

Significance of the Surrounding Tissues for the Development of the Limb-Rudiment in *Triturus pyrrhogaster* (BOIE)

By

Hiroshi TAKAYA

(Zoological Institute, Kyoto Imperial University)

With 4 figures in the text

(Received March 11, 1938)

The experiments so far carried out on embryos of the Amphibian Urodele, give information of the significance of the influence exerted from outside on the developing limbs, especially for the establishment of their symmetry relationship. Evidence is gathered by grafting a rudiment to the heterotopic position of the body, with the subsequent appearance of reduplicated structure, or change of asymmetry, or rotation of the grafted limb as a whole. Concerning the last phenomenon, NICHOLAS (1924) shows the direct surrounding to be primarily significant for the control of the posture of the developing limb. As to the other phenomena, the question is open whether they are also brought about by the influence of the immediate surrounding, or by an effect from other portions of the body, or by the combination of the two. The present experiments, undertaken at the suggestion of Prof. Yô K. OKADA and carried out under his supervision from May to June, 1936, sought an answer to this question. Before going any further, I wish to express my hearty thanks to Prof. Yô K. OKADA for his kind guidance and encouragement throughout the course of the work.

The red-bellied newt, *Triturus pyrrhogaster*, which is the most common in the vicinity of Kyoto, was used as material. Owing to the difficulty involved in replacing the tissues surrounding the limb-disc without disturbing the disc itself, double operations were performed: First in embryos in the tail bud stage a circular disc, the centre of which was occupied by the presumptive limb-rudiment, was cut off about five somites in diameter, and replaced by tissues from such a portion of the body as the flank, or the abdomen (see fig. 1). When the wound was completely healed after this procedure, the limb-disc three somites in diameter was transplanted from another embryo into the prior graft so as to occupy the regular limb-position with

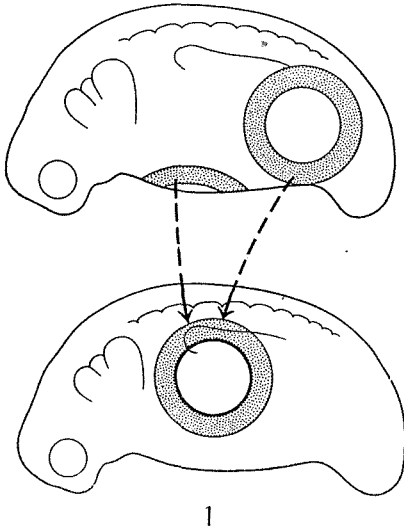


Fig. 1. Diagrammatic representation of the mode of transplantation.

respect to the embryo as a whole. Replacement in the first operation was made in varied orientations with respect to the body, but the limb-rudiment in the second operation was placed always in normal orientation except in one case where disharmonic orientation was given. In this manner the axial relation between limb-rudiment and surrounding tissues was examined in five different combinations; they are indicated in table 1.

In all the operations most of the grafted limbs developed; however, absorption of the grafts occurred in a few cases in each group of the operations (ref. tab. 2). Reduplication or other irregularities of limb-structure were often met with, in spite of the harmonic orientation with which the

Table 1

Series	Grafting of the flank tissues		Series	Grafting of the abdominal tissues	
	Orientation of the tissues	Orientation of the limb-disc		Orientation of the tissues	Orientation of the limb-disc
NES	<i>hom aa dd</i>	<i>hom aa dd</i>	NEB	antero-anterior (<i>aa</i>)	<i>hom aa dd</i>
NESd	<i>hom ap dv</i>	<i>hom aa dd</i>	NEBd	antero-posterior (<i>ap</i>)	<i>hom aa dd</i>
			NEBh	antero-anterior (<i>aa</i>)	<i>het ap dd</i>

Table 2

Experiment	Operation	Survived	Single limb	Double limb	Absorbed
A) Grafting of the flank tissues					
NES	8	7	—	6	1
NESd	8	8	4	3	1
B) Grafting of the abdominal tissues					
NEB	12	9	5	2	2
NEBd	8	4	3	—	1
NEBh	14	8	3	4	1

grafted rudiment was placed. Frequency of the reduplication and degree of the doubleness were not necessarily uniform throughout all the operations: They varied according to the different orientations of the surrounding tissues, as well as to the different positions of the body where the graft originated. These facts apparently manifest the influence of the tissues experimentally applied surrounding the limb-disc, and details will now be given.

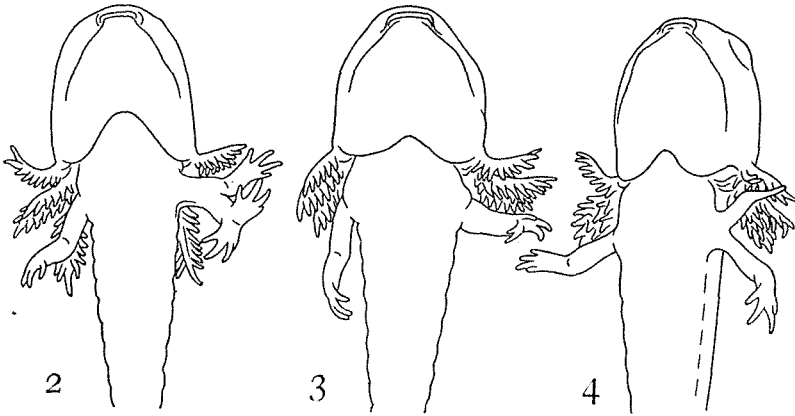


Fig. 2. Reduplicated limb in the harmonic transplantation of the flank tissues (series NEB)

Fig. 3. Irregular formation of limb in the disharmonic transplantation of the flank tissues (series NESd)

Fig. 4. Limb reduplication in the disharmonic orientation of the rudiment enclosed by the harmonically placed abdominal tissues (series NEBh)

When the surrounding area of the limb-disc was replaced by the tissues of the body side, centred about five to six somites posterior to the limb-site, both limb and tissues being placed harmonic to the body (series NES), reduplication took place in all cases so far as any limb was produced. Limbs were divided generally from the proximal portion. Fig. 2 represents one of the specimens in which the ulnar and the palmar mirrorings were clearly established between reduplicants. Other mirrorings such as the radial or the radio-palmar were also met with, but irregularities of the limbs were found only in exceptional cases. On the other hand, if the same tissues were grafted so as to be disharmonic to the body side as well as to the limb-disc (series NESd), a single limb was produced in about half of the successful cases (see tab. 2). Where reduplication occurred, it was limited to the distal portion of the limb, showing more or less irregularity in the whole limb structure (fig. 3). Moreover, the reduplicated limbs were always less developed than those of the preceding series of experiments.

Replacement of the surrounding by the tissues of the mid-ventral region of the body with the secondary transplanted limb-rudiment in the normal

orientation in the centre, resulted in no aberrancy in the limb-growth whether the replacement of the surrounding was done in the antero-anterior (series NEB) or in the antero-posterior (series NEBd) direction. The limbs were always single and normal except in two cases in the first orientation, where a stunted process was found at the radial border of the limb normally developed (ref. tab. 2). Whereas in the antero-anterior orientation of the abdominal tissues reduplication was frequently met with when the secondary transplanted limb-disc was oriented to be disharmonic to the body side (series NEBh). In these cases members of the reduplicated limbs were arranged in the antero-posterior direction. The anterior members were more or less deficient in the distal portion, but assuming a radial mirroring of the posterior members which were always normal and harmonic to the body side (fig. 4).

Microscopic study of some of the specimens showed that the girdle of the operated embryos was always small, being deficient in the peripheral portion. Deficiency was particularly marked in the suprascapula and in the ventral tip of the coracoid. The central portion of the coracoid including the glenoid cavity was the only one commonly found in all of these specimens. According to DETWILER (1918), HARRISON (1918) and others, such incomplete development of the girdle is usual in the heterotopic transplantation of the rudiment. From these facts it can easily be recognized that the influence of the replaced tissues is so strong that the development of the rudiment, even in the regular limb-position, becomes altered just as it would be if it were in an abnormal position.

In the flank region of the body, it is well established by HARRISON (1921) and others that the harmonic combination of the limb-rudiment to its surrounding produces a slight preponderance of reduplications, whereas the disharmonic combination gives a great preponderance of single limbs. The same rule holds true in the results of the present experiments where the limb-rudiment, instead of being grafted to a heterotopic position, is enclosed in its normal position by the substituted tissues, taken from the body side. It seems, therefore, natural at least in this comparison, to recognize the important effect of the immediate surrounding upon the mode of limb-growth.

According to NICHOLAS (1924), a pair of limbs result from a single limb-disc transplanted into the mid-ventral region. He regards this as the typical result of the influence which issues from so peculiar a position as the ventral mid-line. In our cases in which the surrounding of the limb-disc was replaced by tissues of the region corresponding to that into which NICHOLAS transplanted his graft, limbs were single if its orientation was normal with respect to the embryo as a whole. Even if reduplication took place as in the disharmonic orientation of the secondary transplanted limb-disc (series NEBh), two components which were produced, were arranged antero-posteriorly (fig. 4) and not dorso-ventrally as indicated in NICHOLAS' figure. The

results obtained were apparently the same as those found by HARRISON (1921) and others in the orthotopic transplantation of a limb-rudiment; the replacement of the surrounding by the tissues from the abdominal region manifests, therefore, no peculiar influence upon the mode of the limb-growth. Nevertheless, it was noted by the replacement of the abdominal tissues and also by that of the flank tissues that the development of the limb behaved somewhat differently when in the normal surrounding. Postural regulation of the developing limb by means of rotation did not occur in any case of the present experiments. Absorption of the grafted rudiment practically never occurred in the orthotopic transplantation, whereas it did occur, though not frequently, in each series of these experiments (ref. tab. 2).

From the facts so far enumerated, we may come to the following tentative conclusion: The developing limb is quite sensitive to influences from without. The mode of its development is much influenced by the nature of the immediate surrounding. The effect of the less immediate portions, and of the constitution of the body as a whole, comes out only when the influence of the direct surrounding is weak or weakened.

Literature

- DETWILER, S. R. 1918. Experiments on the development of the shoulder-girdle and the anterior limb of *Amblystoma punctatum*. *J. Exp. Zoöl.*, vol. 25.
- HARRISON, R. G. 1918. Experiments on the development of the fore limb of *Amblystoma*, a self-differentiating, equipotential system. *J. Exp. Zoöl.*, vol. 25.
- , 1921. On relations of symmetry in transplanted limbs. *J. Exp. Zoöl.*, vol. 32.
- MANGOLD, O. 1929. Das Determinationsproblem. Zweiter Teil, Die paarigen Extremitäten der Wirbeltiere in der Entwicklung. *Ergeb. d. Biol.*, vol. 5.
- NICHOLAS, J. S. 1922. The effect of the rotation of the area surrounding the limb bud. *Anat. Rec.*, vol. 23.
- , 1924 a. The response of the developing limb of *Amblystoma punctatum* to variation in the orientation of the surrounding tissue. *Anat. Rec.*, vol. 29.
- , 1924 b. Regulation of posture in the fore limb of *Amblystoma punctatum*. *J. Exp. Zoöl.*, vol. 40.
- SWETT, F. H. 1926. On the production of double limbs in Amphibians. *J. Exp. Zoöl.*, vol. 44.
-