For details see April number, Journal American Chemical Society, 1917.

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A contribution to the metamorphosis of skin in amphibians.

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Gudernatsch has shown that thyroid is able to induce metamorphosis in frog and toad larvæ, if they are fed on this gland. The effect was so striking that metamorphosis sometimes started five days after thyroid was fed.

From these experiments it is however not certain that thyroid is the substance which under normal conditions causes metamorphosis, nor has it been proven by these experiments that an agent similar to its physiological character is involved in normal Amphibian metamorphosis.

In a series of experiments it has been shown, that also under normal conditions some agent must be furnished to the organs of the animal in order to bring about metamorphosis of these organs. It has turned out that the organs themselves are unable to produce this agent and that it is the body which produces the agent, *i. e.*, some place or organ in the animal's body remote from the organs examined. If this agent is prevented from reaching the organ, it will not metamorphose.

The general method of these experiments is to remove the organs from the animal's body, before the body begins to produce the agent and to graft it to another animal in which the agent will not be produced for a long time. By means of this metamorphosis of the organ can be inhibited for as long as the new host does not metamorphose; in this way metamorphosis could in some cases be delayed by seven months. Until now the eyes, the skin and the gills—the latter by Kornfeld—were studied in this way. Today we will report mainly on experiments performed on the skin of *Amblystoma punctatum.*

In order to make you familiar with the changes occurring in the skin of this animal during development I will show a few pictures.

The first one (Fig. 1) shows a normally fed animal, still being larval; the skin is then reddish or yellowish brown without any particular patterns (*N* 15). The next stage is brought about by the development of a greenish or yellowish network on the brown background. Color and definiteness of this network depend upon the kind of food and on the amount of light. Animals kept in bright light and fed on thymus show the greatest definiteness of this stage (Fig. 2). When the skin develops the network, the animals are still larvæ no matter how they have been treated, though rare exceptions do occur. The next stage is the separation of this network into single green or yellow spots. Fig. 3, p. 2, shows this stage in an animal which was kept in daylight and fed on thymus; such animals always reach this stage while they are still larvæ. But animals fed on worms or kept in darkness usually do not work out their first spots before they have left the water. In this case another characteristic which appears independently from the stage of separation in animals reaching this stage while they are larvæ appears simultaneously with the separation stage; namely, soon after the animals leave the water, the background changes its color to a dark reddish or sometimes greenish brown and the skin appears leathery. All colors become more dim and faint. We called this stage "Cinnamon." It is shown in Fig. 4, *N* 3. The animal then becomes darker and darker until it is finally black. The yellow spots, which have become reduced in number and size lighten up and are finally bright yellow and shining. But the number of the spots as well as the shade of the yellow are subjected to great variations which of course cannot be discussed here.

I have here a number of formalin specimens which represent different stages of the skin colorations and which may perhaps illustrate these stages better than I could explain it.

The method was then as follows:

For each experiment three larvæ of *A. punctatum* of about the same age were used. From *A* one piece of skin including one eye was grafted to *X* while the other half of the head's skin including the other eye was grafted to *Y*. 23 pairs were operated on in this way. The skin grafts in each pair were continuously compared with each other as well as with their respective hosts.

A number of animals died before results were obtained; but in about ten pairs *X* did not metamorphose when *Y* did. In this case the grafts did metamorphose simultaneously with their respective hosts but in consequence they did not metamorphose simultaneously with each other as they would have done if left with the animal *A*. As *X* and *Y* were about of the same age, the differences were mostly slight, ranging from 3 to 28 days. But in the pair 33–34, the animal 34 did not metamorphose for some reason and was still larval in January, when it was subjected to a new operation and died. In this animal the skin graft as well as the eye graft also remained in an entirely larval condition. The other piece of skin and the other eye originating from the same animal *A* as the grafts of the Exp. 34 had, on the other hand, metamorphosed already at the beginning of September, four months after which time the graft of Exp. 34 was still larval.

On October 19 both animals were photographed and painted. Fig. 5 shows animal 33; it is fully metamorphosed and black and its spots are bright and yellow. The graft is also metamorphosed and has developed three spots. In this case the graft's spots are almost orange and quite different from the host's spots, indicating particularly well their different origins. This is also interesting because it shows that the specific characteristics of the graft have not been changed by the host, though the time of metamorphosis has been so thoroughly influenced by the host. Fig. 6 shows Exp. 34, the other animal of this pair. It is still larval, having not even developed the network. The graft can be plainly seen. It is according to its different origin, slightly different in shade, but in the same color stage—of an even brown color. The eye is also larval, as it still shows the yellow ring unbroken. Both animals have been preserved in formalin. You will easily see the spots of the skin graft in animal 33; the skin graft of animal 34 has been removed from this animal and grafted to a larva of *A. opacum*, which is preserved in formalin also; in examining it you will notice the uniform brown coloration of the graft and the entire lack of any network or spots,—four months after the graft on animal 33 was metamorphosed.

Finally I would like to mention that this agent which causes metamorphosis in the skin is by no means a specific substance;

it can also be furnished to the skin by the body of another species. Fig. 7 shows a specimen of *A. tigrinum*, to which a piece of skin with the eye from a larva of *A. punctatum* was grafted. In four other specimens of *A. tigrinum* which had a similar graft, metamorphosis had occurred from 1 to 6 weeks ago; in these animals also the graft was metamorphosed. The animal shown here was still larval, when painted, as you see from its color and large gills. The graft, as you see, is also larval. The skin is even grayish brown and the eye shows the ring still. It was not until after about three weeks that this animal also metamorphosed and then, simultaneously with its host, also the graft metamorphosed. The animal was painted again on November 24, the ring of the eye is gone and only a few yellow spots, instead of the ring are left (Fig. 8).

Hence what these experiments show is that under normal conditions a certain agent induces metamorphosis in the Amphibian organs; that this agent cannot be produced by the organs themselves, but must be furnished by the animal's body; that metamorphosis can be delayed as long as the agent does not reach the organs and that this agent is a non-specific one, as it also is produced by another species.

According to the method of these experiments it was only possible to delay metamorphosis (by seven months in the case of the eye experiments on *Salamander maculosa*). I am, however, fully aware that it will be necessary to show that metamorphosis can be entirely prevented if the agent is permanently kept away from the organs, as only this will demonstrate that it is actually a substance produced outside the organs—a substance which in no way can be produced by the organ itself. Such experiments have been started several times but could not be finished, as it is very difficult to obtain the proper material and to obtain it just at times when it is needed.