain why thymus-feeding should develop tetany in Salamander larvae and should not in anuran larvae. It suggests itself that metamorphosis in part must depend on a factor similarly being present in one group but absent in the other group. The development of that factor may be induced primarily by processes occurring in a certain stage of differentiation, but also may be influenced and inhibited or disturbed by thymus diet. The action upon this factor of the thymus may be widely different from that upon developmental processes preceding its development; this is indicated by the fact that development while accelerated during the larval period is on the contrary retarded from the time at which metamorphosis should occur. It is this phenomenon which emphasizes the fact that metamorphosis to some degree must occupy a particular place among the processes of development. In this connection, finally, frequent reports may be remembered according to which thymus causes disturbances of the blood circulation; in metamorphosis of the Amphibians the blood circulation undergoes a fundamental change in the course of which the gills are absorbed, and in Salamanders, the absorption of the gills according to Maurer, is a prerequisite for the formation of the parathyroids. It may be worth while to keep these facts in mind during further studies of the influence exerted upon metamorphosis by the thymus.

Though the effect of thymus feeding on development and metamorphosis is very evident, it appears to the writer that similar effects may be produced by other and purely quantitative exter-

nal conditions, such as temperature and quantity of food and in general by all factors which modify growth, rate of growth, size and velocity of development. No doubt such factors are of great importance in determining at what time, at what size and developmental stage of the animal, metamorphosis will occur. Since the relations between these different factors are very complicated and the number of experiments relative to them is rather small, discussion of these conditions must be postponed.

Finally it should be mentioned that the thymus gland apparently contains all substances which are necessary to build up the substance of an Amphibian organism to maintain the animal growing and to sustain life permanently. This is demonstrated by a number of specimens of *A. punctatum* kept at low temperature which have been fed on thymus since about the 14th day of their life and are now about 14 months old; they are increasing in size.