Studies on Metamorphosis in Amphibia (2)

By

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It has been made clear by many studies since Gudernatsch (1912) that the bewildering sequel of appearance and disappearance of various organs and tissues during metamorphosis of the amphibia is governed and regulated by thyroid hormone. However, as to the relation of this hormone to various stages of metamorphosis, only a glimpse was gained by works of Miyashita, Nonaka and Yamamoto (1957), and of Miyashita (to be published), who investigated histological differentiation of the thyroid and various iodine compounds in this gland at various metamorphic stages. It was known by them that the ratio of thyroxine to the total iodine compounds of the gland was 7-8% from the post-external gill stage to the differentiation of hind limbs, then after a transient fall to 2-3%, it rose higher than prior to 12-13%, remained at this level for some period, and with the beginning of the absorption of the tail, showed gradual decline toward the end of the metamorphosis. In this way, reduction of thyroxine was demonstrated in the thyroid gland during the metamorphosis, while histological pictures of hyperfunction such as increase in epithelial cells and decrease in colloid were observed. It seems therefore proper to assume, that the discharge of thyroxine towards the periphery would augment during the metamorphosis, and consequently that a large amount of thyroxine would be needed for abrupt conversion of the system though there is an exception such as the above mentioned. If so, what alterations will be introduced into metamorphic phenomena. (Findings by Miyashita are given in this paper with his permission.)
Materials and Methods

We used larvae of *Bufo vulgaris formosus* immediately after hatching from eggs, collected in a vicinity of Maebashi City on March 16, 1959. They were bred in well-water at room temperature, and beginning on the 47th day after hatching, when the hind limbs were differentiated to such a degree as to protrude the knees laterally and to have the fin-anus between the soles, 2.05μg/cc of $\text{I}^{131}$ was injected for 7 consecutive days, and returned to the well-water on the 53rd day. The majority of the control group completed metamorphosis on the 71st day, whereas the experimental group was delayed, completing it on the 80th day and later. For histological observations, they were fixed in Romeis liquid, embedded in paraffine, and serial transverse sections cut at 6-10μ were stained with azan.

Findings

Morphogenesis:

During and after the administration of $\text{I}^{131}$, there were no morphological differences between the experimental and control group. But by and by growth was gradually retarded in the experimental group. The absorption of the tail commenced nearly on the 58th day in the control group, and about the 70th day in the experimental group, though in the latter, individual difference was rather remarkable. The completion of the metamorphosis was observed in the control group about the 71st day, and on the 80th day and thereafter in the experimental group. Therefore the period between the administration of $\text{I}^{131}$ and the completion of the metamorphosis was nearly 24 days for the control, and more than 33 days for the experimental group. In this way, we could not see any such morphological disharmony that is generally observed in the so-called thyroxine-metamorphosis, that is, artificially accelerated metamorphosis.

Histological findings:

Histological investigation revealed in some organs remarkable difference between the experimental and control group, scarcely any in others, and some difference in still others. The thyroid manifested remarkable change because it selectively took up the labelled iodine. Also other organs are considered to have been affected by radioactivity, though varying in degree. Besides, the abnormality in
the thyroid may secondarily induce alterations in other tissues. So we must be especially cautious in giving unitary interpretation to the histological picture.

Findings in principal organs are as follows:

a) Thyroid: In control larvae, we observed the lumen of follicles filled with colloid, one layer epithelial cells surrounding them, and some of these cells among follicles, while in experimental larvae the glands were completely destroyed, leaving only a little connective tissue and scattered mesenchymal cells. In this way, the transfer into well-water after 7 days consecutive administration of I^{131} could not restore the thyroid, damaged by this isotope.

b) Respiratory organs: There were some differences between the experimental and control group. In controls, the respiratory epithelium of the alveoli was well developed, and smooth muscle remarkably observable, whereas in experimental larvae, the respiratory epithelium was less developed. There was also some difference in the degree of involution of the internal gill; it showed, in controls, almost complete involution together with vessels in it, leaving only agglomerate deposits of pigment here and there, while in experimental larvae, structure of gill lobule was still noticeable. The development of the respiratory tract was also more advanced in controls, and so was the differentiation of laryngeal epithelium and cartilage.

c) Digestive organs: Both in experimental and control larvae, shortening of the intestinal tract, and development of its proper layer and muscular layer was observed, but rather less in the former. Further, in the former remarkable pigment, deposition was recognized in the epithelial cells of the caudal part of the intestinal tract.

d) Liver: Both in the control and experimental group, characteristic pigment deposition was markedly seen. Blood cells among cell cords were quantitatively more pronounced in the experimental group. In the cytoplasm of the epithelial cells, orange G stainable granules were seen in this group, but the degree of differentiation was lower.

e) Gonad: There was a pretty remarkable difference between the two groups. In the experimental group, a pronounced reduction in primordial gonad cells, together with proliferation of interstitial cells were observed, and there were depositions of black brown pigment here and there.

f) Thymus: The atrophic picture of the thymus, ordinarily
seen in metamorphosis, was remarkable in the control group, but not recognized in the experimental larvae. It comprised thymus cells predominantly.

g) No remarkable difference was observed in the pancreas, oral parts, skin, cartilage, nervous and circulatory system between the two groups.

Discussion

The above findings show that metamorphosis was completed both in $\text{I}^{131}$ given larvae and non-treated controls only with a slight difference. According to Shirai, Fukushima, Shimada and Iijima (1960), destructive effects of $\text{I}^{131}$ on the thyroid were as follows: when 2.05$\mu$C/cc, the same dose as in the present experiments, was given, degenerative change first appeared in the epithelial cells, followed by destruction of follicle structure, and the proliferation of connective tissue, which began, concomitantly with it, to proliferate in the periphery of the gland, gradually replaced parenchymal cells. And it was clarified in the present experiments that the thyroid thus injured could not be restored even if the larvae were returned to well-water, being completely lost during metamorphosis. On the ground of this fact it may be assumed that thyroid hormone would not be produced at least during metamorphosis. According to Miyashita's study (to be published), the thyroxine consumption is to be augmented during the metamorphosis. In the present experiments, however, the metamorphosis, once started, was not interrupted despite the complete absence or extreme reduction of thyroxine, but it was only retarded. Previously, Nonaka, Yazawa, Noguchi and Ono (1960) anticipated, in the first report of this work, that alterations of various tissues, which are grouped as metamorphic phenomena, would not be dependent only on passive factors; that is to say, there would not be simple responses primarily elicited by thyroxine, but complicated ones which preliminarily require qualitative changes of the tissues, comprising the reaction system.

But once metamorphic activity has started in the peripheral tissues comprising the reaction system, thyroxine might exert only a quantitative effect on it. At least the possibility of such inference was suggested by the results of the present experiments.

In the histological findings, we must assume the presence of primary changes due to radioactivity intermingled with secondary
changes due to thyroid dysfunction. In the present experiments, the most remarkable change was retarded growth of the digestive and the respiratory organ. The reason is considered to be that, as reported by Mikami, Yoshida, Takano and Suzuki (1956), Sawano, Sato and Sakamoto (1958), the entodermal system is sensitive to radioactivity, and subsequently the organs derived from this system are retarded in their growth. Further, in the present experiments, the thymus was less atrophied in experimental larvae than in the controls. According to Nonaka (1949), this is primarily to be ascribed to reactive hypertrophy due to thyroid hypofunction or deficiency. That no abnormality was found in the cartilage, muscle and skin was in agreement with the absence of disharmony in the morphogenesis.

**Conclusion**

Larvae of *Bufo vulgaris formosus* in the stage of the tadpole were injected with I$^{131}$ (NaI) in a dose of 2.05$\mu$g/cc, and later developmental courses were observed, with the following results:

1) Morphogenetically there was no difference between the experimental and control group, but the former completed metamorphosis in 33 days after the administration of I$^{131}$ as against 24 days for controls. In this way, the administration of I$^{131}$ retarded metamorphosis.

2) The thyroid was completely destroyed during metamorphosis, leaving only scattered connective tissues and mesenchymal cells, subsequently the function of this gland was considered to have been perfectly lost.

3) From 1) and 2) it was concluded that the metamorphosis, once stated, would not be stopped even in the absence of thyroxine.

4) In the digestive and the respiratory system and in the thymus and the gonad, retarded growth, undifferentiated histological pictures, and degeneration were observed, which were considered to be due to radioactivity and the destruction of the thyroid.

**References**

Explanation of figures

Fig. 1. Section of the thyroid gland of control larva. × 450.
Fig. 2. Section of the region, completely being destroyed thyroid gland of experimental larva. A little connective tissue and scattered mesenchymal cells were left. × 450.
Fig. 3. Section of the lung of the control larva. × 200.
Fig. 4. Section of the lung of the experimental larva. The differentiation of the respiratory epithelium and smooth muscle was late. × 200.
Fig. 5. Section of the region, completely involuting the internal gill of control larva. × 200.
Fig. 6. Section of the internal gill of the experimental larva. The structure of the gill lobule was still noticeable. × 200.
Fig. 7. Section of the respiratory tract of the control larva. × 200.
Fig. 8. Section of the respiratory tract of the experimental larva. The differentiation of the laryngeal epithelium and cartilage was late. × 200.
Fig. 9. Section of the intestinal tract of the control larva. × 200.
Fig. 10. Section of the intestinal tract of the experimental larva. The development of the proper layer and muscular layer was late. × 200.
Fig. 11. Section of the caudal part of the intestinal tract of the control larva. × 450.
Fig. 12. Section of the caudal part of the intestinal tract of the experimental larva. Pigments were remarkably found in the epithelial cells. × 450.
Fig. 13. Section of the liver of the control larva. In the intercellular spaces pigments were found and blood cells decreased. × 200.
Fig. 14. Section of the liver of the experimental larva. Many blood cells were remarkably found in the intercellular spaces, and the differentiation of the epithelial cells was late. × 200.
Fig. 15. Section of the gonad of the control larva. × 450.
Fig. 16. Section of the gonad of the experimental larva. The decrease of primordial gonadal cells and the proliferation of interstitial cells were observed. × 450.
Fig. 17. Section of the thymus of the control larva. The atrophy of inanition on metamorphic stage was observed. × 200.
Fig. 18. Section of the thymus of the experimental larva. The atrophy of inanition was not observed. × 200.

Abbreviations used in the figures

c: colloid

e: epithelial cell

c: cartilage
c: connective tissue

sm: smooth muscle

p: pigment

in: involuted internal gill

bv: blood vessel

er: erythrocyte

stm: striated muscle

pl: proper layer
dc: digestive canal
h: hepatic cell
g: primordial gonad cell
i: interstitial cell
t: thymus cell
m: mesenchymal cell
mt: mitosis
Plate II

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Plate III

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Plate VI

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