MEMOIRS OF THE COLLEGE OF SCIENCE, UNIVERSITY OF KYOTO, SERIES B.
Vol. XX, No. 1, Article 3. 1951.

Studies on Situs Inversus Viscerum in Amphibia. II. Production of S. I. V. through extirpation or exchange of parts of the neural plate.

By

Hirosi TAKAYA

Zoological Institute, University of Kyoto.

(Received May 12, 1951)

In Amphibia the twisting of digestive tract and of heart may be reversed by means of transplanting a square piece of the neural plate in reversed orientation. This was first found by SPEMANN (1906) and later confirmed by MEYER (1913). Also the author (1944) repeated the same experiment on Japanese salamander and frogs with similar results. From this experience, however, the author was suggested that there might be other ways than the reversed transplantations of the neural plate to bring about the visceral reversion. This supposition was tested by means of extirpation and exchange of parts of the neural plate. These operations were found effective to produce the reversed viscera, and the results will be given in the following. As for the materials, embryos of *Triturus pyrrhogaster*, of *Hynobius nebulosus* and of *Rana japonica* were employed.

A. Extirpation of the neural plate.

The operation was to cut out a rectangular piece of the neural plate about one third of its total length. This was done at the middle of the plate. As this area of the plate is narrower, the neural fold was also cut off in most cases of the operation. It goes without stating that not only the neural ectoderm but also the underlying roof of the archenteron was removed. Healing of the wound was easy and complete in embryos of *Triturus*, but was incomplete in *Hynobius* and *Rana*. The extirpation was tested in two different ways, that is, either in the whole width or in the half width of the neural plate.

a) *Extirpation in whole width.* By the extirpation, reversion of the visceral organs was actually brought about. This was common in Urodela and Anura, and the results are given in Table I. Perfect reversion of the visceral organs was met with in 3 cases out of 37 successful operations (8%) in *Triturus*, in 3 cases out of 20 (15%) in *Hynobius* and in 1 case out of 12 (8%) in *Rana*. These frequencies of occurrence were a little lower as compared with those obtained in the reversed transplantation of the neural plate. They were, as reported in a former paper (TAKAYA, 1944), 31 per cent with grafts of whole width

and 17 per cent with grafts of half width of the plate in *Triturus* embryos. In the present experiments, there occurred also abnormalities which are shown in the accompanying table as partial or imperfect reversal. Those cases where the twisting of the heart is normal while that of the digestive tract is reversed, or the contraly, cases where the twisting of the heart is reversed and the digestive tract is normal, are briefly designated as partial reversal. On the other hand, there were other cases where the visceral organs showed no asymmetrical arrangement. Presumably this is brought about by imperfect reversion, and the cases are called imperfect reversal. In such cases, the stomach and the liver did not stand side by side, but were piled up dorso-ventrally, and the intestine was streched out straightly without bending in any direction. Also the heart, in most of these cases, was reduplicated probably on account of failure of the union of both sides of the rudiments. In some of these reduplicated hearts, the twisting of respective members was in the opposite direction with the result of producing enantiomorphism. But frequently either one of the two hearts, mostly the right one, was underdeveloped to be deficient in structure. Partial reversion was met with in *Hynobius*, whereas imperfect reversion occurred quite often in *Triturus* and in *Rana* (see Tab. I, A).

Inasmuch as these abnormalities are scarcely met with in nature, they are duely referable as brought about by the operation. When we consider these cases together with those of the perfect reversion as manifesting influence of the operation, frequency of the influence amounts to 32 per cent (12/37) in the case of *Triturus*, to 50 per cent (10/20) in *Hynobius* and to 75 per cent (9/12) in *Rana*. These values are equal or even higher than those obtained in the reversed transplantation of the neural plate. In the latter operation of the author (TAKAYA, 1944), influence was observed in 32 per cent in *Triturus* and in 28 per cent in *Rana*. In the other respects, there was no particular difference noticed in the results between the two operations mentioned above. It is apparent, therefore, that the extirpation of a rectangular piece of the neural plate exerts the same influence as in the transplantation of similar piece in reversed orientation. If this is correct, it emerges that the reversed piece of the neural plate cannot be considered as the seat of a cause to produce visceral reversion, because thre is no piece reversed in the case of the extirpation. The true cause of the occurrence must be concealed in a condition common to the both operations.

Other organs besides the viscera were also influenced by the operation. However, the degree and extent of the influence appeared to have no intimate connection with the visceral reversion. There were cases where visceral reversion occurred while other parts of the body were quite normal, or conversely, where viscera were normal notwithstanding extensive abnormalities of the other parts. Under these conditions, it

Table 1. Results of extirpation of parts of the neural plate.

Stage of embryo	Available cases	Perfect reversion	Partial reversion: cordis normal viscerus reversed	Partial reversion: cordis reversed viscerus normal	Imperfect reversion	Percent
A. Extirpation in whole width.						
a. *Triturus pyrrhogaster*						
15	4	-	-	-	2	50
16	5	1	-	-	2	60
17	12	1	-	-	2	25
18	8	-	-	-	2	25
17-18	8	1	-	-	1	25
Total	37	3	-	-	9	32
b. *Hynobius nebulosus*						
17-18	20	3	2	5	-	50
c. *Rana japonica*						
	12	1	-	-	8	75
B. Extirpation in half width.						
a. *Triturus pyrrhogaster*						
Right	40	3	-	-	3	15
Left	36	2	-	-	-	6
Total	76	5	-	-	3	11
b. *Rana japonica*						
Right	14	-	1	1	2	29

may be conjectured that the irregularities of viscera and of the other parts of the body, though brought about by a single operation, are realized through mechanisms quite different from one another.

b) *Extirpation in half width.* The operation was the same as in the preceding experiments except that the size of a piece extirpated was small, being either right or left half of the neural plate. By the diminution of the extirpated area, operative injuries appeared to be much decreased in the external. Especially in *Triturus* most embryos were normal or nearly normal in their external appearance. Internally, however, influence of the operation was not necessarily diminished. Among 76 operations in *Triturus* perfect reversion of the viscera was realized in 5 cases, 2 of them belonging to the left-side operation and the other 3 to the right-side operation (cf.

Tab. 1,B). Also in these experiments. imperfect reversion was met with in 3 out of 40 operations on the right side. To sum up, extirpation of the half plate showed influence in 8 cases out of 76 (11 %). Although this value is a little lower as compared with those obtained in the preceding operations, the resultant structures of the viscera were quite similar and no peculiarity was noticed in other respects. On embryos of *Rana* the operation was tested merely on the right side. In this case perfect reversion was realized in no specimen, but partial reversion was found in 2 specimens and imperfect reversion in 2 out of 14 operations. From the results enumerated above, it becomes apparent that the occurrence of the reversed viscera is attained also by extirpation of merely half the width of the neural plate, either right or left half being found to have the effect.

B. Exchange between right and left halves.

In the same area of the neural plate where the extirpation was carried out, exchange was tried between right and left halves. This was made by replacing the right half with the left one and vice versa. The replaced pieces of the plate were placed either in normal or in reversed orientation. Results of the experiments are summarized in Table II. Also in this case

Table II. Results of exchange between right and left halves of the neural plate.

Operarion	Orientation	Available cases	Perfect reversion	Partial reversion cordis normal viscerus reversed	Imperfect reversion	Percent
a. *Triturus pyrrhogaster*						
R→L	normal	10	3	-	2	50
	reversed	13	3	-	1	30
L→R	normal	10	2	-	3	50
	reversed*	18	2	2	2	33
Total		51	10	2	8	40
b. *Rana japonica*						
R→L	normal	14	-	-	2	14
	reversed	13	1	-	6	55
L→R	normal	14	1	-	1	14
	reversed	13	-	-	2	16
Total		54	2	-	11	24

R→L : Replacement of left half with right one.
L→R : Replacement of right half with left one.

the operation was effective to produce visceral reversion, and it was realized in 10 out of 51 specimens (20%) of *Triturus* and 2 out of 54 specimens (4%) of *Rana*. These frequencies were a little higher than those obtained in the preceding operations, apparently indicating that the replacement of the half plates, like the reversed transplantation, bears a more powerful influence than the mere extirpation. In this experiment it was further noted that no marked difference was brought about in the result by altering the orientation of the replaced pieces and the side of the neural plate on which the operation was carried out. By replacing the right half with the left one, the operated area of the plate became to be composed of the materials merely of the left side, and conversely, the plate consisting of the materials of the right side alone was produced by replacement of the left half with the right one. In these two oparations, in spite of such a striking difference of the materials composing of the neural plate, the effect to produce visceral reversion was quite similar (cf. Table II). From this fact, it may be surmised that there may be no qualitative difference between the right and left sides of the neural plate.

Discussion

The present experiments together with those reported in former occasion (TAKAYA, 1944) disclosed that the neural plate including the underlying roof of the archenteron bears significance for the reversion of visceral organs. Actually, however, the visceral reversion was realized by such operations upon the neural plate as reversed transplantation, extirpation, and exchange between right and left halves. Between the extirpation and the reversed transplantation, there may be no factor common as is referable to the cause of the visceral reversion. The fact that the same effect is exerted also by exchange of the half-plates, appears to complicate further the solution of the problem. Under these conditions, we are inclined to consider that a particular part of the neural plate or a particular condition given by the operation should not be consulted, but general injuries of the operation may be referable as a cause. In this connection, further it is mentioned that, all through the experiments on the neural plate, the occurrence of the visceral reversion was in comparatively low frequencies. Excepting few operations where the reversion was realized a little more than 50 per cent, frequencies were within the range between 10 to 30 per cent, the average being 33 per cent in *Triturus* and 34 per cent in *Rana*. These values are considered too low to assune that the neural plate alone is the seat of a factor or factors to bring about the visceral reversion. It may be presumed that there exist regions other than the neural plate which have influence upon the visceral organs, and that the neural plate as one of the members takes part in the occurrence of reversion.

In the experiments with the half-plates, operation was effective on

either side of the plate. As pointed out in the previous paper (TAKAYA, 1944), this was also a rule in the reversed transplantation of half-plates. Concerning the frequency of occurrence, however, results were not necessarily the same between the sides of operation. In the reversed transplantation on *Triturs* reversion took place in 20 per cent with right halves and in 40 per cent with left halves, and on *Rana* it was in 25 per cent with right halves and in 9 per cent with left halves (cf. TAKAYA, 1944). In the case of extirpation experiments on *Triturus*, reversion was found in 15 per cent on the right side and in 6 per cent on the left. From these results, it is impossible to decide which side of the neural plate is more effective to produce visceral reversion. On the other hand, the results of exchange between right and left halves clearly indicate that the operation on either side has the same effect. We are inclined, therfore, to consider that as far as the occurrence of visceral reversion is concerned, no difference exists between right and left sides of the neural plate. On the contrary, many authors who have reference to this problem, maintain the existence of a qualitative difference between the two sides of the body (SPEMANN, 1919; WILHELMI, 1921; GOERTTLER, 1928; HUXLEY and de BEER, 1934; LUDWIG., 1932: ZWANZIG., 1938). Concerning the cause of this qualitative difference, however, nothing is pointed out by any one of these authors. Under these conditions, it may be claimed that, at least within the neural plate, no such difference exists between right and left sides. Whether this difference exists in the other part of the body or not is still a question to be solved.

Literature

GOERTTLER, K. 1928. Anat. Anz., *66*.
LUDWIG, W. 1932. Rechts-Links-Problem., Berlin.
MORGAN, Th. 1927. Experimental Embryology., New York.
SPEMANN, H. and FALKENBERG. H. 1919. Roux' Arch., *45*.
TAKAYA, H. 1944. Zool. Mag., *56*.
TAKAYA, H. 1949. 実験形態学, *5*.
WILHELMI, H. 1921. Roux' Arch., *48*.
ZWANZIG, H. 1938. Z. wiss. Zool., *150*.