METAMORPHOSIS OF *HYNOBIUS* LARVAE FOLLOWING REMOVAL OF FOURTH BRANCHIAL AND ARTERIAL ARCHES[1)]

BY

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(*With 7 Figures in Text*)

Introduction

According to FIGGE ('30), the absence of the fourth arterial or pulmonary arch, the spiral valve, and septa in the truncus arteriosus was responsible for the failure of *Necturus* to metamorphose. And moreover FIGGE claimed that he had succeeded in preventing metamorphosis after ligating the fourth branchial arches with the arterial arches running in them on both sides in the larvae of *Amblystoma tigrinum*, a metamorphosing form.

In the experimental study which was undertaken by GABER ('30), FIGGE's ligation of the fourth branchial arch was replaced with the removal of the lungs of axolotls.

In the latter case the experimental animals had been made to metamorphose completely without lungs. Metamorphosis was induced by feeding some desiccated thyroid glands to the animals.

It could be considered that the GABER's experimental animals were quite unable to complete pulmonary respiration without the lungs. But FIGGE's ligation of the fourth arterial (pulmonary) arch was to leave the possibility of some pulmonary function, since the dorsal part of the arch (ductus arteriosus) which joined the pulmonary artery to the dorsal aorta persisted in urodela.

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It was not only to know the effect of pulmonary respiration on metamorphosis, but also to analyse the causal effect of ligating of the fourth branchial and arterial arches on the morphogenesis of urodelan metamorphosis from the mere morphological point of view.

In the present paper, metamorphosis of *Hynobius* larvae* after the removal of the fourth ceratobranchials together with the arterial arches running in them will be reported.

I wish to express my heartfelt thanks to Prof. Dr. K. OGUMA, whose valuable criticism has been to me no doubt of great help throughout this study.

Material and Technique

For the present study, larvae of the salamander were collected from their natural habitat in an advanced larval condition in July of 1930. After the specimens were brought to the laboratory, they were kept in rather large glass-vessels. In the vessels, larvae of the salamander were very voracious, and were kept well nourished by feeding with earth-worms.

When operated on, they were about 45-50 mm. in length, with fully grown external gills and five-toed hind limbs.

Eight completely metamorphosed individuals, numbered 46 to 53, are here reported. About fifty of the animals had been used previously during the study.

Among various stages of metamorphosis, such as the disappearance of dorsal and caudal fins with simultaneous rounding of the tail, gill atrophy, protrusion of the eyes, shedding of the skin—resulting several times in epidermal changes to terrestrial or adult condition, the author took the development of vomerine teeth of adult characteristics as a final stage of completion of metamorphosis.

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* *Hynobius retardatus* DUNN (synonym of *H. lichenatus* BOUL.) which are for the present considered unique species of *Hynobius* in Hokkaido Island.

After complete appearance of the vomerine teeth and the completion of metamorphosis in general, the experimental animals were autopsied.

During the actual process of operation no anaesthetic was used. The operated animal was first tied up with a damp bandage so as to expose only its head region. Slight postero-anterior incision was made in the cutaneous fold on either side, growing back from the hyoid arch. The animal was then laid on its back on the paraffin block mounted in a thin circular glass-dish with vertical wall.

Further operation was performed under a binocular dissection microscope. The field of operation was illuminated with a strong electric lamp.

The opercular cutaneous fold from the hyoid arch was turned up along the postero-anterior incision previously made, and the branchial and arterial arches exposed. The fourth branchial arch was seized and removed by means of a fine straight forceps. After both of the fourth ceratobranchial cartilages which represented the fourth arch were removed one after another, the removed parts were strictly examined in order to determine whether the cartilage had been completely taken out.

This operation involved considerable bleeding because the afferent part of fourth arterial arch, although small, had been torn off.

After the operation the animals were placed in glass-vessels of fresh water through which oxygen gas was bubbled for 24 hours. Each individual was kept in a separate jar.

This operation was made on nine larvae of the salamander on July 23 of 1930 of which only one was found dead on the next day after the operation; the other eight, however, were completely safe from the effects of operation, and on the following day they were very voracious.

Among the eight heads No. 48 was first autopsied on September 19. The animal took at that time quite a terrestrial or adult condition.

Corrigendum

Page 101, line 6 from bottom, for 'July' read 'August'.

Results

The branchial apparatus in the larva of the studied species consisted of four branchial arches as is usual in the genus of *Hynobius*. The first and the second branchial arches consisted of a pair of hypo- and a pair of ceratobranchials respectively. These arches hang directly on a median basal element. The third and the fourth branchial arches were represented by a pair of single ceratobranchials respectively. The ceratobranchial cartilage of the fourth was more delicate than the third. The anterior ends of ceratobranchial cartilages of 2nd, 3rd and 4th arches adhered closely to each other with the consecutive median edges.

The vascular arches of the larva of the above named species consisted of four branches of a ventral aorta on each side. Of these, the first three passed to the first three branchial arches and supplied their gills. The fourth did not supply a gill. Running in the fourth branchial arch, this arterial arch made a network of capillaries (vestigial gill).

The branchial skeleton of the young terrestrial form of the species which was about 55 to 60 mm. in length underwent considerable changes. In short, the third and the fourth were lost and the second ceratobranchial cartilages were considerably reduced. The hypobranchials of the second arch had become longer and slenderer than in larval condition. In this stage the fourth arterial arch had developed vigorously.

With the terrestrial form the first arterial arch had become a carotid. The second had developed as the systemic arch which joined its fellow to form a dorsal aorta. The third remained vestigial, running along the anteroventral surface of the fourth or pulmonary arch.

The pulmonary arch was a markedly well-developed vessel in the adult and was continued as the pulmonary artery. Ductus arteriosus joined the pulmonary artery to the dorsal aorta.

No. 48:—The most median pair of ceratobranchials which were the representative of the fourth branchial arch were removed on August 23. Thus at the same time the fourth arterial arches running in them were extirpated. Then the complete removal of the cartilages was verified.

On September 3 the operated part was examined and it was ascertained that neither new-formation of the cartilage nor regeneration of the afferent portion of the arterial arch had occurred. The animal was still larval.

On September 9 the animal was found showing a marked tendency to swim to the surface of the water for air. General appearance approached metamorphosis. The larval pigment pattern had been sloughed; tail rounded, gill atrophied to the vestigial rods with dark pigmentation. On this day the animal was transferred from aquatic to a terrestrial environment.

The control animals, consisting of normal unoperated larvae kept in the laboratory, began the metamorphic processes which coincided with the above in time and the sequence of phenomena.

On September 19 No. 48 was fixed and the next day, was dissected in order to determine whether or not regeneration of the afferent portion of the pulmonary arch had been brought about. As shown in Fig. 1 such regeneration was not detected. The small distal remains of the right afferent vessel of the arch passed forwards and directly anastomosed to dorsal surface of the distal end of the third vascular arch.

The specimen was 47 mm. in length and had the adult vomerine teeth.

In normal unoperated controls which had completed metamorphosis in the laboratory, the pulmonary arch had attained a size as large as the rest.

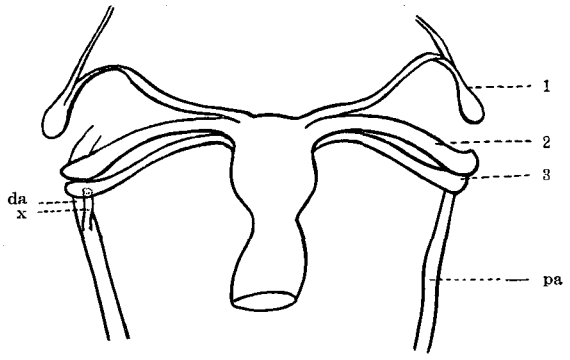

Fig. 1. Ventral view of the arterial arches in No. 48 experimental animal slightly diagrammatic. Left and right fourth arches were lacking.
x, direct anastomosis between pulmonary artery and third arterial arch; 1, 2, 3, first, second and third afferent branchial vessels; pa, pulmonary artery; da, ductus arteriosus.

Fig. 2. Metamorphosed experimental animal, No. 48. 47 mm. Photograph taken 16/IX/30.

The sequence of events of the metamorphic process above mentioned which was to be observed in the experimental animal had been established in the following individuals. Hereafter daily records during the growth of the animals will be omitted.

No. 50:—On November 26 the animal was fixed and the next day was dissected. The specimen was about 53 mm. in length. The stout hind limb had a well-developed fifth toe. Eyes, rather large and pro-

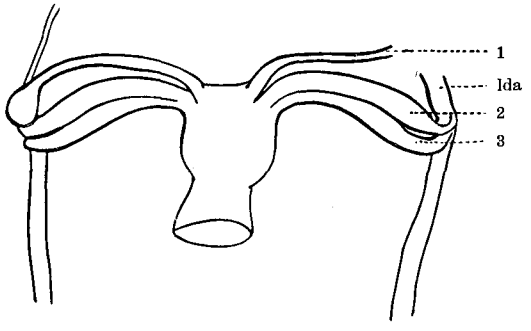

Fig. 3. Ventral view of the arterial arches in No. 50 slightly diagrammatic. Both fourth arterial (pulmonary) arches were lacking. lda, lateral dorsal aorta; 1, 2, 3, first, second and third afferent branchial vessels.

truding, were prominent. The vomerine tooth was well developed. In general, the animal was a typical, well-nourished young salamander.

The vascular arches of the specimen are shown in Fig. 3. The first arch has become a carotid. The second arch (systemic arch) and the third appear similar. The fourth is quite absent.

This condition clearly shows that the extirpation of the larval fourth arterial arch had ended in success and no regeneration had ever occurred.

No. 51 :—On December 7 the animal was fixed in 10% formalin as usual. This young salamander measured 62 mm. in total length.

In this case the right pulmonary arch had been regenerated. The regenerated vessel was as vigorous as the third.

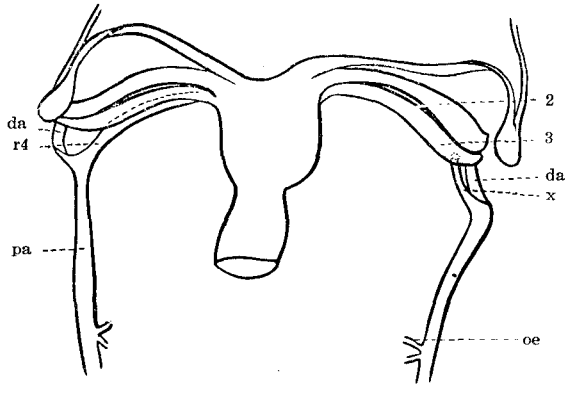

Fig. 4. Ventral view of the arterial arches in No. 51 slightly diagrammatic. Right afferent vessel of fourth arch has been regenerated (r 4). oe, oesophageal branch of pulmonary artery. (For lettering see fig. 1)

The left pulmonary arch was absent. From the junction whence the fourth continued as the pulmonary artery, a small vessel passed forwards and directly joined the distal portion of the third arch dorsally (Fig. 4).

No. 46 :—Fixed on December 16. The specimen was 62 mm. in length.

The right and left fourth afferent branchial vessels (pulmonary arches) were lacking. But on the right, arose a small anastomotic branch from the joint whence the fourth arterial arch was ordinarily continued as the pulmonary artery and opened into the distal portion of the right third afferent branchial vessel. On the other hand, from the similar junction of the left side a very small branch arose. It tapered off in surrounding tissue. These branches would be the remainder of the removed afferent vessel of the fourth arch (Fig. 5).

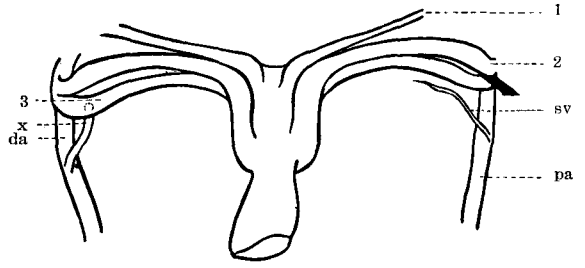

Fig. 5. Ventral view of the arterial arches in No. 46 slightly diagrammatic. Both fourth arterial arches were lacking. Left remnant is a small vessel (sv) tapered off in surrounding tissue. Right remnant is a short anastomotic vessel (x) between pulmonary artery and third arch. (For lettering see fig. 1)

No. 52:—Fixed on December 17. This young salamander measured 65 mm. in length.

The right pulmonary arch had been regenerated. The left was lacking. But direct anastomosis was found between the distal portion of the third aortic arch and the most anterior part of the pulmonary artery.

No. 53:—On March 15 of the following year this specimen had reached the length of 70 mm. On this day it was fixed in 10% formalin. It was about six months after the animal had completely metamorphosed.

The right pulmonary arch was lacking. From the joint whence the fourth arterial arch was continued as the pulmonary artery, arose an extremely small vessel and joined into the proximal part of the third arch dorsally.

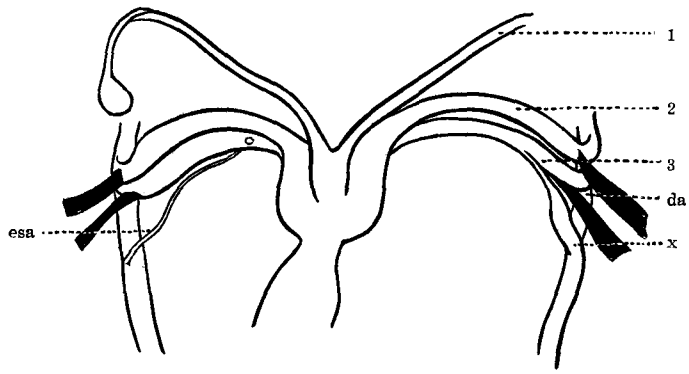

Fig. 6. Ventral view of the arterial arches in No. 53 slightly diagrammatic. Both pulmonary arches were lacking.
esa, extremely small anastomosis between pulmonary artery and proximal part of the third arch. (For lettering see fig. 1)

The left also had no pulmonary arch, but the direct anastomosis between the distal portion of the third arch and the most anterior part of the pulmonary artery was found. In this case the anastomotic vessel had gained a considerably large size (fig. 6).

No. 47 :—This young salamander was killed on March 16th, sixth months after it had been first put in terrestrial environment. It measured 60 mm. in length. Though rather small in comparison to No. 53, the animal was well-nourished.

The right pulmonary arch had been regenerated. The vessel seemed vigorous enough to do its function.

The left pulmonary arch was lacking. But, as was the case with the right side of No. 53, an extremely small anastomosis, which linked up the pulmonary artery with the proximal end of the third aortic arch dorsally could be detected.

Fig. 7. Fully metamorphosed experimental animal, No. 53. 70 mm. Photographed while under a chloretone anaesthetic on 13/III/31.

No. 49 :—On October 3 of 1930 this specimen was found quite dried up on the floor of the laboratory. The specimen had completed a terrestrial form. To the author's regret, the autopsy was impossible.

Discussion and Summary

From the results obtained and presented above, we find that the larvae of the present species of *Hynobius* metamorphosed quite normally in the actual time and the sequence of events of the morphogenesis, despite the total absence of the fourth branchial and arterial arches.

The complete removal of the fourth ceratobranchials with the vessels running in them was more reliable than the ligation of the fourth arterial arch performed by FIGGE in order to produce specimens in which aortic arches conform to the condition found in *Necturus*.

Since the foregoing experimental animals metamorphosed, FIGGE's conclusions regarding his ligation experiments that *Necturus* as well as the other perennibranchiates are larval forms because of the absence of the afferent portion of the pulmonary arch, are quite doubtful.

The results of the present study indicated strongly that the pulmonary arch was, morphologically speaking, not necessary for metamorphosis.

The results of the investigation are briefly as follows:

1. When the fourth ceratobranchials were removed entirely in the larval stage of fully grown external gills, new-formation of the cartilage did not take place. The transition of the hyobranchial apparatus from the larval condition to that of the adult was brought about normally.

2. The absence of the fourth arterial or pulmonary arch had no effect on the morphogenetic processes of amphibian metamorphosis.

3. The young salamanders without the fourth arterial arch developed normally.

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