

Studies on Situs Inversus Viscerum in Amphibia  
V. Production of S. I. V. following the replacement  
of the presumptive mesoderm

By

**Hiroshi TAKAYA**

Zoological Institute, University of Kyoto

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The fact that the reversion of the visceral asymmetry is brought about by means of an operation done to the presumptive mesoderm of gastrula, was shown by the previous experiment of the author (Takaya, '52). In that experiment the lateral mesoderm of gastrula was extirpated or transplanted in reversed orientation. It was also found out that the operation was successful in producing reversed viscera no matter which side of the body was tried. In other words, there was no apparent tendency to indicate the operation of which side was more effective. However, whether there is really no difference between operated sides of embryo still remains to be ascertained, because there are many authors who maintain, concerning the occurrence of the visceral reversion, preponderant influence of the left side over the right (Huxley and de Beer, '34; Ludwig, '32; Wilhelmi, '21; Zwanzig, '38). In the present experiment, in order to test the verity of the question, replacement of the presumptive mesoderm was carried out. The replacement was made between left and right, and between lateral and median areas. Also replacement of the mesoderm between embryos in different developmental stages was examined. In all these operations the grafted piece of mesoderm was always placed normally with respect to its anteroposterior direction. As for materials, embryos of *Triturus pyrrhogaster*, *Hynobius nebulosus*, *Rana nigromaculata* and *R. japonica* were employed.

**I. Replacement of mesoderm between opposite sides of embryos.**

In embryos of middle gastrulae a rectangular piece of the presumptive mesoderm was cut out close to the blastopore and was replaced by the mesoderm of the opposite side. This was done in the direction either from the left side to the right or from the right to the left. The operated piece of the mesoderm normally underwent invagination except in a few cases in which retarded invagination took place resulting in an imperfect closure of the blastopore. In consequence, there were embryos which showed abnormalities in the posterior half of the body. But extent and degrees of the abnormalities

brought about by the present operation, were generally slight as compared with those obtained in the earlier experiments in which extirpation or transplantation of the mesoderm was examined (Takaya, '52). The present operation had an effect in producing the reversed viscera in embryos of *Triturus*, *Hynobius* and *Rana nigromaculata* (ref. Tab. 1). As found in earlier experiments, the visceral reversions produced this time were either perfect or imperfect, but the perfect reversion was found more frequent. In the imperfect reversion the visceral organs were symmetrical without presenting asymmetry in any direction. But there was one exception in which the endodermal organs, i. e., digestive tracts were reversed, while the mesodermal organ, i. e., the heart remained normal (ref. Tab. 1). Considering the fact that the present operation was confined to the area of the presumptive mesoderm, this specimen is peculiar in that the reversed organs were all those of the endodermal origin and the mesodermal organ remained unreversed.

Table 1 Results of the replacement of mesoderm between left and right sides.

Operation	Stages	Available cases	Visceral reversion		%		Total
			Perfect reversion	Imperfect reversion	Perfect reversion	Imperfect reversion	
A. <i>Triturus pyrrhogaster</i>							
L → R	11~13	30	3	(1)+⊕	10	6	17
R → L	11~13	28	6	(-)+⊖	21	-	21
Total		58	9	(1)+⊕	14	3	19
B. <i>Hynobius nebulosus</i>							
L → R	12	14	-	(2)+⊖	-	14	14
R → L	12	11	1	(2)+⊖	9	18	27
Total		25	1	(4)+⊖	4	15	20
C. <i>Rana nigromaculata</i>							
L → R	11	5	-	(1)+⊖	-	20	20
R → L	11	5	-	(-)+⊖	-	-	-
Total		10	-	(1)+⊖	-	10	10

L → R represents the replacement of the left mesoderm by the right one and R → L the inverse replacement. Numerals within parentheses indicate cases where, symmetrical disposition of the visceal organs is found, and those within circle, cases where the digestive organs are reversed and the heart is normal.

In the present experiment occurrence of the visceral reversion was found, on an average, in 20 per cent in urodeles and in 10 per cent in anuran (ref. Tab. 1). This percentage is rather low as compared with that already obtained in the case of extirpation and transplantation of the mesoderm (ref. Takaya, '52). Probably exchange of the mesoderm between opposite sides of embryo has an influence which is not so powerful as found in the case of extirpation or transplantation. However, there was some difference in the rate of reversion between the two sides of embryo on which the operation was carried out. The left-side operation on *Triturus* and *Hynobius* produced reversions a little more frequently than the right-side operation. But, as is shown in Table 1, the difference was not so great that it seemed too difficult to make this conclusion.

## II. Replacement of mesoderm between lateral and median areas.

On *Triturus* embryos of middle gastulae, the lateral area of the presumptive mesoderm was replaced by the median area, or conversely the median area by the lateral one. The median area used in this experiment corresponds to the chorda-mesoderm, i. e., the organizer. In spite of this fact, the embryos developed were normal or nearly normal externally, and there was none in which reduplication of the axial organs was brought about. Internally,

Table 2 Results of the replacement of mesoderm between median and lateral areas.

Operation	Stages	Available cases	Visceral reversion		%		Total
			Perfect reversion	Imperfect reversion	Perfect reversion	Imperfect reversion	
A. Replacement of the lateral area by the median							
M → L	13	14	4	(1)+[-]	28	7	36
M → R	13	14	1	(1)+[1]	7	14	21
Total		28	5	(2)+[1]	18	10	29
B. Replacement of the median area by the lateral							
L → M	13	13	5	(1)+[-]	38	8	46
R → M	13	9	4	(1)+[-]	44	11	55
Total		22	9	(2)+[-]	40	9	50

M → L represents the replacement of the median area by the left-side mesoderm and M → R the one by the right-side mesoderm. Numerals within smooth parentheses indicate cases of the symmetrical disposition of the visceral organs, and those within angular parenthesis, cases where the digestive organs are normal while the heart is reversed.

however, reversion of the visceral asymmetry was taking place. This occurrence was found common in the two replacements examined. In the replacement of the lateral mesoderm by the median one, the left-side operation produced more reversions than the right-side one (ref. Tab. 2A). In the inverse replacement the operation was done on the median area of embryo, and there was no disparity given as regards the left or the right of the embryo. Nevertheless, the reversions were brought about in comparatively high rates, and moreover, the rates were variable according to the kind of mesoderms replaced. The replacement with the right-side mesoderm produced more reversions than that with the left-side one (ref. Tab. 2B). From this result only, a preponderant influence of the right-side over the left-side one may be assumed. But this assumption is in apparent contradiction with the result of the other replacement in which the left-side operation, that is, the replacement of the left-side mesoderm by the median one is shown to be more effective. Thus the present experiment has failed to give any clue to the difference of the flank of embryo.

### III. Replacement of mesoderm between embryos of different developmental stages.

The lateral mesoderm of the middle gastrulae was replaced by the corresponding area of the late gastrulae, or conversely the mesoderm of the late gastrulae by that of the middle gastrulae. The replacement was always done on the same side of embryos, but the operation was tested on either side. As a result of this replacement topographical relation of the mesoderm with respect to its prospective fate may change, but there is no change as to the placement of the left and the right of the body. In this replacement reversion of the visceral asymmetry occurred. The occurrence was more frequent in the replacement of old mesoderm by young one than in the inverse replacement. On the other hand, rates of the occurrence changed according to the difference of the sides of embryo on which the operation was carried out. In the replacement of older mesoderm by younger one the left-side operation yielded reversions as many as 88 per cent of the operated specimens, whereas in the right-side operation reversions were found in only 33 per cent (ref. Tab. 3A). However, it was different in the inverse replacement. In this case no marked difference was noted between the operations of left and right, the rates obtained being similar in either case (ref. Tab. 3B). From these results of the experiment, it is impossible to assume any difference of the influence of the body-sides upon the production of the visceral asymmetry. On the contrary, it was revealed that the age difference between replaced mesoderm and the host one was rather important in the act of producing the visceral reversion.

Table 3 Results of the replacement of mesoderm between different development stages.

Operated side	Stages	Available cases	Visceral reversion		%		Total
			Perfect reversion	Imperfect reversion	Perfect reversion	Imperfect reversion	
<i>Triturus pyrrhogaster</i>							
A. Replacement of old mesoderm by younger one							
Left	12→13	8	6	(1)+[-]	75	12	88
Right	12→ <sup>13</sup> / <sub>14</sub>	12	3	(1)+[-]	25	8	33
Total		20	9	(2)+[-]	45	10	55
B. Replacement of young mesoderm by old one							
Left	13→11	16	3	(1)+[-]	19	6	25
Right	13→11	11	2	(1)+[-]	18	18	27
Total		27	5	(2)+[-]	18	7	26
<i>Rana japonica</i>							
Replacement of old mesoderm by young one							
Left	11→13	7	-	(3)+[1]	-	57	57

Numerals within smooth parentheses represent cases of the symmetrical disposition of the visceral organs and those within angular parenthesis, cases where the digestive organs are normal while the heart is reversed.

#### IV. General remarks and conclusions.

In the present experiment, replacement of the presumptive mesoderm was examined in three different ways. All the replacements examined were found to be effective in producing the visceral reversion. Therefore, the present result demonstrates the importance of the presumptive mesoderm to the production of the visceral asymmetry, substantiating the conclusion already arrived at in the preceding experiment of the author (Takaya, '52). Regarding the influence of the replaced mesoderm, however, results of the experiment were not necessarily the same. As represented by different rates of the visceral reversions, different operations seemed to give different effects. Of all the operations done, the replacement of the mesoderm between embryos of different developmental stages was found to be the most effective, while the replacement between opposite sides of the body to be the least effective in producing the reversion. Inasmuch as the replacement between mesoderms of different developmental stages was made on the same side of the body, it may be stated that the age difference of the replaced mesoderm

bears stronger influence on the production of visceral reversion than the change of the body-sides. As regards the age difference in question, Goerttler ('28) has shown that the lateral mesoderm of the left side is, in the normal course of development, a little advanced as compared with that of the right side. But the present experiment has disclosed that it is merely the age difference that is significant, the replacements either by older or younger mesoderms being found to have influence in producing the reversion. Therefore, no apparent connection seems to exist between the present result and the result of Goerttler.

In some of the operations carried out in this experiment, there was a noticeable difference between the operations of the left side and those of the right side. But the difference was hardly noted in the other operations. Under the circumstances, it is difficult to find out constant tendency which is issued from the body-sides on the production of the visceral reversion. The present experiment has shown that the replacement bears effect even when it is carried out on the median area of the body. The replacement on the median area proved more effective in producing reversions than the replacement on the lateral area. From these results we are inclined to conclude that, as far as the mesoderm is concerned, no definite difference between the right and left sides exists to produce the visceral reversion.

### Literature

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