

ON RELATIONS OF SYMMETRY IN TRANSPLANTED LIMBS

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ONE HUNDRED AND THIRTY-SIX FIGURES

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INTRODUCTION

The circumstance that originally suggested the present study was the apparent difference in the results obtained by Streeter ('07) and by Spemann ('10) in their respective experiments with the amphibian ear vesicle. According to the original account of Streeter, the otocyst, when taken out of an embryo just after closure and replaced after having been rotated 180° on any of its axes, develops in normal posture, though a right vesicle placed on the left side remains true to its side of origin. According to Spemann, the inverted vesicle develops in inverted position, the rudiments of the constituent parts being localized at the time of operation. Although subsequent work by Streeter ('14) seems to have shown that the normal development of the inverted vesicles, found in his cases, was due to their regaining normal posture by rotation as a whole, the original divergence of results nevertheless had raised theoretical questions of great interest, which Spemann has ably discussed. The main question was whether we might have in the otic vesicle an 'harmonic equipotential system' with its future asymmetry in some way stamped upon its intimate structure. Though Spemann's analysis answers the question in the negative, as far as the closed ear vesicle is concerned, it is nevertheless important to determine how far, if at all, systems of this kind are present in the embryo, for their study would throw light upon the question of the mode of representation of adult form characters in the germ, giving evidence from a new quarter with regard to the great problems of development which have usually been approached by way of experiments upon the unsegmented egg and the early stages of cleavage. The method of embryonic transplantation obviously

affords a means of studying this question in any organ or part that in the adult lacks a plane of symmetry. It was with this purpose in view that the present experiments with the limbs of *Amblystoma* were begun. Limb buds were implanted in both normal and abnormal location, oriented in various ways with respect to the main axes of the embryo-host, and the form and posture of the resulting limbs were studied.

It became evident, after the first experiments were made, that the rudiment of the fore limb behaved differently from the auditory vesicle, no matter whether Streeter's original interpretation or Spemann's was accepted as correct. While it was found that a certain tendency did exist for inverted limb buds to rotate back to normal posture during development, this was not the usual result, nor did the rotation take place in the sense meant by Streeter in his later publication ('14). Furthermore, many irregularities of development were produced by the operations, due largely to the power of the limb rudiment to duplicate itself by budding.¹ On the other hand, it often occurred that buds transplanted from one side of the body to the other developed in harmony with their new surroundings, a right limb bud, for example, placed on the left side, giving rise to a normal left limb.

The earlier experiments which were made in 1911 and 1912 led to no satisfactory general conclusion, so that publication of the work was deferred pending further investigation. Subsequently numerous additional experiments were made, in which more effective precautions against regeneration of the limbs from the host were taken.² The situation began to clear when in some of the cases in which the asymmetry of the limb was reversed by

¹ Cf. Barfurth ('94), who showed that supernumerary limbs could be produced in amphibians by regeneration after irregular amputation; Tornier ('05), who obtained multiple appendages by cutting into the limb bud of tadpoles; Braus ('04, '05, and '09) and Harrison ('07), who found that transplanted limb buds frequently give rise to double limbs.

² The first experiments were reported to the National Academy of Sciences in November, 1912, at the New Haven meeting. Later reports were made before the American Association of Anatomists in December, 1915 ('16), and before the American Society of Zoologists in the following year ('17 a). The main results have been stated somewhat more fully in the Proceedings of the National Academy ('15 and '17 b).

transplantation it was observed that the reversal came about by a process of reduplication or twinning. By following closely the history of individual cases, it became evident that the double formation was not infrequently obscured by the preponderance of the reduplicating limb bud over the original, so that the former grew into a member of opposite asymmetry, while the original bud was reduced to a mere spur or nodule, which might readily be overlooked. The tendency to produce duplicities thus proved to be even greater than the actual number of fully developed cases indicated. In other cases the reversal appeared to be more direct; at least, a limb of the side of origin often failed to appear as such on the surface, though slight irregularities in the early stages of development, coupled with an appreciable delay in the process, showed that some internal readjustment of the grafted tissue was taking place.

It seemed that the functional activity of the limb, when transplanted to its normal environment, might accentuate the apparently anomalous results just described. In order to eliminate this factor, a series of experiments in which the limb bud was grafted on some other part of the body was undertaken. Here the proper nervous connections did not become established, functional activity was usually lacking or was at best but slightly developed, and the undisturbed effect, upon development, of the relative orientation of the tissues of graft and host could be observed. The latter experiments led to the formulation of the three following simple rules,³ which hold for implantations either in normal or in abnormal location:

Rule 1. A bud that is not inverted (dorsodorsal) gives rise to a limb of the side of origin of the bud, whether implanted on the same or on the opposite side of the body.

Rule 2. An inverted bud (dorsoventral) gives rise to a limb of reversed asymmetry, whether implanted on the same or on the opposite side of the body.

Rule 3. When double limbs arise, the original one (the one first to begin its development) has its asymmetry fixed in accord-

³ These rules were phrased somewhat differently in two preliminary communications ('17 a and '17 b).

ance with rule 1 or 2, while the other is the mirror image of the first.

Experiments previously reported⁴ have shown that the limb bud is an 'harmonic equipotential system,'⁵ and additional experiments with inverted buds (p. 87) and with half buds (p. 83) confirm this result. We must assume, then, that the potencies of the cells of the limb bud to form the fore limb are in the last instance represented in their intimate structure and not merely in their arrangement. The above rules show, however, that not all essential features are stamped upon the constituent elements of the rudiment at the time of transplantation. For example, the difference between the right bud and the left is not an absolute one, since a right limb bud upside down behaves like a left one right side up and vice versa. From this the conclusion has been drawn that the elements making up the limb bud are differentiated in an anteroposterior direction, i.e., along the anteroposterior axis, but are not yet differentiated, at least not irreversibly, along the dorsoventral axis at the period of development at which the transplantations are made. In this one respect the differentiation of the limb is dependent upon its orientation with reference to the dorsoventral axis of the embryo; otherwise as regards its specific form, the limb bud constitutes a self-differentiating system.

These questions will be considered more fully in the concluding section (p. 85).

METHODS AND TERMINOLOGY

All experiments were made upon embryos of *Amblystoma punctatum* in stages that have been previously defined.⁶

In performing the operations the embryo which is to receive the implanted limb bud is first made ready. If the bud is to be placed in normal location, the wound is prepared as in the extirpation experiments referred to above. A circular incision, hav-

⁴ Harrison, '18.

⁵ "Jedes kann Jedes und alles Einzelne steht in Harmonie zu einander." (Driesch, '02, p. 229.) See also: '99, p. 72; '05, p. 679, and '08 b, p. 120.

⁶ Harrison, '15 and '18.

ing the diameter of three and a half somites (ca. 0.9 mm.) and ventral to the third, fourth, fifth, and half of the sixth myotome, is made, and the disc of tissue, including both mesoderm and ectoderm, is lifted, after which the remaining mesoderm cells are carefully cleaned off. This may all be done without injury to the pronephros, which lies immediately dorsal to the limb rudiment, though if this organ is injured or even extirpated, there is no noticeable effect on the subsequent development of the limb. The embryo thus prepared is held in readiness for the grafting, being secured in position by pieces of silver wire or glass rod bent into proper shape. The limb bud which is to be transplanted is removed from another embryo, as described above, care being taken in lifting it from its bed to take all of the mesoderm possible. It is then transferred on the point of the scissors to the first embryo and fitted into the wound. The orientation of the bud is important and may be carried out as desired by noting the position of pigment markings in the ectoderm of the graft. After it has been properly placed, it is held in position for an hour or more by a single piece of glass rod, bent into such shape that it straddles the embryo, exerting a light pressure upon the grafted tissue. The healing of the wound takes place readily, though frequently a small area of underlying yolk may be left exposed on the border of the wound. This usually heals in a day or two and does not seem to influence the result of the experiment, unless the yolk begins to disintegrate, in which case death of the embryo usually follows.

At the time when the first experiments were made, the conditions necessary to prevent regeneration had not been determined, so that in a number of cases the extirpated area was too small or the wound bed was insufficiently cleaned of scattered mesoderm cells for the result to be conclusive. These experiments have not been included in the tabulations, but will be referred to separately in so far as they are of special interest. In all of the later experiments the size and character of the wound in the host was such as to preclude regeneration of the limb from that source; the resulting limbs must, therefore, have arisen from the engrafted tissue. Even in the cases where the wound was

not especially cleaned, there is no evidence, aside from certain exceptional cases, that the tissue of the wound bed displaces the transplanted bud, though the possibility of its participation in the make-up of the limb cannot be excluded, and it probably actually does take place to some extent.

In transplanting the limb bud to a location other than the normal, the recipient embryo is first prepared as in the other experiments. A wound of proper size is made, usually in the flank just below the ventral border of the myotomes, and the bud grafted in the same manner as described above. In doing this it is well to avoid injury to the pronephric duct (p. 15).

Three different factors regarding the placement of the limbs have been considered in these experiments, viz.: 1) location of the graft in the embryo; 2) the side of the body on which it is placed (whether the same from which it was taken or the opposite); and 3) orientation with respect to the cardinal points of the embryo. The experiments thus fall into eight categories, as follows (fig. 1):

A. Limb buds placed in abnormal location—heterotopic transplantation.

1. On the same side of body as origin—homopleural (*hom.*).

a. Dorsal border of limb bud dorsal with respect to embryo—dorsodorsal (*dd.*).

b. Dorsal border of limb bud ventral with respect to embryo—dorsoventral (*dv.*).

2. On side of body opposite to origin—heteropleural (*het.*).

a. Dorsal border of limb bud dorsal with respect to embryo—dorsodorsal (*dd.*), in which case the anteroposterior axis is reversed.

b. Dorsal border of limb bud ventral with respect to embryo—dorsoventral (*dv.*), in which case the anterior and posterior points of the graft correspond, respectively, to those of the embryo.

B. Limb bud placed in natural location—orthotopic transplantation.

The several categories under this head as under A.

According to the rules stated on page 4, two of the combinations (homopleural dorsodorsal and heteropleural dorsoventral) yield limbs which are of the same side of the body as that on

which they are placed (fig. 2), for the non-inverted limb bud from the same side (*hom.dd.*) does not have its prospective asymmetry changed while the inverted limb bud from the opposite side (*het.dv.*) does. The limbs which develop in these combina-

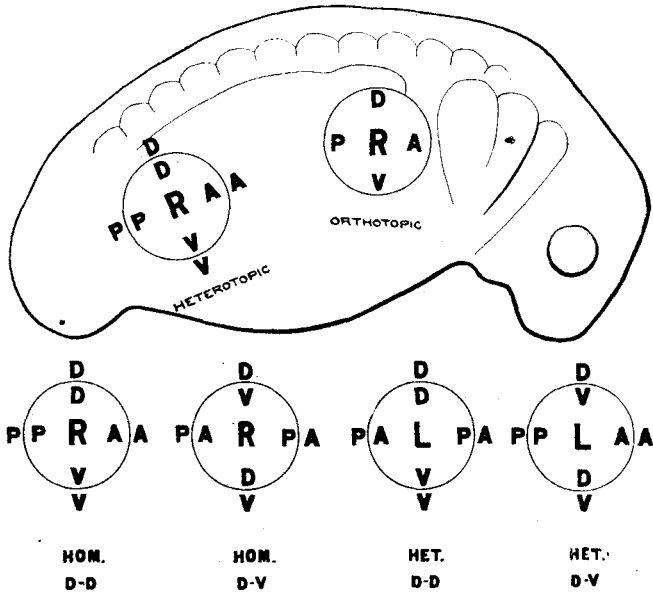


Fig. 1 Diagram showing the eight different operations. The outline of an Amblystoma embryo in the operating stage is shown above. The circles within it represent the limb bud, in the normal (orthotopic) and the abnormal (heterotopic) location. The four circles below represent the four different ways in which limb buds may be oriented with reference to the cardinal points of the embryo; the letters (*D*, dorsal; *V*, ventral; *A*, anterior, and *P*, posterior) within the circles designate the original cardinal points of the transplanted limb, those outside the corresponding points of the embryo. The operations are represented to be on the right side. *R*, right limb bud; *L*, left limb bud; *hom.*, homopleural; *het.*, heteropleural.

tions thus fit in with their surroundings; they have therefore been called harmonic. The other two combinations (homopleural dorsoventral and heteropleural dorsodorsal) give rise to limbs of the side opposite to that of their seat of implantation, for the inverted bud from the same side (*hom.dv.*) has its asymmetry reversed, while the non-inverted bud from the opposite side

(*het.dd.*) remains as it was. The limbs which develop here are not primarily in harmony with their surroundings, so that these combinations have been termed disharmonic.

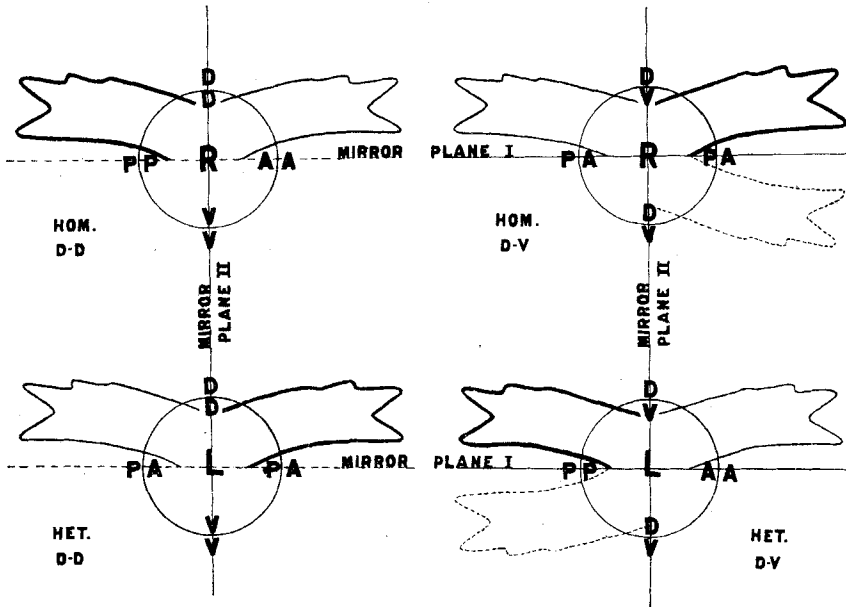


Fig. 2 Diagram showing the results of the four operations, heterotopic or orthotopic, represented as on the right side of the embryo. The circles indicate the transplanted limb buds, the letters having the same significance as in figure 1. Thus the two upper figures in the diagram represent homopleural, and the two lower ones heteropleural transplantations. The two on the left show the transplanted bud in upright (dorsodorsal) orientation while the two on the right are inverted (dorsoventral). The limbs which develop are shown in profile, the ulnar border being uppermost (dorsal) in all which actually develop. A heavy outline indicates the primary member, a light outline the reduplicating one. It is to be noted, however, that the latter develop in by no means all cases, while the former may be resorbed in the heteropleural dorsoventral combination, leaving only the reduplicating member present. The broken outlines show the posture that the limb would have assumed, had it developed as a self-differentiating member totally independent of the influence of its surroundings.

The transplanted limb bud is a flattened disc of tissue, and it is theoretically possible to make eight further combinations by placing the medial surface of the graft corresponding to the lateral surface of the embryo. This is impracticable, however, because

the mesoderm would thus be brought to the surface and the ectoderm buried beneath it. The same effect might be obtained, however, by transplanting the mesoderm alone. While the difficulties in this procedure are great, they have now been in a large measure overcome. The positive experiments are too few in number to warrant any very definite statement, but they do indicate that it is immaterial which surface of the mesodermal disc faces laterally.

A much greater variety of experiments could be had by experimenting with positions intermediate between the upright and inverted positions, i.e., with limb buds turned, say, 90° instead of 180° . Such experiments may yield very interesting results, but as yet there has not been sufficient time to carry them out, nor has the effect of implanting the limb exactly in the midline been studied.

The experiments with superposed buds were made in the same way as the above, except that the mesoderm of the host was not excised. In the case of half buds, more combinations are possible, as described in the section dealing with this group. Both here and in the superposition experiments all possible positions with regard to the placement of the graft within the limitations stated above were experimented with. Relations of harmony and disharmony proved to be the same here as in the case of whole buds.

The total number of cases of which records have been kept is 462. The analysis is based, however, upon the 271 individuals which yielded positive results. The identity of the individual cases has been maintained by rearing each in a container by itself and keeping a separate history of each. These histories consist in notes and in sketches made from time to time directly from the living specimens, mostly with the aid of the camera lucida.

In dealing with so large a mass of material it has of course been necessary to select typical cases for presentation, and in order not to interrupt the continuity of the general account, the individual histories, as far as given, have been gathered together in an appendix. The main body of the paper has been divided in

accordance with the outline presented above. The larger groups of experiments have been considered apart from each other, and each subgroup is treated in a special section. The peculiar features of each of the larger groups have been considered at the beginning, and the results of the experiments summarized separately at the end of each main section. The more general questions are treated in the final chapter.

It has been thought best to provide numerous illustrations in order to avoid lengthy descriptions. Since it was not possible to keep a complete pictorial history of each case, those were selected for drawing that promised typical or otherwise interesting results. Unfortunately, however, it was not always possible to predict what the outcome of an experiment would be, so that some important cases were not drawn in early stages, while others of less interest were.⁷

GENERAL FEATURES OF THE DEVELOPMENT OF THE TRANSPLANTED BUDS

The development of the transplanted limb buds must now be considered in comparison with normal development. When the normal limb bud appears it is a round prominence just below the pronephros. It soon becomes more sharply marked off from the background and begins to 'point' dorsoposteriorly.⁸ The radial border of the fore arm and hand is at first ventrolateral, then ventral, and the first digits to arise are the first and second. The third and fourth digits appear later on the dorsal border of the hand, so that there is never any difficulty in distinguishing the ulnar from the radial border unless the third and fourth digits are entirely suppressed. The palmar surface of the hand faces at first ventromedially and later medially.

The transplanted limbs, both heterotopic and orthotopic, give evidence of their orientation early in development, inasmuch

⁷ Almost all of the preliminary sketches and many of the finished drawings were made by Miss Lisbeth Krause. The former, which were pencil sketches, had to be redrawn for reproduction. For this part of the work and also for a number of the original drawings I am indebted to Mr. A. Hemberger and Mr. H. D. Rhynedance.

⁸ Harrison, '18, p. 419.

as their direction of 'pointing' is determined principally by the bud itself. In two of the combinations (homopleural dorsoventral and heteropleural dorsodorsal), they point anteriorly or dorsoanteriorly; in the other two (homopleural dorsodorsal and heteropleural dorsoventral) posteriorly or dorsoposteriorly like the normal. The subsequent development in the latter case is normal, but in the former there is a tendency for the limb to stick out more sharply to the side or to rotate more or less from the position in which it would be found were the position determined entirely by the orientation of the bud itself. Nevertheless, the palm tends to face ventromedially, or else the limb is so rotated that it faces more ventrally or anteriorly. In order to determine whether the limb is right or left, it is necessary to be able to distinguish between the palm and the back of the hand, which is not always so simple as it might seem. It can usually be done, however, by noting the digits, which are frequently slightly flexed. When there is uncertainty, it is necessary to resort to sections, in which case there is no difficulty in distinguishing between the two faces, because of the much greater thickness of the soft parts on the flexor surface of the skeleton.

The duplicities that arise are of all grades and kinds, and occur in very different proportions in the several experiments. Sometimes they make their appearance very early, sometimes late in development. In the orthotopic grafts reduplication is far more common when the developing limb and the substratum are of opposite sides. In such cases the doubling member nearly always appears as a bud posterior to the main limb, growing there into a limb of proper asymmetry. The extent of reduplication may include the whole limb from the shoulder down, or only certain of the digits. The duplicate limb is as if it were mirrored from the original in a plane which is perpendicular to the plane of the proximodistal axes of the two limbs⁹ and which cuts the axes of the two limbs at their junction, at an angle which varies from almost 0° to 90°. In the former case the two members are almost parallel, in the latter they diverge in the opposite direction at almost 180°, the mirror plane bisecting the angle between them

⁹ Bateson, *Materials for the Study of Variation*, p. 479.

(fig. 3). In the present paper the relation of the mirror plane to the long axis of the limb has not been taken into account for purposes of description, the relation only to the dorsopalmar and the radioulnar axes being stated; i.e., the degree of divergence of the two members is not taken into account. Thus, when the mirror plane is parallel to the radioulnar axis, the limb is said to

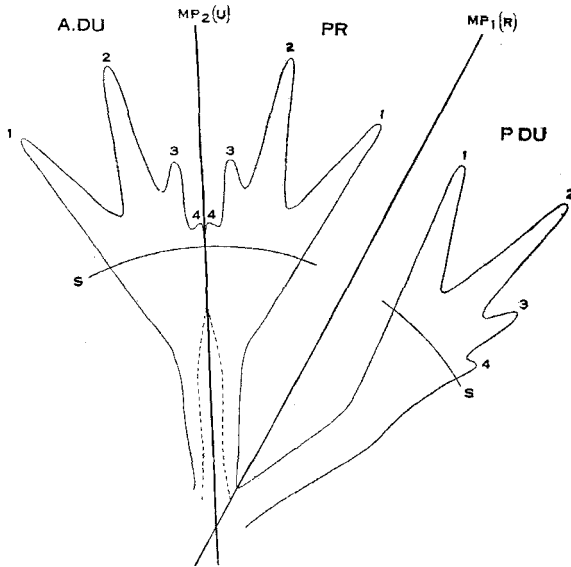


Fig. 3 Diagram showing mode of reduplication. PR , primary limb; $P.DU$, posterior reduplicating member; $A.DU$, anterior reduplicating member; $MP_1(R)$, primary (radial) mirror plane; $MP_2(U)$, secondary (ulnar) mirror plane; 1 to 4, first to fourth digits, respectively. S , location of section shown in figure 4B. Dotted lines show the outlines of limbs as they would have been had there been no coalescence.

be mirrored in a palmar or a dorsal plane, according as the palms or the backs of the hand face one another; when the plane is parallel to the dorsopalmar axis, the mirroring is in a radial or an ulnar plane, according as the radial or ulnar borders of the limb face one another (fig. 4, *A*). Intermediate planes are described as radiodorsal, ulnopalmar, etc. (fig. 4, *B*). No attempt has been made for the present to measure accurately the angles of mirroring. It has been found, in agreement with Bateson, that

when there is a double reduplication, then the two mirror planes intersect at the bifurcation in a line perpendicular to the proximo-distal axes; i.e., so that with reference to the radioulnar and dorso-palmar axes the planes of reflection face one another (fig. 4). Considerable deviation from this rule has, however, been noted in certain cases, and the amphibians do not seem to follow it with the same regularity as the arthropods, according to Bateson.¹⁰

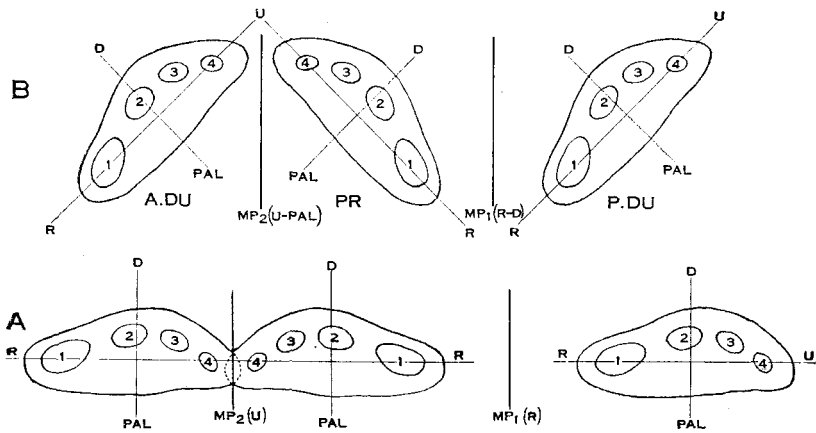


Fig. 4 Diagram of reduplication, sectional view. In A the mirror planes are radial (MP_1) and ulnar (MP_2), and a certain amount of coalescence between the primary and the anterior reduplicating members is shown, as in figure 3. In B the mirror planes are radiodorsal (MP_1) and ulno palmar (MP_2). D, dorsal; PAL, palmar; R, radial; U, ulnar.

EXPERIMENTAL

A. Limb buds implanted in abnormal location—heterotopic transplantations

In nearly all of the experiments in this group the limb bud was implanted on the flank of the embryo at the ventral border of the myotomes between the region of the fore and hind limbs. In a few cases it was placed on the side of the head between the eye and the ear, but the grafts were absorbed in all of these except

¹⁰ Op. cit., p. 552.

one, which yielded an imperfect appendage. They need not be considered separately here, though a more extensive series of experiments of the latter type would probably yield different and more interesting results.

The limb buds transplanted to the flank of the embryo are placed in an environment similar to that of the normal fore limb, as far as relations to the body wall and muscle plates are concerned, though they lack the specific blood supply and innervation of the limb region. Consequently, a very high percentage

TABLE 1
Heterotopic transplantations. Summary of experiments

OPERATION	NUMBER OF EXPERIMENTS		SINGLE LIMBS NOT REVERSED		SINGLE LIMBS REVERSED		REDUPLICATED	
	Total	Positive ¹	Number	Percent	Number	Percent	Number	Percent
Hom. dd.	19	7	3	42.8	0	00.0	4	57.1
Hom. dv.	31	12	0	00.0	11	91.7	1	8.3
Het. dd.	28	10	8	80.0	0	00.0	2	20.0
Het. dv.	60	16	1(?) ²	6.3(?)	7	43.8	8	50.0
Total.	138	45	12	26.7	18	40.0	15	33.3
Average of percentages				32.3		33.9		33.9

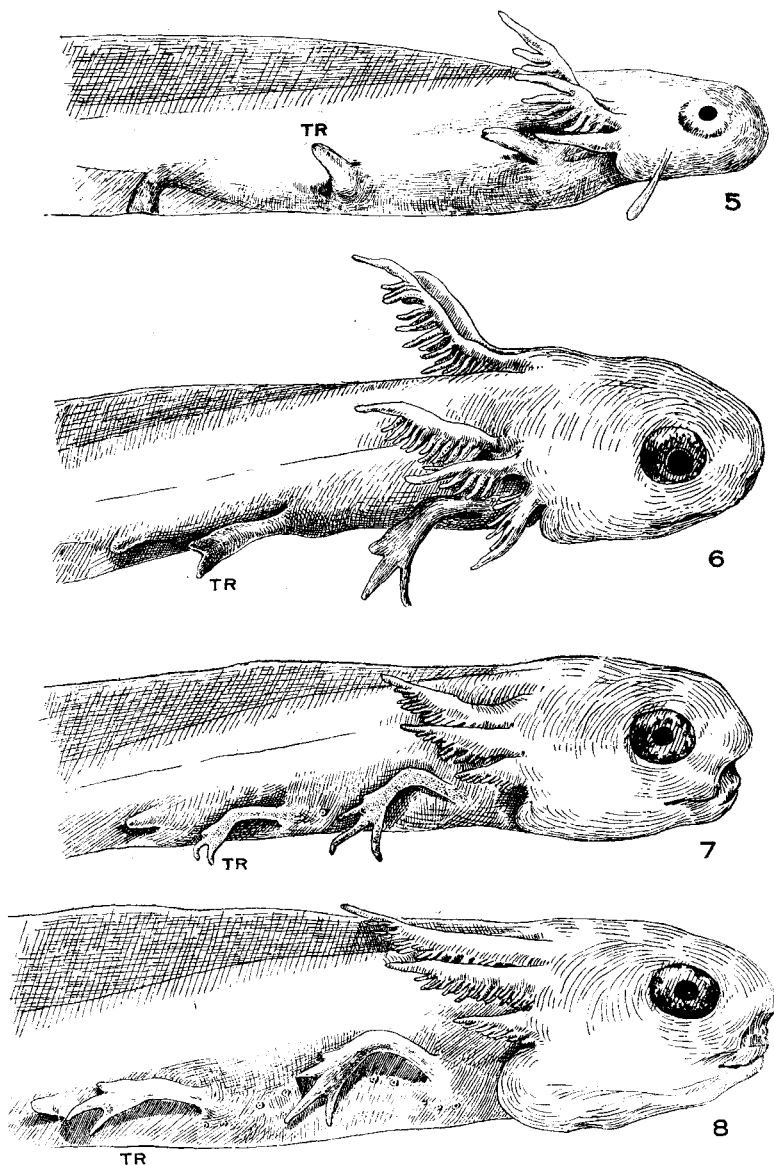
¹ Excluding all cases where death occurred prematurely or where the grafted limb was resorbed or remained rudimentary. Percentages in all tables have been calculated on the basis of positive experiments.

² There is evidence that in this case there was an error in the orientation of the bud and that it should therefore be classed in the group het. dd.

of cases yielded only abortive limbs, and those that did develop rarely showed any functional activity.¹¹ There is also greater difficulty in securing good healing of wounds in the intermediate region, so that a larger proportion of the cases died early. In many of these cases there is obviously some interference with the development of the pronephric duct, which becomes blocked. The secretion which accumulates causes the formation of a cyst of considerable size, which may interfere with the development of the limb bud.

The results of the experiments are summarized in table 1.

¹¹ Cf. Detwiler, '19 and '20.



Figs. 5 to 8 Heterotopic transplantation of fore limb; right limb to right side (*hom.dd.*). *TR*, transplanted limb. $\times 10$.

Fig. 5 Exp. Tr. E. 148, eight days after operation.

Fig. 6 Same, twenty days after operation.

Fig. 7 Same, twenty-eight days after operation, drawn from preserved specimen.

Fig. 8 Experiment Tr. E. 154, drawn from preserved specimen, killed twenty-two days after operation.

1. *Homopleural transplantations, normal or dorsodorsal orientation.* Nineteen cases were operated upon in this way (table 1). In all of the cases where observations are recorded (thirteen in number), the limbs, in the course of their development, gave evidence of their original orientation, in that they pointed posteriorly or dorsoposteriorly when they first began to grow out (fig. 5). In the three cases that gave rise to single limbs they contin-

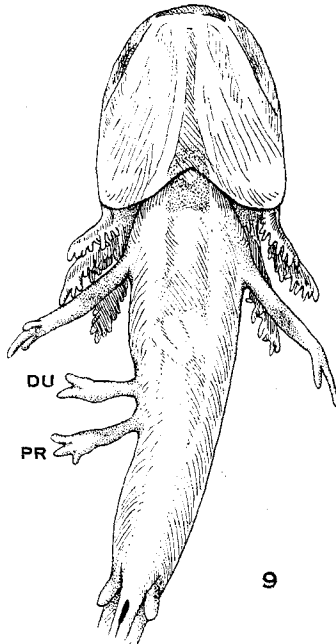
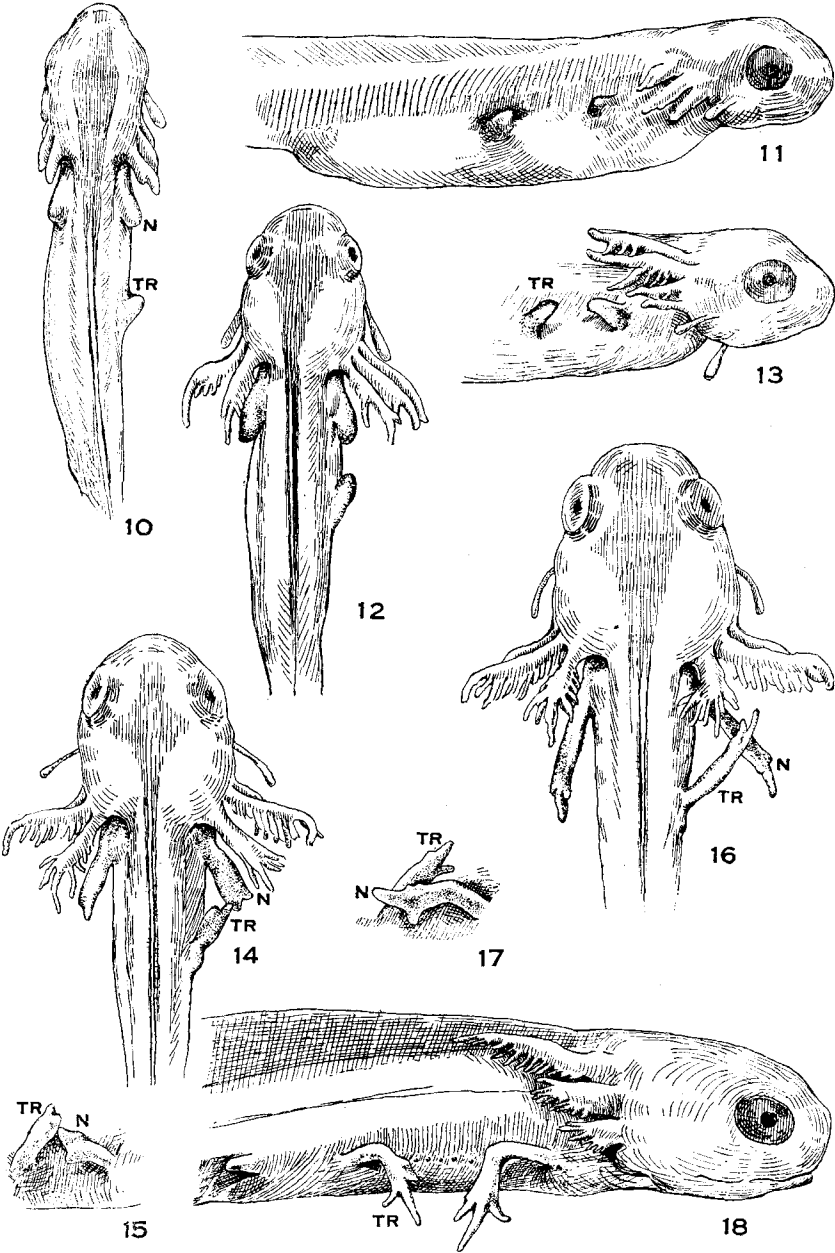


Fig. 9 Heterotopic transplantation (*hom.dd.*), showing twin limbs from one implanted bud; *PR*, primary member; *DU*, reduplicating member. Exp. Tr. E. 182. $\times 10$.

ued their growth in this direction, developing almost exactly like the normal (figs. 6, 7, and 8). Likewise in the four cases that gave rise to double appendages, the transplanted buds first began to grow in a dorsoposterior direction, and only later did the reduplicating buds appear on the anterior border of the original limb. The original bud developed in each case into a limb of the same side, and the reduplicating buds became limbs of opposite asymmetry (fig. 9). Histories of typical cases are given in the appendix (p. 119).



2. *Homopleural transplantations, inverted or dorsoventral orientation.* Thirty-one experiments of this kind were made, with results as shown in table 1. Of the twelve cases yielding positive results, one¹² gave rise to a pair of limbs and the others to single limbs in which the asymmetry was reversed; i.e., the right limb bud when placed upside down on the right side of the body gave rise directly to a left limb. Even in the case which showed reduplication the primary limb of the pair became reversed. In all of the cases where limbs resulted, the initial direction of pointing was anterior or dorsoanterior (figs. 10 and 11); i.e., nearly the opposite of normal. In four other cases this was also true. In only four cases is the direction of pointing recorded as posterior, and from these nothing definite was developed. All limbs which developed continued their growth in the same general direction, sometimes being directed more dorsally and sometimes more sharply anteriorly (figs. 12 to 17). They also showed the tendency to project more directly to the side than the normal limbs. The final posture assumed by these appendages varies considerably and does not seem to be dependent upon the degree of development attained by the appendage. Two cases, each having perfectly developed hands, exhibit the following extreme conditions: One¹³ is practically a perfect mirror image of the normal right limb both as regards form and posture (fig. 18). The

Figs. 10 to 17 Heterotopic transplantation of fore limb; right limb bud to right side inverted (*hom.dv.*), Exp. Tr. E. 219. *N*, normal limb, right side; *TR*, transplanted limb. $\times 10$.

Fig. 10 Dorsal view, five days after operation.

Fig. 11 Lateral view, same age.

Fig. 12 Dorsal view, eight days after operation.

Fig. 13 Lateral view, same age.

Fig. 14 Dorsal view, twelve days after operation.

Fig. 15 Lateral view of limbs only, same age.

Fig. 16 Dorsal view, sixteen days after operation.

Fig. 17 Lateral view, same age.

Fig. 18 Heterotopic transplantation; right limb to right side inverted (*hom.dv.*), Exp. Tr. E. 139; drawn from specimen preserved twenty-eight days after operation. $\times 10$.

¹² Tr. E. 220.

¹³ Tr. E. 139.

upper arm runs dorso-anteriorly and laterally. The elbow bend is somewhat less than 90° and the fore arm and hand extend antero-ventrally and laterally. The extensor surface of the elbow-joint faces dorsally and slightly anteriorly and medially. The palm of the hand faces medially, anteriorly, and slightly ventrally.

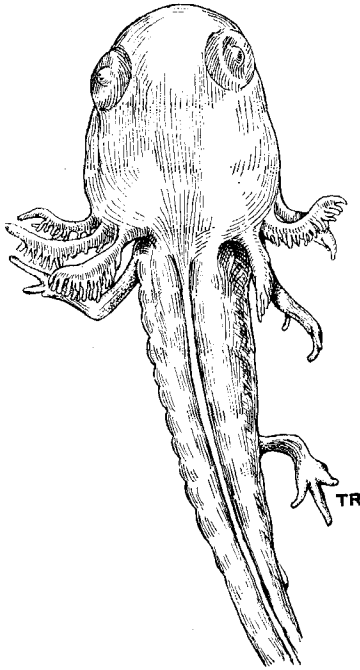


Fig. 19 Heterotopic transplantation (*hom.dv.*), Exp. Tr. E. 140; drawn from specimen preserved twenty-eight days after operation. *TR*, transplanted limb. $\times 10$.

The other case¹⁴ has its upper arm transverse and horizontal, and its fore arm extends ventroposteriorly at an angle of less than 45° to the horizontal axis (fig. 19). The palm looks ventrally and anteriorly. In order to bring this limb into the position of the former, it would have to be rotated about the axis of the humerus 45° or more and then adducted dorsoanteriorly at the shoulder-joint through about 30° . The difference in position assumed by the limbs in the various cases is thus due to differ-

¹⁴ Tr. E. 140.

ences in the amount of rotation, etc., undergone during the later stages of development.

Histories of these cases are given in the appendix (p. 120).

3. *Heteropleural transplantations, dorsodorsal orientation.* Twenty-eight experiments in this class have been made (table 1). Five of these died prematurely, and in twelve the tissue was either resorbed or failed to develop beyond the nodule stage. In one case¹⁵ the bud developed into a stump about as long as the upper arm, but without digits. Two cases gave double limbs and eight developed into limbs which preserved their original prospective asymmetry. Two other cases may belong in this category, one in which the original orientation of the bud is recorded as uncertain¹⁶ and another¹⁷ in which it is recorded as dorsoventral probably by mistake.

In the development of the limb buds in this group twenty-one, in addition to the two doubtful cases just mentioned, are recorded at first as pointing in an anterodorsal direction, thus preserving their original tendency in this respect. In the eight cases in which the pointing was slight and in the five in which no definite pointing was observed the limbs were abortive or resorbed.

In the eight cases where single limbs of the side of origin developed they retained their posture, developing as nearly exact mirror images of the normal fore limb of the side to which they were transplanted (figs. 20 to 23). The elbow-joint points dorso-anteriorly, though varying somewhat, and the palm of the hand faces ventrally, medially, and anteriorly (figs. 24 and 25). Individual cases show variations similar to those observed in the previous group. It is a striking fact that the general type of development is the same here in the heteropleural non-inverted buds as in the homopleural inverted bud, which shows that both the posture and the asymmetry of the limb depend upon some reaction between the bud and its new environment. (For case histories see p. 121.)

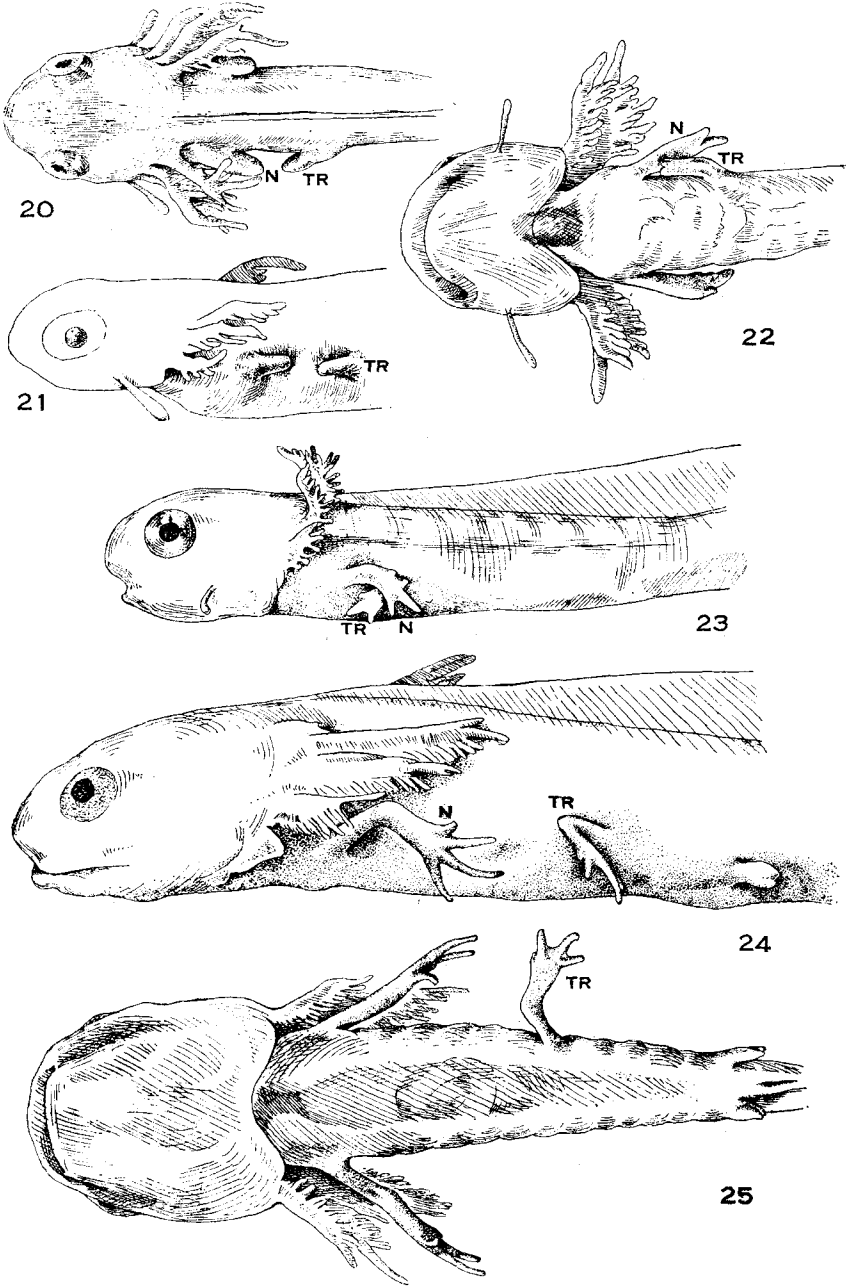
The cases which showed reduplications, but two in number, differ considerably from one another. In the first¹⁸ growth was slow and the resulting limb short with irregular reduplications

¹⁵ Tr. E. 118.

¹⁶ Tr. E. 117.

¹⁷ Tr. E. 113.

¹⁸ Tr. E. 119.



in the hand, so that right- or left-sidedness could not be determined. In the other¹⁹ the limb developed promptly and formed a duplicate member (fig. 26), which was first seen at ten days and

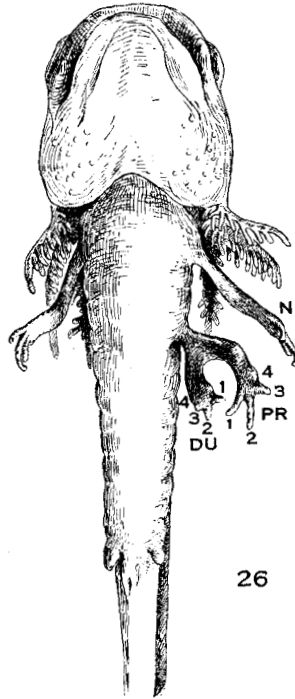


Fig. 26 Heterotopic transplantation (*het.dd.*), Exp. Tr. E. 127. Drawn from specimen preserved twenty days after operation. *PR*, primary; *DU*, reduplicating member; 1 to 4, digits.

Figs. 20 to 23 Heterotopic transplantation of fore limb; right limb bud to left side (*het.dd.*), Exp. Tr. E. 227. *N*, normal left limb; *TR*, transplanted limb. $\times 10$.

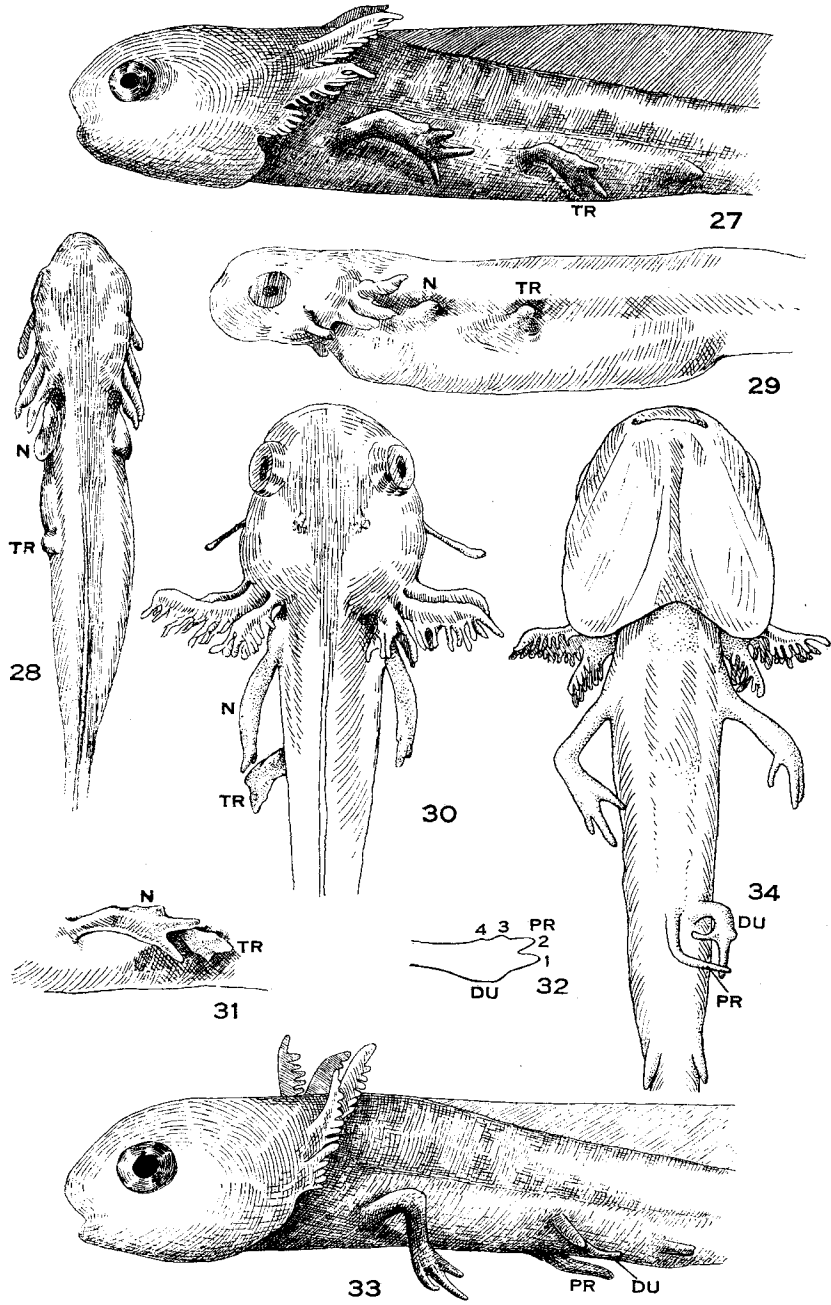
Figs. 20 and 21 Dorsal and lateral views, respectively, eight days after operation.

Fig. 22 Ventral view, thirteen days after operation.

Fig. 23 Lateral view, seventeen days after operation.

Figs. 24 and 25 Heterotopic transplantation (*het.dd.*), Exp. Tr. E. 107. Lateral and ventral views, respectively, of preserved specimen, twenty-six days after operation. $\times 10$.

¹⁹ Tr. E. 127.



which developed ultimately into a left limb, the mirror image of the primary member, having been reversed from the original prospective asymmetry of the transplanted bud.

4. *Heteropleural transplantations, dorsoventral orientation.* Sixty operations were done in this series. For some unknown reason a very large proportion (twenty cases) died prematurely and sixteen of the survivors yielded only abortive limb buds, leaving only twenty-four available for consideration. Eight of these are recorded as imperfect, six produced reduplications to some degree, and nine, single limbs of reversed asymmetry. Several of the latter were somewhat defective and others showed slight reduplications. Several cases which are exceptional will be considered below.

In the cases where single limbs arose, development took place in a manner fundamentally like that of the limb buds normally oriented (*hom. dd.*). As the buds grew out, they began to point in a posterior direction, and so continuing, developed into limbs in normal posture (fig. 27). There was, however, less regularity than in the homopleural dorsodorsal group. The direction of pointing was not always dorsoposterior, as in the normal limb, but was in many cases inclined more ventrally. There are records of pointing in all of the positive experiments and in many of the negative. In only three cases is the direction recorded

Fig. 27 Heterotopic transplantation of fore limb; right limb bud to left side inverted (*het.dv.*), Exp. Tr. E. 193. Preserved specimen killed twenty-four days after operation. $\times 10$.

Figs. 28 to 32 Heterotopic transplantation of fore limb; right limb bud to left side inverted (*het.dv.*), Exp. Tr. E. 217. *N*, normal left limb; *TR*, transplanted limb; *PR*, primary member; *DU*, reduplicating member; 1 to 4, numbers of digits.

Fig. 28 Dorsal view, five days after operation.

Fig. 29 Lateral view, five days after operation.

Fig. 30 Dorsal view, fifteen days after operation.

Fig. 31 Lateral view of limbs, fifteen days after operation.

Fig. 32 Limb showing beginning of reduplicating digits (*DU*) on ventro-anterior border (from a free-hand sketch nineteen days after operation).

Figs. 33 and 34 Heterotopic transplantation (*het.dv.*); right limb bud to left side. Exp. Tr. E. 163. Anomalous result. Primary member (*PR*) defective; reduplicating member (*DU*) reversed. Lateral and ventral aspects, respectively, drawn from specimen preserved thirty-nine days after operation. $\times 10$.

as dorsoanterior; one of these died early and the other two gave rise to imperfect limbs with indeterminate asymmetry.²⁰

The individual cases in which limbs of opposite asymmetry developed were rather more irregular than in the preceding groups, though the best cases gave perfect reversed appendages. In addition to the ones included in the tabulation, there is one other case that probably belongs in this category. It is one in which the orientation of the bud at the time of transplantation is recorded as uncertain.²¹ The limb that developed is a perfect one of reversed asymmetry in nearly the same posture as the normal limb of the side to which it was transplanted. It showed an unusual amount of motility. In one case, included in the records of this group,²² the transplanted bud developed into a normal limb of the side from which it was taken. It is believed, however, that a mistake was made in recording the operation in this case, and that probably in reality the orientation of the limb was not inverted. The direction of pointing, as observed on the third and fifth days after the operation when the limb bud is recorded as pointing anteriorly, is evidence, though not absolutely conclusive, that an error has been made. If this interpretation is correct, the case would not be exceptional, but would accord with the eight cases described in the previous section.

In the eight cases in which reduplications occurred, the early stages of development were like the normal (figs. 28 and 29); the reduplicating buds not being noted until at least twelve days after the operation. Three individuals showed distinctly that the primary limb was of reversed asymmetry. In one case it was so imperfect that it could not be determined to which side it belonged, but the reduplicating limb was sufficiently developed to show that it was of the same side as the bud was originally, indicating that the original member was in all probability reversed. Another case²³ gave a limb with nearly symmetrical reduplication in the hand without anything to indicate which member was primary (figs. 31 and 32). Two long radial digits are present in the middle and two short ulnar digits on each side. Still another case²⁴ gave a very peculiar result. The primary

²⁰ Tr. E. 108 and 203. ²¹ Tr. E. 109. ²² Tr. E. 113. ²³ Tr. E. 217.

²⁴ Tr. E. 163.

limb bud developed into a long almost filiform structure, without digits, that grew posteriorly on the ventral side of the body not far from the midline. Twenty days after the operation a second bud was noticed dorsal to the original, and this developed into a somewhat peculiarly placed limb. The upper arm runs transversely and the palm of the hand faces dorsomedially (figs. 33 and 34). This limb is clearly a left; i.e., its original prospective asymmetry has been reversed. It therefore constitutes an exception to the rules, not only because of the position of the hand, but also because of its particular asymmetry; for the original (filiform) member should have been reversed (a left), and the second one reversed back again to the original asymmetry. However, the fact that the latter developed at such a considerable distance from the original member, might be regarded as indicating that it was beyond its sphere of influence, perhaps having been split apart from it at an early stage, and that it remained therefore as of the same side. Several cases of regeneration after extirpation of half buds and of transplantation of half buds gave analogous results (fig. 132).²⁵

5. *The shoulder-girdle in heterotopic transplantations.* The limb-girdle in the heterotopic transplantations is developed in more or less reduced condition, as was first shown by Braus ('09) in the anurans. Detwiler ('18) has studied this question in *Amblystoma*, and has found that the degree of development of the girdle is dependent upon the size of the graft and the region from which it is taken, the scapula and suprascapula being localized in the tissue dorsal to the normal limb bud and the coracoid in that ventral to it.²⁶ The form of the reduced girdle derived

²⁵ Cf. Harrison, '18, p. 441 (Exp. Rem. E. 17 and H. R. E. 10), and page 135 of the present paper (Exp. H. R. E. 20).

²⁶ It is a curious fact that in the embryo the limb-girdle has undoubtedly the character of a mosaic, without totipotency of its parts, while in the adult Triton, according to Tornier ('06), Fritsch ('11), and Kurz ('12), a small portion of the shoulder-girdle can regenerate the whole, including the fore limb. According to the two last-named investigators, even if the whole girdle is removed, it will be regenerated together with the free appendage. Kurz has found that this holds for both shoulder and pelvic girdles but that removal of the sacral portion of the vertebral column prevents regeneration. In the anurans, according to Braus ('06), there is considerable variation in the regenerative powers of the limbs in early stages.

from the usual round disc (limb bud) is roughly triangular, as shown in the figure of Detwiler's model (his figure 28), with a ventral process projecting anteriorly, to be identified as a rudimentary coracoid, and a dorsal process, which includes the rudiment of the scapula. In the normally oriented grafts (homopleural dorsodorsal) these processes point anteriorly, with a single process projecting posteriorly slightly behind the glenoid cavity. This shows clearly in two cases.²⁷ The question now arises whether the girdle follows the rules governing the asymmetry of the free limbs. The results, in the main indicate that such is the case, though the girdle developed is often so small and rudimentary, that it is not possible to determine to which side it belongs. In the inverted homopleural grafts, which give rise to reversed limbs, the girdle also seems to be reversed. This is true in four cases out of the five examined in serial sections.²⁸

Among the heteropleural dorsodorsal transplantations, five cases have been examined in sections. In two of them²⁹ with well-developed glenoid cavity, the girdle cartilage is mostly ventral and posterior to the joint. This probably represents a coracoid with asymmetry corresponding to that of the free limb. One case,³⁰ with the cartilage projecting both anteriorly and posteriorly from the cavity, gives no evidence as to the side to which it belongs. One is too rudimentary,³¹ and one seems to have had its asymmetry reversed,³² though the limb is not reversed. In the two dorsoventral heteropleural transplants which have been studied in sections, the side to which the girdle belongs cannot be determined. Other cases from among the earlier experiments, where in most instances the size of the transplanted bud was small, are inconclusive. On the whole, the cases where the asymmetry can be determined with any degree of certainty seem to follow the rules. Only a single case thus far examined is clearly exceptional.

²⁷ Tr. E. 148 and 154.

²⁸ Tr. E. 135, 136, 139, and 140.

²⁹ Tr. E. 124 and 169.

³⁰ Tr. E. 120.

³¹ Tr. E. 127.

³² Tr. E. 107.

6. *Summary of the results of heterotopic transplantations.* A survey of all the experiments in this group brings out the following facts:

Implanted in dorsodorsal orientation, a limb bud gives rise to an appendage of its original prospective asymmetry, whether placed on the same or opposite side of the body. Such appendages have a normal posture when placed on the same side of the body from which they were taken, but when placed on the opposite side they mirror approximately the limb of that side, though they often become rotated to quite different postures. Implanted in inverted (dorsoventral) position, a limb bud gives rise to an appendage of reversed asymmetry whether placed on the same or opposite side of the body. When placed on the same side, such appendages mirror the normal limb of that side, but when grafted on the opposite side, they assume a posture approximately identical with that of the limb of that side.

Limbs implanted in any of the four positions here studied may produce reduplications. As far as it has been possible to determine, the primary limb of the pair is then of the same side as a single limb would be according to the foregoing rules. The reduplicating limb has been found to be, with a single exception, the mirror image of the first.

Limbs that are grafted in abnormal location have at best very incomplete function and are often apparently entirely immobile. They usually do not become so large as those that are implanted in normal location, and they show defects and evidences of atrophy much more frequently.

B. Limb buds implanted in natural location—orthotopic transplantation

In these experiments the limb bud of the host was first removed and then either put back in place, or else a bud from another embryo was grafted into the wound. In all of the earlier cases the wound bed was not cleaned after removal of the bud, so that some cells from the host were left to mingle with the tissues of the transplanted limb rudiment. The later experiments, with

but few exceptions, were carried out under precautions necessary and sufficient to preclude contamination of this kind: the extirpated area was three and a half somites in diameter, and the bed of the wound was carefully scraped after removal of the bud.³³ The results were somewhat different (proportionately) in the

TABLE 2
Orthotopic transplantations. Summary of experiments

OPERATION	NUMBER OF EXPERIMENTS		SINGLE LIMBS NOT REVERSED		SINGLE LIMBS REVERSED		REDUPLICATED	
	Total	Posi- tive	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent
A. Wound bed cleaned and wound not less than 3½ somites								
Hom. dd.....	9	9	9	100.0	0	00.0	0	00.0
Hom. dv.....	61	38	10 ²	26.3	1	2.6	27 ¹	71.1
Het. dd.....	49	31	1	3.2	5 ³	16.1	25	80.6
Het. dv.....	26	16	0	00.0	15	93.8	1	6.3
Total.....	145	94	20	21.3	21	22.3	53	56.4
Average of percentages.				31.6		28.8		39.6
B. Wound bed not cleaned								
Hom. dd.....	0	0	0		0		0	
Hom. dv.....	37	20	19 ⁴	95.0	0	00.0	1	5.0
Het. dd.....	17	13	2	15.4	3	23.1	8	61.5
Het. dv.....	21	15	0	00.0	8	53.3	7	46.7
Total.....	75	48	21	43.8	11	22.9	16	33.3

¹ Including three cases in which the primary bud righted itself by rotation and the duplicate is disharmonic.

² Limbs which became normal by rotation, including one case (I. E. 101) of hyperdactyly.

³ Normal by resorption of original member of pair.

⁴ One case included in which the posture of the limb was abnormal.

two classes of experiments and have been summarized separately in table 2 (A and B). The differences will be taken up in connection with the consideration of each of the subgroups.

7. *Homopleural transplantations, dorsodorsal orientation.* This is in reality merely a control experiment and is a test of the effect

³³ Harrison, '15 and '18, p. 422.

of the operation as such on the development of the limb. A fore limb bud is carefully excised and either replaced in the same wound or else engrafted in normal position in another embryo from which the limb bud had been previously removed.

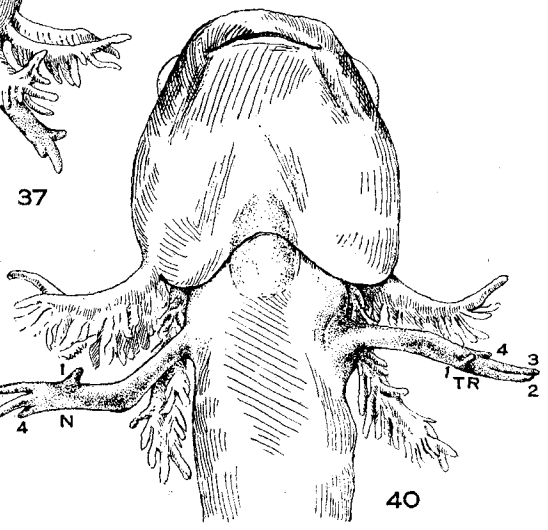
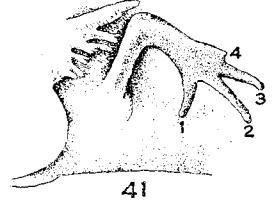
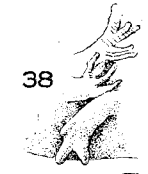
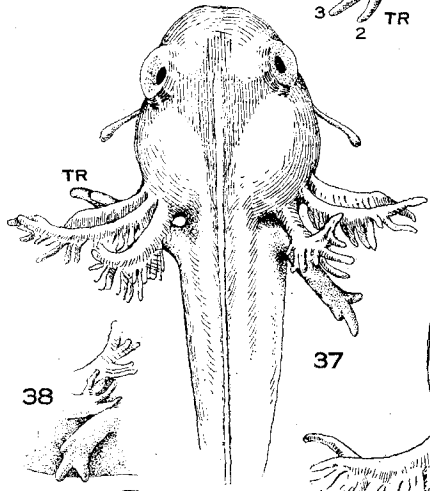
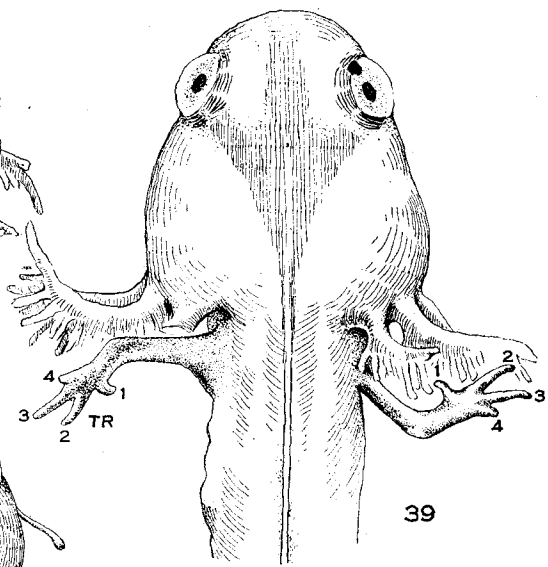
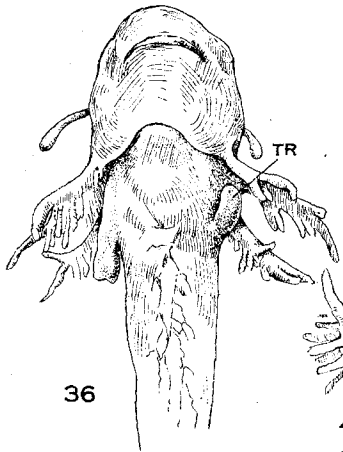
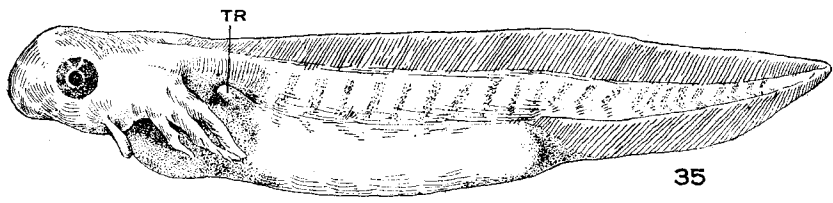
Only nine individuals were operated upon, in all of which the wounds were carefully cleaned. Normal limbs developed in all cases, though they were slightly retarded in the earlier stages of development in comparison with the unoperated limb of the opposite side. In six of the cases the pronephros was removed and in the other three it was left in. No difference was noted between the two sets. It may be safely concluded that the effect of the operation itself upon normal development is practically negligible.

8. *Homopleural transplantations, dorsoventral orientation.* In some of the cases of this series, as in the last, the limb bud was simply lifted and replaced after rotation through 180° . In the others the wound bed was first prepared in one embryo and the bud taken from another. The latter method is preferable and it was employed in all the later experiments.

The total number of experiments is one hundred and four, of which sixty-one were with cleaned wounds of proper size. The latter will be considered first, since the conditions of experimentation are more definitely known and there can be no doubt that the limbs were derived exclusively from the transplanted tissue.

Leaving out of consideration the twenty-three cases which died prematurely or gave rise merely to abortive or rudimentary limbs, there are thirty-eight cases which yielded positive results, as recorded in table 2A. The single limbs are in the minority and are of two kinds, reversed and non-reversed. The most remarkable case³⁴ (history on p. 124), which really gives the clue to the interpretation of the experiments of this group, is the one in which a limb of reversed asymmetry developed, a right limb on the left side, perfectly normal in form, function, and posture, as far as the last is possible on the wrong side of the body (figs. 35 to 41). The shoulder-girdle of this limb is also reversed and

³⁴ I. E. 64.



is quite separate from the rudimentary girdle developed from the tissues of the host (p. 59).

The ten cases in which normal non-reversed limbs developed are clearly contrary to the rule (p. 4). The records of these cases show that the end result is reached by a process of rotation at the shoulder-joint during development (figs. 56 to 58). They will be considered below (p. 40). The reduplicated limbs, of which there were twenty-seven, fall, like the single, into two groups. In the first the original bud developed into a limb of reversed asymmetry, while in the second it is not reversed.

Observations upon the earlier stages of the operated limbs show that in those cases which give reduplications, as well as in the case of the simple limb with reversed asymmetry (figs. 35, 42, 43, 49, 51, and 52), the original direction of pointing is either dorsal, anterior, or dorsoanterior, and more sharply lateral than normal. Likewise in the case of those that develop into single non-reversed limbs, the first pointing is more sharply lateral than normal, and also more dorsal, though only two³⁵ are recorded as pointing slightly anteriorly from the dorsal direction. This shows that the original tendencies of growth, immanent in the bud at the time of transplantation, are by no means inactive when it is in its new position. One or the other of two consequences of this growth tendency now ensues, indicating a sort of antagonistic reaction between the organization of the transplanted rudiment and that of the surrounding parts. The limb either continues to grow in an anterior or anterodorsal direction, in

Figs. 35 to 41 Orthotopic transplantation; left limb to left side inverted (*hom.dv.*), resulting in a normal right limb on the operated side. Exp. I. E. 64. *TR*, transplanted limb. $\times 10$.

Fig. 35 Lateral view, five days after operation.

Fig. 36 Ventral view, ten days after operation.

Fig. 37 Dorsal view, sixteen days after operation; transplanted limb covered by gills.

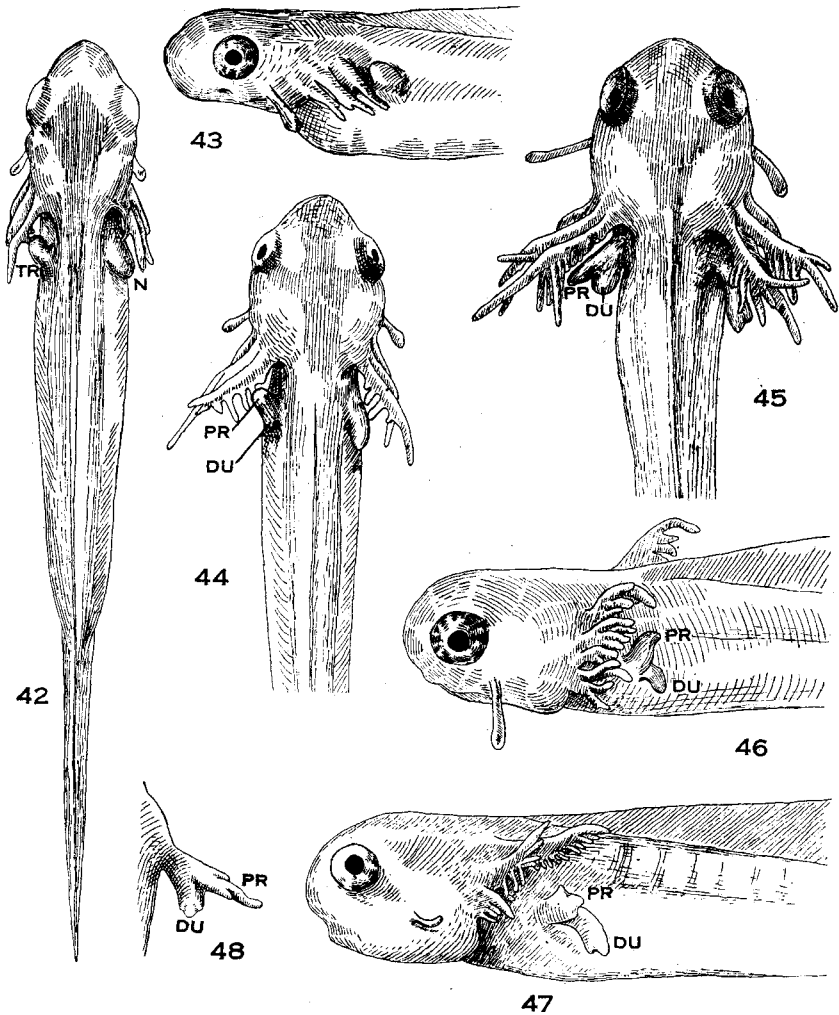
Fig. 38 Lateral view of limb, sixteen days after operation.

Fig. 39 Dorsal view, twenty-three days after operation.

Fig. 40 Ventral view, twenty-three days after operation.

Fig. 41 Lateral view, twenty-three days after operation.

³⁵ I. E. 49 and 94.



Figs. 42 to 48 Orthotopic transplantation; left limb bud to left side inverted (*hom.dv.*), resulting in duplicate limbs. Exp. I. E. 60. *N*, normal right limb bud; *TR*, transplanted bud; *PR*, primary member; *DU*, reduplicating member. $\times 10$.

Fig. 42 Dorsal view, five days after operation.

Fig. 43 Lateral view, five days after operation.

Fig. 44 Dorsal view, seven days after operation.

Fig. 45 Dorsal view, twelve days after operation.

Fig. 46 Lateral view, twelve days after operation.

Fig. 47 Lateral view of specimen preserved eighteen days after operation.

Fig. 48 Ventral view of limb, preserved specimen.

which case its asymmetry is reversed (figs. 36 to 39), just as in the corresponding class of heterotopic transplantations, or it gradually rotates towards its normal position while retaining its original prospective asymmetry (figs. 56 to 59). In the former alternative duplicate limbs are nearly always formed. Only in the one case, referred to above, did a perfect single limb arise. In the other alternative single limbs usually arise, though some of the cases of reduplication certainly belong to this group.

In the duplicities belonging to the first group the original limb bud continues to grow in an anterior direction and ultimately becomes a reversed limb. After a time a reduplicating bud appears on the posterior border of the original bud (fig. 44) and in the clearer cases grows into a homopleural limb in approximately normal posture (figs. 45 to 48). The original bud becomes a reversed limb which, together with the reduplicating member, may form an almost symmetrical complex.

Twenty-four of the thirty-one³⁶ cases of reduplicated limbs are probably of this type. Fifteen are certainly so,³⁷ and in three others³⁸ that are very similar all that is lacking to place them unequivocally in this group is a definite observation as to which bud was the primary one; six more cases³⁹ may also be interpreted in the same manner, though they are not sufficiently clear to insure that this is the only possible interpretation.

The degree of reduplication varies here, as in the other groups of experiments, from the condition where almost the whole arm is involved to that in which the hand is only partly double. In three cases⁴⁰ the anterior bud was much reduced (p. 49), the posterior bud becoming a somewhat irregular homopleural limb. In eleven cases there is only one reduplicating appendage, which is always posterior to the primary (figs. 43 to 48), while in the remaining twelve⁴¹ there are evidences of further doubling, usu-

³⁶ Four cases are considered here which are not included in the tabulation on account of the fact that the wound was only 3 somites in diameter (I. E. 39, 41, 44 and 45).

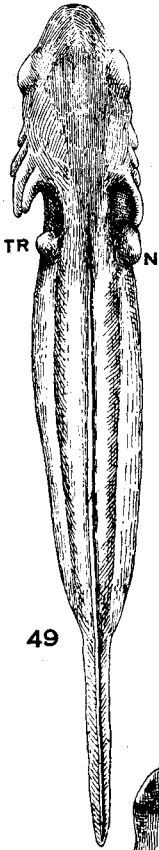
³⁷ I. E. 48, 60, 62, 63, 66, 72, 74, 75, 81, 85, 87, 89, 91, 92, and 96.

³⁸ I. E. 44, 45, and 52.

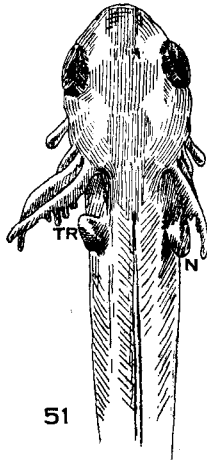
³⁹ I. E. 39, 68, 70, 93, 100, 102.

⁴⁰ I. E. 92, 93, 100.

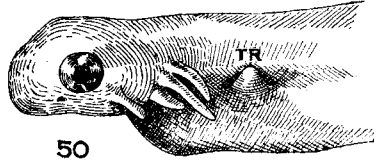
⁴¹ I. E. 39, 45, 62, 63, 66, 72, 75, 81, 85, 87, 91, and 93.



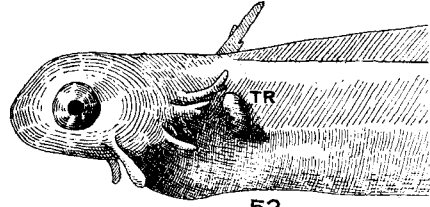
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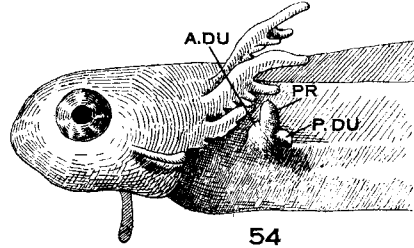
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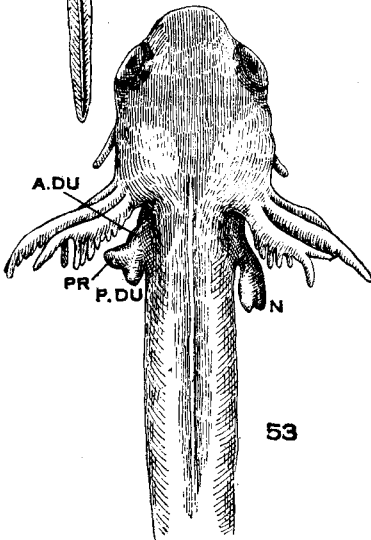
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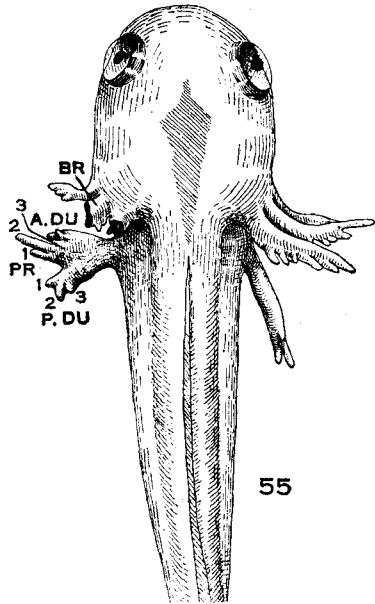
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55

ally on the anterior side of the original (figs. 49 to 55 and 61). When the latter condition arises and the anterior reduplicating member is sufficiently developed, it is seen that it, too, is mirrored from the original member and is homopleural (fig. 55). In one case there are three complete hands, one of which has two of its digits doubled.⁴² The plane from which the posterior reduplicating member is mirrored in the final form of the limb varies from radial to dorsal (figs. 3 and 4) and is usually intermediate between these two extremes (p. 13). Nineteen cases follow this rule, three are indeterminate and there is only one positive exceptional case, in which the mirror plane is ulnodorsal.⁴³ When there is also an anterior reduplicating member, it is generally mirrored from a plane 180° around the limb axis from the first; i.e., ulnar, ulnopalmar, or palmar.

The reduplications belonging to the second group are more restricted and less certain of diagnosis. The limb bud retains its original prospective asymmetry, reaching an approximately normal position by rotation, and reduplication is much less extensive, involving in most cases the digits only (fig. 62). Three cases⁴⁴ almost certainly belong to this group, and there may be two others.⁴⁵

Of the two remaining cases of reduplication, one died too young; in the second⁴⁶ the supernumerary limb was of the same side as the primary and was quite distinct from it. This is a very unusual condition, but the transplanted bud in this case was

Figs. 49 to 55 Orthotopic transplantation; left limb bud to left side inverted (*hom.dv.*), resulting in limb with two reduplicating members. Exp. **E**. 63. *N*, normal right limb bud; *TR*, transplanted left bud; *PR*, primary limb; *A.DU*, anterior, and *P.DU*, posterior reduplicating members. $\times 10$.

Fig. 49 Dorsal view, four days after operation.

Fig. 50 Lateral view, four days after operation.

Fig. 51 Dorsal view, seven days after operation.

Fig. 52 Lateral view, seven days after operation.

Fig. 53 Dorsal view, ten days after operation.

Fig. 54 Lateral view, ten days after operation.

Fig. 55 Dorsal view of specimen preserved seventeen days after operation. Gills (*BR*) removed to show limb. 1 to 3, numbers of digits.

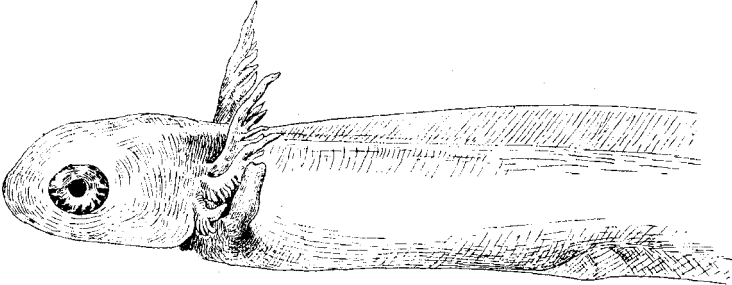
⁴² I. E. 87.

⁴³ I. E. 72.

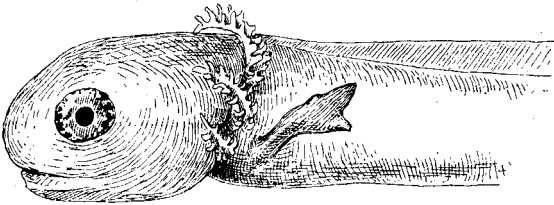
⁴⁴ I. E. 86, 88, 90.

⁴⁵ I. E. 41, 59.

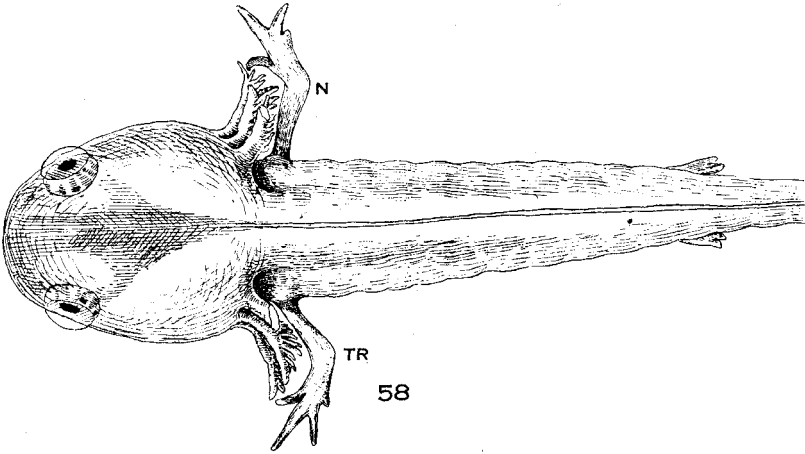
⁴⁶ I. E. 38.



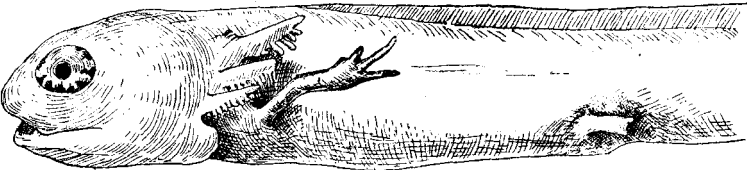
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larger than usual (four somites in diameter), and it is possible that the reduplicating bud, growing from near its anterior border, was uninfluenced by the primary limb and hence was not mirrored.

There remain for consideration those cases in which a single non-reversed limb developed. As in the other cases, the limb bud in these showed at first the consequences of abnormal orientation. When first observed it pointed more sharply laterally and more dorsally (less posteriorly) than normal. Two even pointed dorsally and slightly anteriorly. In the course of development the limb gradually changed its posture and ultimately came to a perfectly normal posture by a process of rotation at the shoulder-joint (figs. 56 to 59). Ten such cases were obtained,⁴⁷ though in several of them⁴⁸ it is possible that reversal may have been brought about by early reduplication and suppression of the original bud, as described in the next section (p. 49). In one of these⁴⁹ a supernumerary radial digit was present, but this is to be regarded as a case of hyperdactyly rather than one of mirrored reduplication. In one case⁵⁰ the limb which originally developed showed irregularities in the digits. The arm was then amputated above the elbow, and the appendage which regenerated was in every respect normal. This case is of considerable interest in showing that the abnormal condition which produces reduplication is not necessarily stamped upon the whole structure, but may be due to some local mechanical disturbance.

In reviewing this group of experiments, it is clear that the first two results, i.e., single reversed limbs and most of the reduplications, come under the same scheme. There is a primary reversal of asymmetry, without reduplication in the first case and accom-

Figs. 56 to 59 Orthotopic transplantation; left limb bud inverted (*hom.dv.*). Limb reaches normal posture by rotation. Exp. I. E. 49. *N*, normal (right) limb; *TR*, transplanted (left) limb.

Fig. 56 Eleven days after operation.

Fig. 57 Twenty-one days after operation.

Fig. 58 Thirty-eight days after operation.

Fig. 59 Preserved specimen, killed at thirty-nine days.

⁴⁷ I. E. 49, 55, 69, 71, 73, 77, 84, 94, 99, and 101.

⁴⁸ For instance, I. E. 77.

⁴⁹ I. E. 101.

⁵⁰ I. E. 71.

panied by reduplication in the second. In the latter the secondary bud, being the mirror image of the other, is again reversed back to the original prospective asymmetry of the transplanted bud. This then occupies an approximately normal position and may function to a considerable extent as a normal limb, though impeded by the connection with its mate. These two results are directly comparable to those of the heterotopic transplantations of the corresponding class (fig. 2).

The third result, in which normal homopleural limbs develop, reaching their normal position gradually by rotation, is fundamentally different. Here no reversal occurs; the limb bud begins its development as a self-differentiating system, but later, under the stress of the changed relation to its environment, it comes again into normal posture.

What determines whether the limb bud shall reverse its asymmetry or rotate back to its normal posture? The earlier experiments of this series afforded no satisfactory answer to this question. It was certainly not due to the size of the wound, the mode of preparation of the wound, the presence or absence of the pronephros, or the age of the embryo. What seemed most likely was that there were minor accidental differences in the amount of rotation to which the limb bud was subjected at the time of operation. It was conceivable, for instance, that if the disc were rotated anteriorly around the dorsal semicircumference of the wound a little less than 180° (fig. 60), the reversing effect of its organic environment might be lessened and the rotation back to normal position facilitated; in this case a normal non-reversed limb would result. If, on the other hand, the grafted bud were rotated 180° or slightly more, the reversing effect might be at a maximum and rotation most impeded, in which case a heteropleural limb or twin limbs would arise.

Experiments made in the spring of 1917 had for their main purpose the testing of this hypothesis. Operations were done in pairs; in one case the limb disc was rotated about the dorsal circumference in a posteroanterior direction slightly more than 180° and in the other slightly less, extremes being probably not more than 190° and 170° , respectively (see histories on pp. 125-6).

The results are not altogether conclusive, though they point to the correctness of the hypothesis. Twenty-three operations were done, of which seventeen yielded positive results. Ten cases harmonize with the hypothesis; four are doubtful, and three are contrary to it (figs. 61 and 62). When the difficulty of exactly estimating the degree of rotation is considered, many apparent exceptions must be expected, and a far greater number of experiments would be necessary to eliminate statistically the effect of this uncertainty. As a matter of fact, the records of the cases classed as surely exceptional give evidence that the amount

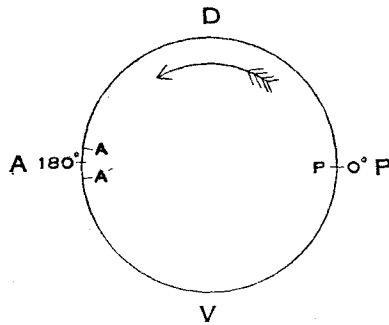


Fig. 60 Diagram showing difference in amount of rotation in two sets of experiments. The circle represents the left limb bud and the arrow the direction of rotation. A, D, P, V, the direction of the cardinal points of the embryonic body, anterior, dorsal, posterior, and ventral, respectively.

of rotation at the time of operation was probably not correctly estimated, for the first direction of pointing (p. 11) in all of these cases is not according to expectation.

The thirty-seven cases in which the wound bed was not entirely cleaned of mesoderm may now be considered. These show a marked contrast to those with cleaned wounds, inasmuch as there are very few reduplications and a very large proportion of normal non-reversed limbs. Thus out of twenty cases which yielded positive results eighteen or 90 per cent are normal, as compared with 23.8 per cent (ten cases) in the clean-wound class. Only one case (5 per cent) had a reduplicated limb, as compared with thirty-one duplicities (73.8 per cent) in the clean-wound class.

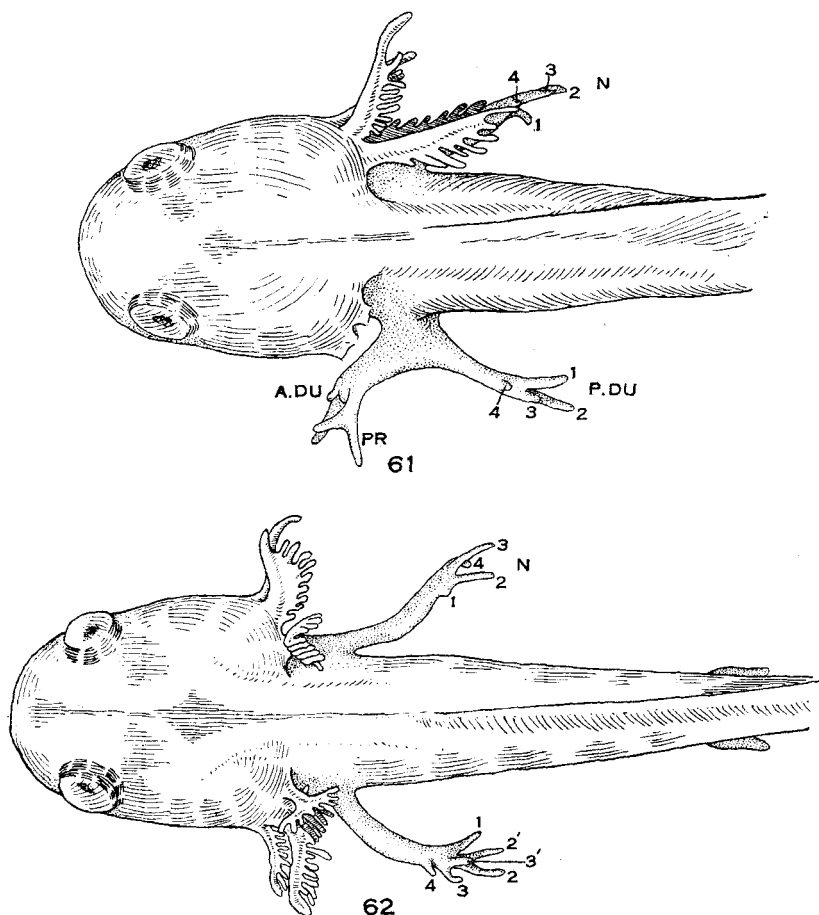
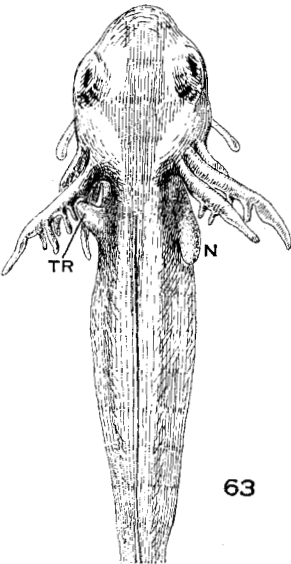


Fig. 61 Orthotopic transplantation; left limb bud to left side (*hom.dv.*), rotated slightly more than 180° from its normal position, *i.e.* P to A' in fig. 60. Exp. I. E. 85. Primary member (*PR*) is a right; posterior reduplicating member (*P.DU*) is a nearly normal left; anterior reduplicating member (*A.DU*) partly coalesced with primary.

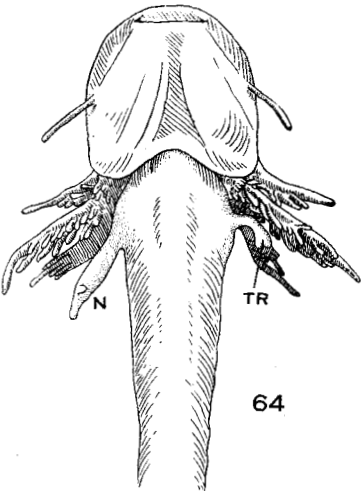
Fig. 62 Orthotopic transplantation; left limb bud to left side (*hom.dv.*), rotated slightly less than 180° (*i.e.* P to A in fig. 60). Exp. I. E. 86. The transplanted limb is primarily a left, having reached its normal posture by rotation; second (2') and third (3') digits, reduplicated. $\times 10$.

Since these differences can scarcely be accounted for on the ground of different degree of rotation of the limb buds at operation (p. 40), it would seem that the few mesoderm cells remaining in the wound bed must have exerted some influence upon the developing limb. This does not mean that the limbs which do develop in such cases arise solely by a process of regeneration from the host. In fact, the rate of development, which is only slightly retarded below the normal, precludes such an interpretation. What probably does take place is an intermingling of cells from the host and the graft, with the result that the former, acting in the same sense as the environment with which they are in harmonic relation, counteract the tendency of the inverted elements to reverse their asymmetry. This was, however not shown to the same degree in the corresponding experiments with superposed limbs (p. 65).

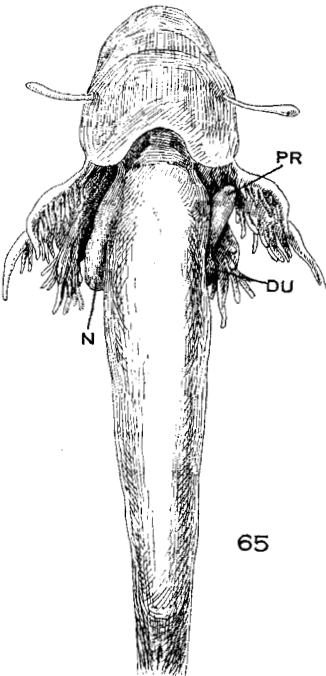
9. *Heteropleural transplantations, dorsodorsal orientation.* Forty-nine cases were operated upon in this way and thirty-one lived long enough to yield definite results (table 2). By far the largest number of these (twenty-five) developed reduplications of one kind or other. Five cases gave rise to limbs with reversed asymmetry, i.e., to limbs which developed to fit their new surroundings, though one of these was considerably underdeveloped. One yielded a somewhat imperfect non-reversed limb and four were rudimentary. These results seem altogether divergent from the corresponding heterotopic transplantations. An examination of them shows, however, that fundamentally they accord with the latter, complete agreement being modified, by a second factor, which may suppress the original bud in favor of the reduplicating member. The normal environment of the transplanted bud and the concomitant normal functioning seem to facilitate this transformation. Moreover, there is no hard and fast line between the different results just enumerated, and the individual cases may be taken as forming a series, beginning with the single non-reversed and ending with the single reversed limb. The reduplications are intermediate. They will be considered in this order.



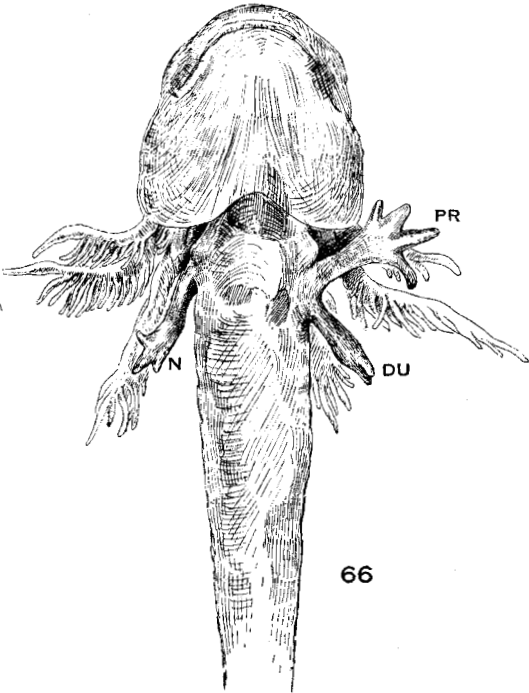
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The history of the case in which a limb of original prospective asymmetry developed⁵¹ is given on page 126. In this individual the limb bud, as it began to grow, pointed anteriorly (fig. 63), and continued to grow in that direction. Though it remained small and imperfect (fig. 64), it is clearly a right limb on the left side (not reversed).

The cases which formed reduplications began their development in the same manner. The first direction of pointing is recorded as anterior in nine cases, anterodorsal in eight, and anterolateral in five. Three are described as pointing dorsally and one laterally. Thus these limbs all show in greater or less degree the initial effect of their original growth tendency. Growth of the bud continues then for some days in a general anterior direction, but sooner or later a reduplicating bud appears, usually at the posterior border of the original bud, and this grows in most cases into an appendage equal to or exceeding the original in size. If the reduplicating bud does not appear until late, then the original one may attain considerable size and remain, for some time at least, the principal member (figs. 65 and 66). If it appears earlier, but not until the original bud has a good start, then the two members may remain of almost equal size (figs. 67 to 71). In other cases, where the reduplicating bud begins to grow early, it soon gains the upper hand, and the original may be reduced to an atrophic or rudimentary limb (figs. 72 to 74). This condition leads over to the single reversed appendage in which the original bud is reduced to a spur or nodule

Figs. 63 and 64 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 87. Resulting limb, though defective, is reversed. *N*, normal right limb; *TR*, transplanted limb. $\times 10$.

Fig. 63 Dorsal view, seven days after operation.

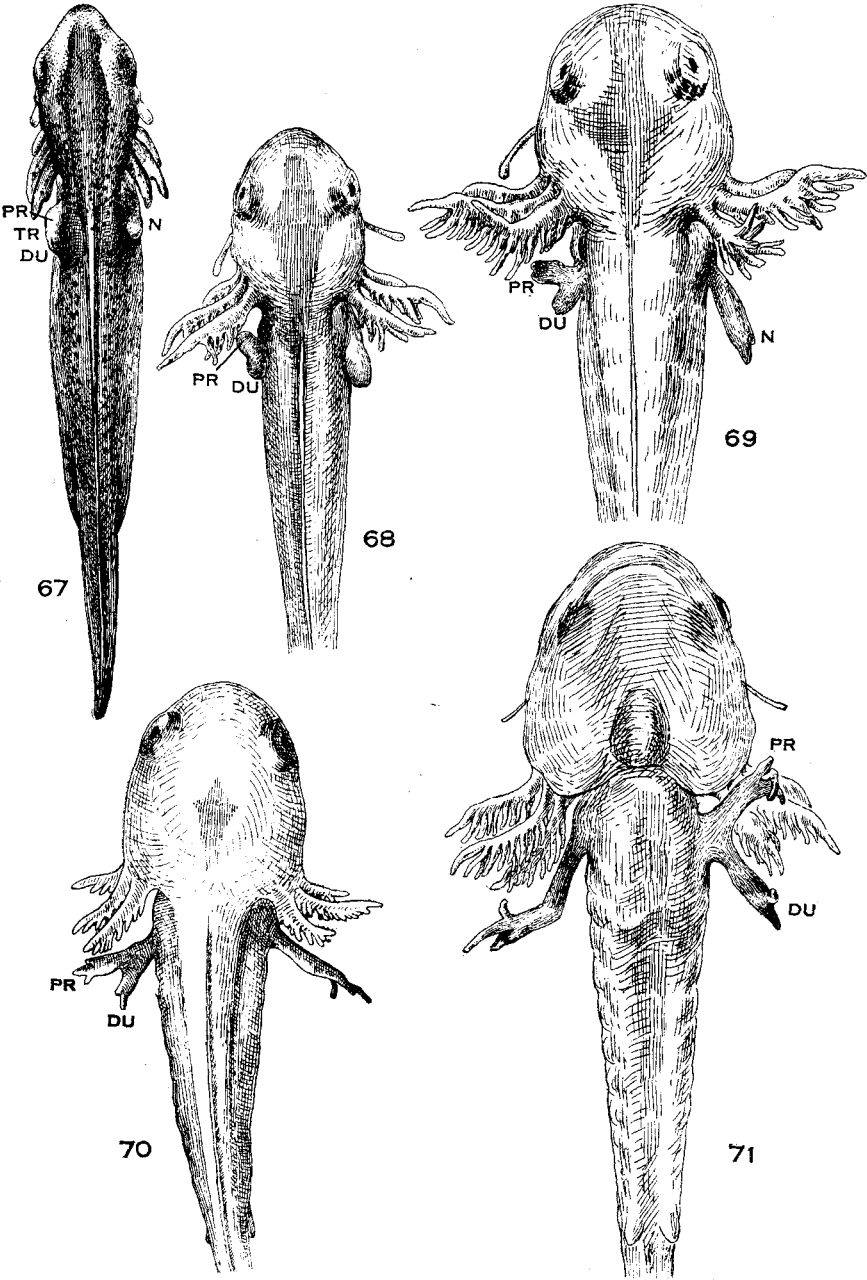
Fig. 64 Ventral view of specimen preserved sixteen days after operation.

Figs. 65 and 66 Orthotopic transplantation; right limb to left side (*het.dd.*). Exp. R. E. 96. Resulting limb reduplicated.

Fig. 65 Ventral view, ten days after operation; primary limb (*PR*) points into gills; reduplicating bud (*DU*), just appearing. $\times 10$.

Fig. 66 Ventral view, nineteen days after operation; primary member (*PR*) shows evidence of reduplication of hand; reduplicating member (*DU*) is in approximately normal position. $\times 10$.

⁵¹ R. E. 87.



(p. 49). The reduplicating limb is, of course, mirrored from the original and hence corresponds to the side of the body on which it is grafted.

Being placed in the position of the normal limb, the reduplication is favorably situated with regard to blood and nerve supply, and it is probably on this account that so many of them develop into functional appendages. In this respect the experiments of this group differ considerably from the preceding (homopleural inverted), where there is a greater tendency for the original member to retain its predominant condition. Otherwise the course of development in the two groups is strikingly alike.

For the study of the details of reduplication, nineteen cases are available, including one case with small wound not considered in the table. Seven others were preserved at relatively early stages in order to investigate the internal processes involved. Histories of several typical cases are given in the appendix.

Seventeen of the cases conform to the main type and do not differ materially from those considered in the last section. As in the homopleural inverted limbs (p. 35), the degree and character of the reduplication vary much from case to case. In some the digits alone are doubled, and at the other extreme we find two almost entirely separate limbs. In thirteen individuals a second reduplicating limb formed on the anterior side of the original. These usually did not develop so completely as the limbs arising from the posterior buds, and the reduplication often involved only the distal part of the manus, with the digits more or less symmetrically placed. The anterior reduplications are mirrored from the ulnar or ulnopalmar surface and occasionally from the

Figs. 67 to 70 Orthotopic transplantation; right limb to left side (*het.dd.*). Exp. R. E. 70. Resulting limb reduplicated. *N*, normal right limb bud; *TR*, transplanted limb bud; *PR*, primary member; *DU*, reduplicating member. $\times 10$.

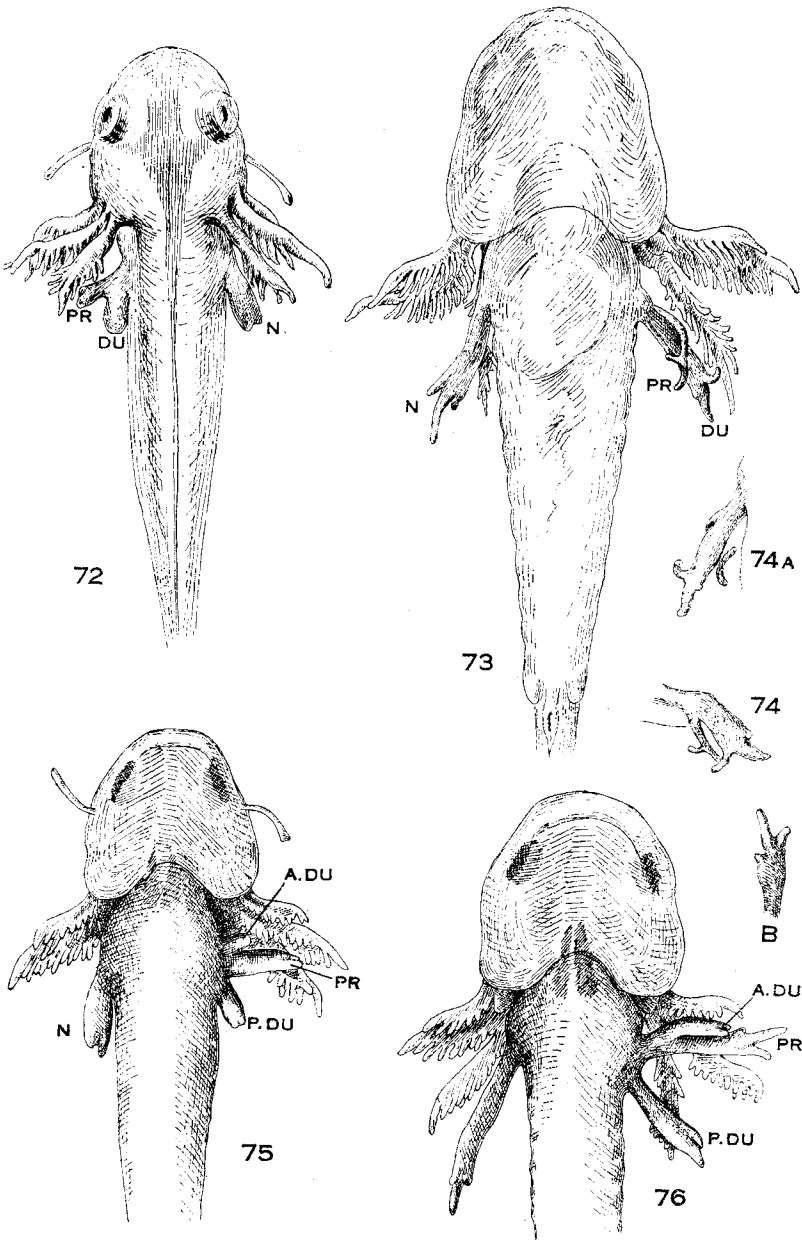
Fig. 67 Dorsal view, five days after operation. The transplanted bud already gives evidence of reduplication.

Fig. 68 Dorsal view, ten days after operation. Reduplicating bud is large and in normal position.

Fig. 69 Dorsal view, sixteen days after operation.

Fig. 70 Dorsal view of specimen preserved thirty days after operation.

Fig. 71 Similar case (Exp. R. E. 71); ventral view, twenty-two days after operation. $\times 10$.



palm. One case⁵² had three almost complete separate appendages (figs. 75 and 76).

Two cases of the nineteen⁵³ gave rise to an anterior reduplicating bud only, which in both individuals was mirrored from the ulnopalmar surface. Owing to the position of the reduplicating bud in front of the original heteropleural limb, it could not be brought into normal posture (fig. 77).

There remain to be considered the five cases in which the asymmetry of the transplanted bud was reversed. These are of the utmost interest in showing how a secondary factor (reduplication) may so modify the result that the rules of symmetry seem not to hold. They show more than any others the necessity of having complete histories in each case, for the manner in which the end result is reached is of cardinal importance for the correct interpretation of the process. As stated above, these cases gradate into those in which duplicate limbs arise, so that the classification is somewhat arbitrary, the single-limb condition being a masked reduplication. Like the others, they begin their development with growth of the bud in an anterior direction (figs. 83 and 86). Then a posterior reduplicating bud makes its appearance, and the original bud is rapidly reduced (figs. 84 and 85 and 87 to 89) in relative importance, becoming a spur or nodule attached to the latter. The history of a typical case is given on page 128.

Figs. 72 to 74 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 74. Reduplication with atrophic primary member. *N*, normal right limb; *PR*, primary transplanted limb; *DU*, reduplicating member. $\times 10$.

Fig. 72 Dorsal view, twelve days after operation; the primary limb already appears as an appendage of the reduplicating member.

Fig. 73 Ventral view, twenty-one days after operation.

Fig. 74 Lateral view of transplanted limb.

Fig. 74A Dorsal view of same.

Figs. 75 and 76 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 133. Two almost perfect reduplicating members, one anterior (*A.DU*) and one posterior (*P.DU*) to the primary (*PR*). The relations of these limbs are just as in the diagram, fig. 4B. $\times 10$.

Fig. 75 Ventral view, eleven days after operation.

Fig. 76 Ventral view, nineteen days after operation. *B*, lateral view of posterior reduplicating member.

⁵² R. E. 133, p. 127.

⁵³ R. E. 120 and 134.

In one individual⁵⁴ the growth in an anterior direction was well marked before the posterior reduplicating bud appeared (figs. 78 to 80). The anterior one was finally reduced to a spur, which, however, was considerably longer than in the next two cases of the series.⁵⁵ This is in reality the border-line case and might be

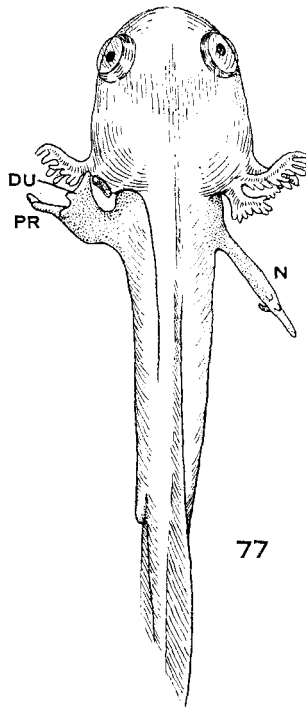


Fig. 77 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 134. The reduplicating member (*DU*) is anterior to the primary (*PR*). Preserved specimen, eighteen days after operation. $\times 10$.

classed equally well as a reduplication. In one individual⁵⁶ the anterior bud was reduced to a slight scar, while in another⁵⁷ (figs. 90 to 92) it had only a very slight development and was soon entirely resorbed.

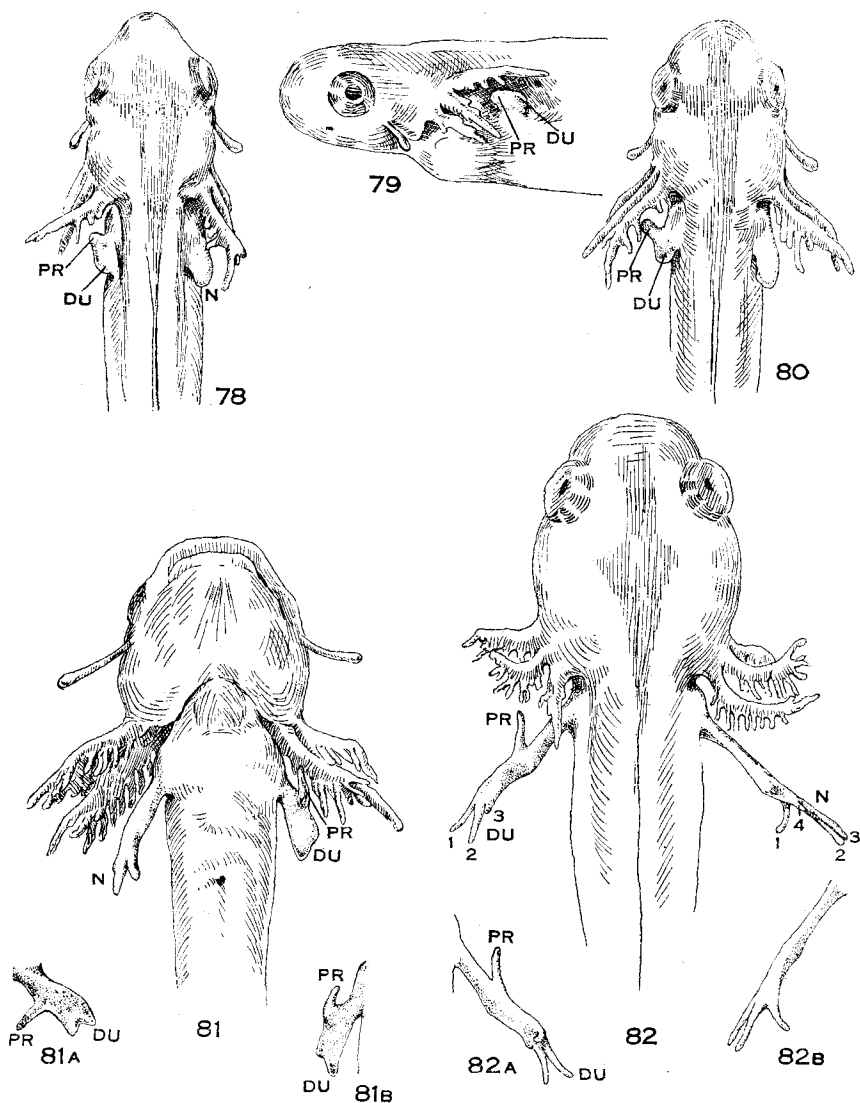
The cases in which the wounds were not cleaned (for the most part the earlier experiments made in 1911 and 1912) were seven-

⁵⁴ R. E. 108.

⁵⁵ R. E. 77 and 69.

⁵⁶ R. E. 91.

⁵⁷ R. E. 95.



Figs. 78 to 82 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 108. Reduplicating member (*DU*) in normal position, developed at expense of original (*PR*), which is reduced to a long spur. *N*, normal right limb; 1 to 4, numbers of digits. $\times 10$.

Fig. 78 Dorsal view, six days after operation. Reduplicating bud already more massive, though less prominent, than the primary.

Fig. 79 Lateral view, six days after operation.

Fig. 80 Dorsal view, eight days after operation.

Fig. 81 Ventral view, fifteen days after operation.

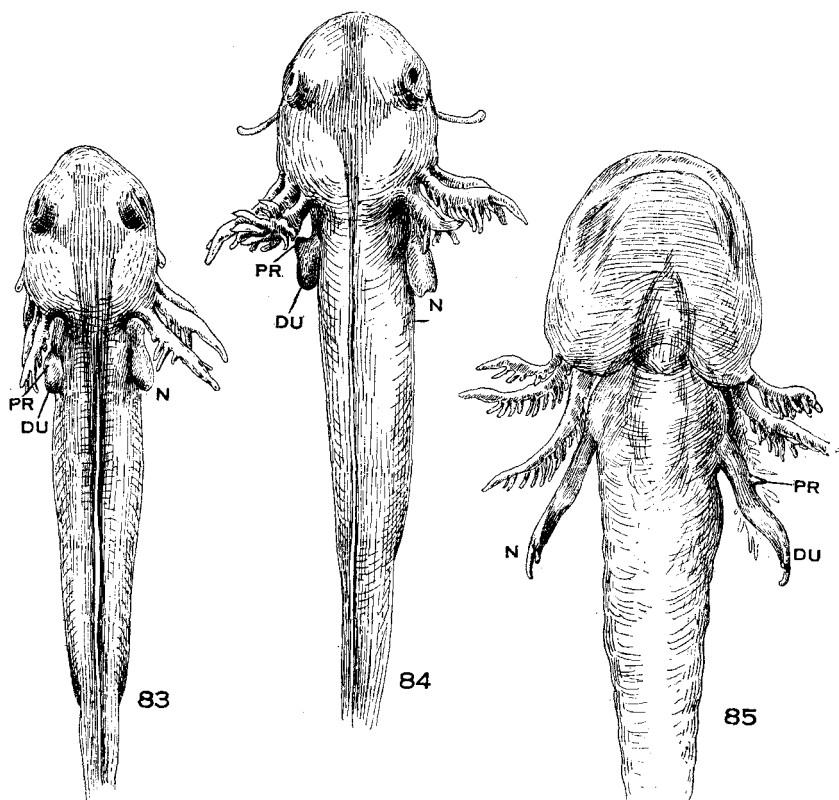
Fig. 81A Lateral view of limb.

Fig. 81B Dorsal view of same.

Fig. 82 Dorsal view, thirty-three days after operation.

Fig. 82A Ventral view of transplanted limb.

Fig. 82B Ventral view of normal right limb.



Figs. 83 to 85 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 77. Primary member (*PR*) reduced to a small spur on the reduplicating member (*DU*) which has become a normal left limb. $\times 10$.

Fig. 83 Dorsal view, eight days after operation. Reduplication of transplanted bud already visible. $\times 10$.

Fig. 84 Dorsal view, eleven days after operation. Reduplicating bud larger than primary.

Fig. 85 Ventral view, twenty-six days after operation.

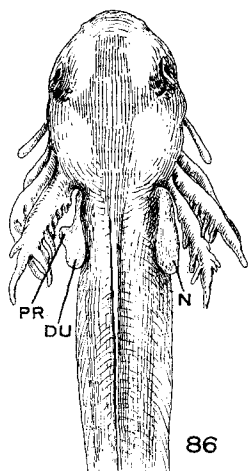
Figs. 86 to 89 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 69. Primary member (*PR*) reduced to a nodule on the reduplicating one (*DU*). $\times 10$.

Fig. 86 Dorsal view, nine days after operation.

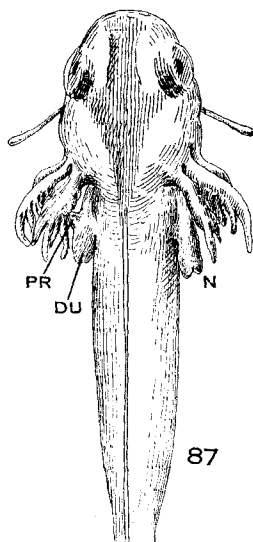
Fig. 87 Dorsal view, twelve days after operation.

Fig. 88 Dorsal view, sixteen days after operation.

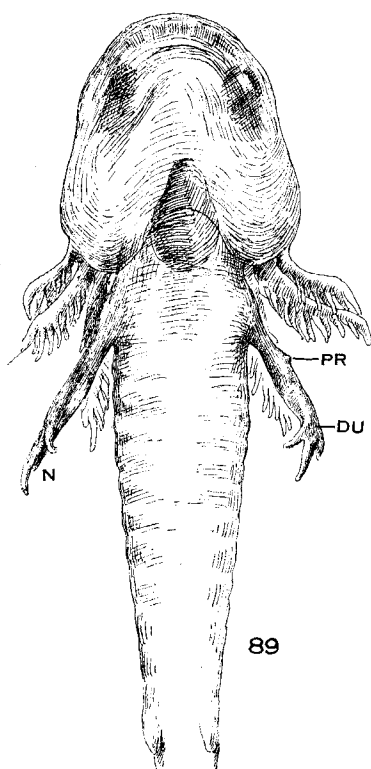
Fig. 89 Ventral view, twenty-six days after operation.



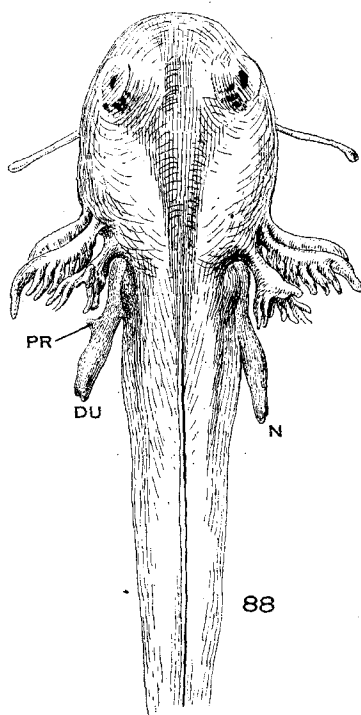
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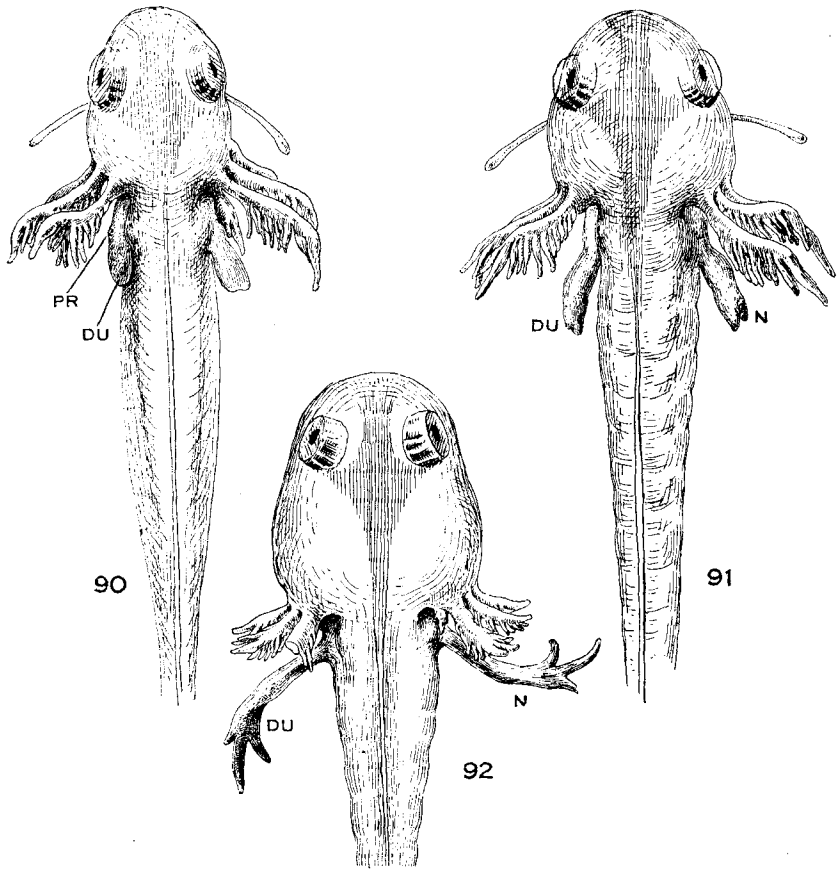


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teen in number, in thirteen of which limbs developed. The distribution of these in the various groups does not show any significant differences from the cases with cleaned wounds. Eight (61.5 per cent) gave reduplications and three (23.1 per cent)



Figs. 90 to 92 Orthotopic transplantation; right limb to left side (*het.dd.*). Exp. R. E. 95. Primary bud (*PR*) entirely obliterated; the reduplicating member (*DU*) a normal left limb. *N*, normal right limb. $\times 10$.

Fig. 90 Dorsal view, ten days after operation; the primary bud (*PR*) shows as a slight nodule.

Fig. 91 Dorsal view, thirteen days after operation.

Fig. 92 Dorsal view, thirty-three days after operation. Owing to weakness of wrist and hand extensors, the larva has difficulty in bringing its hand to normal posture.

developed into reversed limbs. In at least two of the individuals reversal was brought about by reduplication. The third is uncertain. Of the two cases (15.4 per cent) recorded as developing without reversal, only one is clear. The other died at fifteen days and was lost, so that the notes made from the living specimen could not be verified.

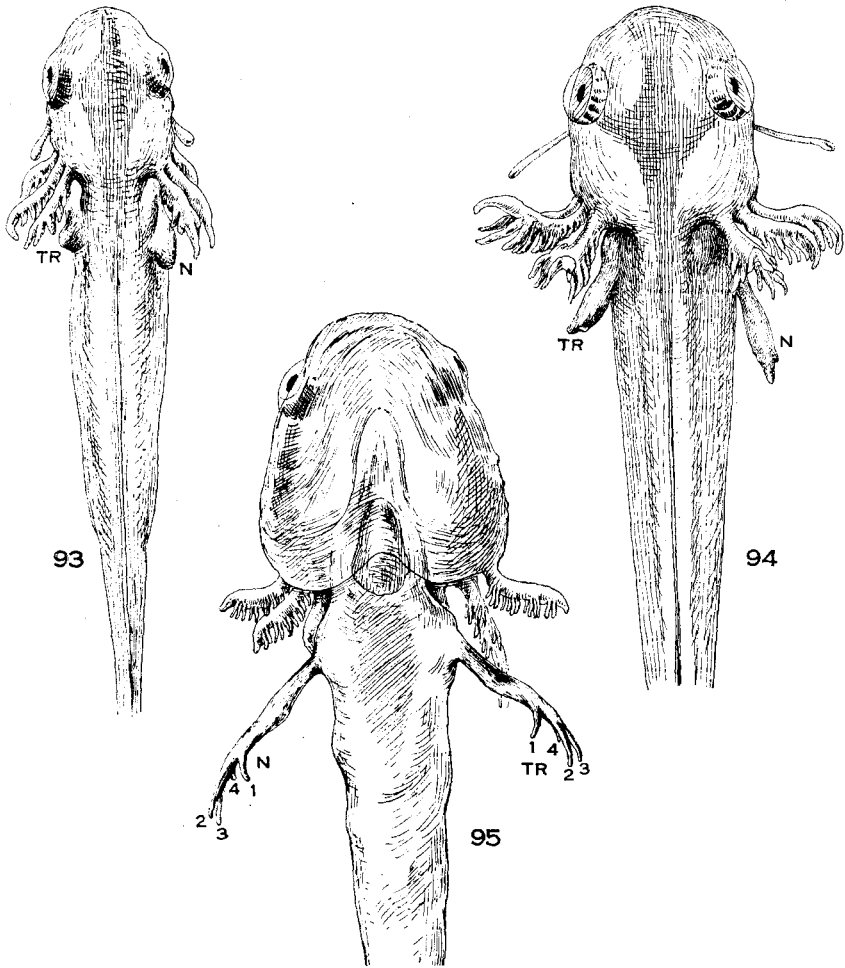
10. *Heteropleural transplantations, dorsoventral orientation.* Twenty-six experiments were made in this group. In five out of the twenty-three individuals that lived the transplanted tissue was resorbed, and in two others the resulting appendage was imperfect or rudimentary, so that sixteen positive cases are available. Single limbs with reversed asymmetry developed in fifteen, and only one gave rise to a duplicate structure (table 2).

This group of cases shows that from the first the transplanted limb buds behave differently from those implanted in dorsodorsal orientation. When they begin to become prominent, they point dorsoposteriorly in most cases, though sometimes more sharply dorsally and frequently more laterally than the normal bud (fig. 93). As the bud grows, it thus occupies a nearly normal position, though it may continue for some time to project more sharply to the side or more dorsally than the normal limb (figs. 94 and 96). When the third and fourth digits develop, they are, however, not formed on the ventral border of the appendage, as they would be if the original asymmetry were preserved, but they come in on the dorsal border, just as in the normal hand of the side to which they were transplanted (figs. 2, 95, 98, 99, and 102). The palm of the hand, as in the normal individual, faces the body of the larva. Besides the one case in which reduplication actually occurred, there were three others in which slight indications of doubling appeared, only to disappear later, the more ventrally lying bud soon being resorbed. Histories of typical cases are given on page 128.

In all of these cases there was some retardation of development, and in some⁵⁸ it was very marked. A somewhat greater amount of tissue is lost by disintegration when the limb is placed dorso-ventrally than when placed otherwise, since the bud does not

⁵⁸ *E. g.* R. E. 90.

fit into the wound so exactly. Besides this there seems to be a time factor involved in the reversal, which would indicate that the dorsoventral axis of the limb elements is slightly differentiated, though not irreversibly so.



Figs. 93 to 95 Orthotopic transplantation; right limb to left side (*het.dev.*). Exp. R. E. 80. Transplanted limb (*TR*) becomes a normal left. *N*, normal right limb; 1 to 4, numbers of digits.

Fig. 93 Dorsal view, six days after operation. Transplanted bud much smaller than normal.

Fig. 94 Dorsal view, fourteen days after operation.

Fig. 95 Ventral view, forty-two days after operation.

The sole case in which a double appendage resulted⁵⁹ is interesting, inasmuch as it shows that the primary bud grows into a reversed limb, while the reduplicating bud has the original asymmetry (figs. 103 to 105). This is the opposite of the result obtained when the bud is implanted in dorsodorsal orientation. (History on p. 129.)

In the experiments with wounds not cleaned the proportion of reduplications is considerably larger—seven out of fifteen, or 46.7 per cent, as against one case in sixteen in the clean-wound group. There were eight cases (53.3 per cent) in which normal limbs with reversal of asymmetry developed, as against fifteen (94 per cent) in the case of the clean-wound experiments.

11. *The shoulder-girdle in orthotopic transplantations.* The above account has dealt only with the external features of the limb. The shoulder-girdle is likewise of interest.

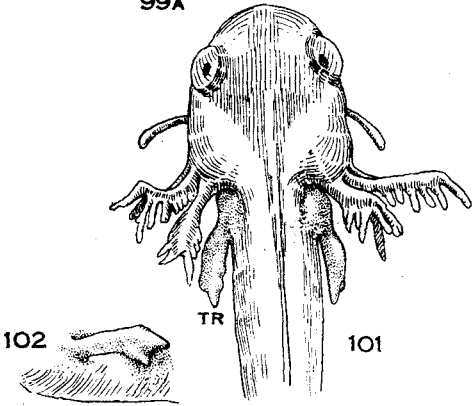
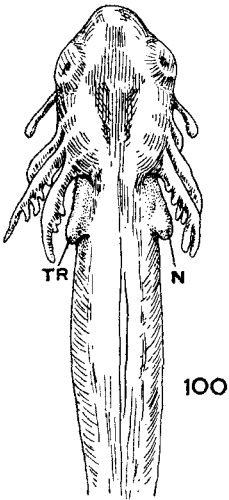
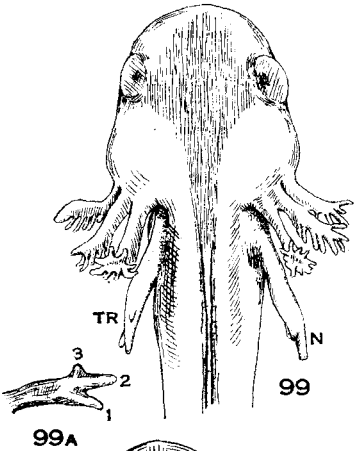
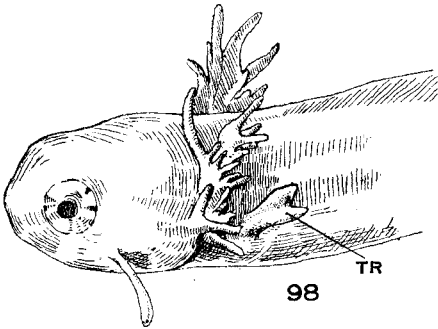
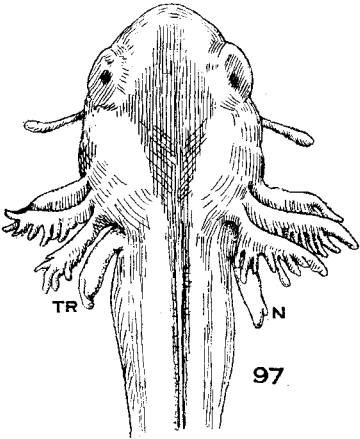
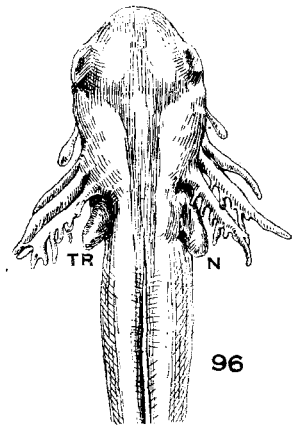
As the heterotopic transplantations show, a small portion of the girdle surrounding the glenoid cavity always develops in connection with the grafted limb. After extirpation of the limb bud, however, the outlying regions of the girdle, including the suprascapula and portions of the procoracoid and coracoid, develop from cells that are left in the host.⁶⁰ It was to have been expected, therefore, that relations of harmony or disharmony would manifest themselves in the shoulder-girdle in orthotopic grafts. Study of serial sections of some of the cases shows that this is usually the case. Twelve individuals, belonging to three different groups have been examined in this way.

The three harmonic grafts (*het.dv*) all show girdles that are normal, except that they are somewhat underdeveloped. There has obviously been a union of host and graft tissues to form a normal whole, in spite of the fact that the transplanted bud was from the opposite side of the body.

The nine disharmonic grafts all show some form of irregularity, and in nearly all cases there is some sort of double girdle with reversal of the part that is derived from the graft. The condition of the girdle is complicated by the reduplication of the free

⁵⁹ R. E. 93.

⁶⁰ Cf. Detwiler, '18, p. 503, and Harrison, '18, p. 429.



limb which takes place in most cases (table 2). It is more readily understood in the two cases in which a single limb of opposite asymmetry is present.

In the first of these,⁶¹ in which a limb bud from the same side of the body was implanted in inverted position (p. 32, figs. 39 to 41), there are two entirely distinct shoulder-girdles. The anterior one has no connection with the other and is undoubtedly derived from the host, having the characteristics of girdles which develop after extirpation of the limb bud. The scapula and suprascapula are already joined in cartilaginous union with the procoracoid, but the coracoid is connected with the latter by ligament only. The girdle belonging to the transplanted limb is mainly posterior to the other, though there is some overlapping. It is large to have developed from a transplanted bud, but it has the characteristics of such. There is a distinct procoracoid process as well as a large coracoid, both of which project posteriorly from the glenoid cavity. This girdle is clearly reversed, as is the transplanted limb which is connected with it.

The other single disharmonic limb is the one developed from a bud taken from the opposite side of the body.⁶² The limb itself is atrophic (fig. 64). The girdle is double, but the ventral parts of the two members are fused. The suprascapula, which is single and belongs to the host, is not connected with the rest. The

Figs. 96 to 99 Orthotopic transplantation; right limb to left side (*het.dv.*). Exp. R. E. 107.

Fig. 96 Dorsal view, eight days after operation. Transplanted bud (*TR*) smaller than normal (*N*) and more pointed.

Fig. 97 Dorsal view, thirteen days after operation.

Fig. 98 Lateral view, thirteen days after operation.

Fig. 99 Dorsal view, nineteen days after operation.

Fig. 99A Lateral view of transplanted limb. 1 to 3, numbers of digits.

Figs. 100 to 102 Orthotopic transplantation; right limb bud to left side (*het.dv.*). Exp. R. E. 116. Transplanted limb (*TR*) becomes a normal left. $\times 10$.

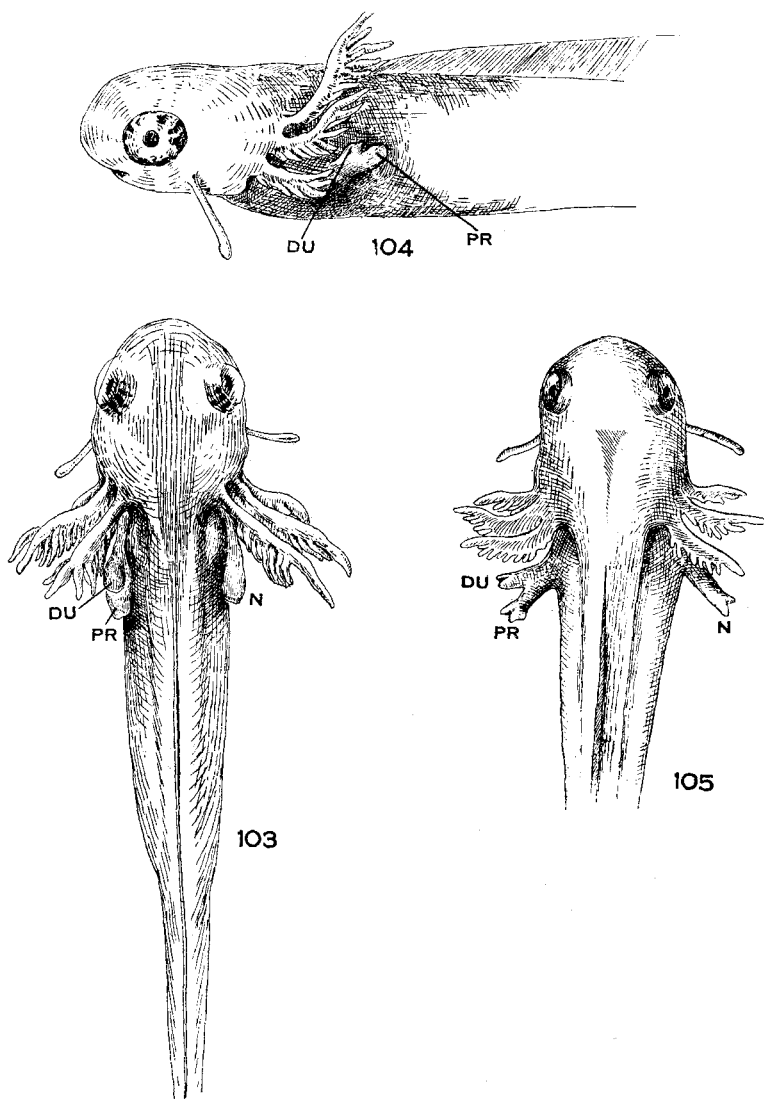
Fig. 100 Dorsal view, seven days after operation. Transplanted bud only slightly smaller than normal.

Fig. 101 Dorsal view, thirteen days after operation.

Fig. 102 Lateral view, same age.

⁶¹ I. E. 64.

⁶² R. E. 87.



Figs. 103 to 105 Orthotopic transplantation; right limb bud to left side (*het.dv.*). Exp. R. E. 93. Reduplication, the primary (*PR*) member being a left (reversed). *DU*, reduplicating member. $\times 10$.

Fig. 103 Dorsal view, nine days after operation.

Fig. 104 Lateral view, same age.

Fig. 105 Dorsal view, preserved specimen, age fifteen days.

ventral part, which forms the glenoid cavity, is in fore and aft symmetry, with a coracoid and procoracoid process pointing in each direction. The posterior half of this cartilage has almost certainly developed in connection with the grafted limb and is reversed, while the anterior half is derived from the host.

In the disharmonic cases which have reduplicated limbs, the shoulder-girdles are on the whole less regular, owing to the complex articulations of the double appendages. One of them⁶³ (*hom.dv*) is, however, similar to the one first described in having two entirely separate girdles, one derived from the host and one from the graft. The suprascapula, procoracoid, and coracoid of the former are separate chondrifications, situated directly opposite the corresponding parts of the normal limb. The girdle of the transplanted limb has a broad flat glenoid cavity for articulation with the massive humerus. There is a large coracoid running ventrally from the joint, though without any very well-marked procoracoid. This girdle is placed some distance posterior to that of the host. Another of these cases⁶⁴ (*hom.dv*) is more like the second case described above, inasmuch as the dorsal element (suprascapula) is separate, while the two coracoids (from host and graft, respectively) are fused. The procoracoid of the host is a separate cartilage in this case. Two other cases⁶⁵ are of the same general type with fused coracoids, though they are rather too young to show all characteristics. Again, two others⁶⁶ have two scapulae with coracoids fused. There is only one case⁶⁷ that shows in sections practically no sign of doubling of the girdle, though even in this the coracoid region is thicker than normal and the glenoid cavity is large in correspondence with the more massive humerus.

To sum up: The shoulder-girdle in orthotopically grafted limbs is derived in part from the host and in part from the transplanted tissue. The former portion retains its normal asym-

⁶³ I. E. 81.

⁶⁴ I. E. 93.

⁶⁵ I. E. 68 (*hom. dv.*) and R. E. 129 (*het dd.*).

⁶⁶ R. E. 77 (*het. dv.*) and R. E. 96 (*het. dv.*).

⁶⁷ I. E. 60 (*hom. dv.*).

metry, while the latter behaves in accordance with the rules governing the asymmetry of transplanted limbs. In the disharmonic combinations the portions of the girdles derived, respectively, from the two sources may fuse together or may remain entirely separate. In the harmonic combinations they unite to form a single normal girdle.

12. *Summary of the results of orthotopic transplantations.* The orthotopic transplantations develop according to the same rules as the heterotopic. In the homopleural dorsodorsal and the heteropleural dorsoventral groups rules 1 and 2 (p. 4) are very closely followed. In the former the limb buds, being right side up, retain their normal asymmetry; and in the latter, being upside down, they reverse it. In both groups this results in limbs which correspond to the side on which they are implanted (harmonic combinations).

In the other two groups the primary single limbs which develop do not correspond to the organic environment, since the homopleural graft, when placed upside down, becomes reversed, and the heteropleural graft right side up retains its original prospective asymmetry. In these combinations, which have been called disharmonic, single limbs are, however, the exception. It is here that rule 3 comes into play. Reduplications occurred in 71.1 per cent of the cases in the homopleural dorsoventral group and in 80.6 in the heteropleural dorsodorsal. The former includes only one case of single limb reversed. In this class are also five cases of reversed single limbs, which are fundamentally the same as reduplications, the original limb having been suppressed or resorbed. The disharmonic relation thus augments immensely the tendency to reduplicate. In the case of the heterotopic grafts, on the contrary, the greater proportion of reduplications occurs in the harmonic combinations. This curious fact will be discussed below (p. 107). The ten cases of non-reversed single limbs which resulted from homopleural inverted buds are, as already pointed out, exceptional in that the limb regained its normal posture gradually during development by rotation at the base.

C. Superposed limb buds

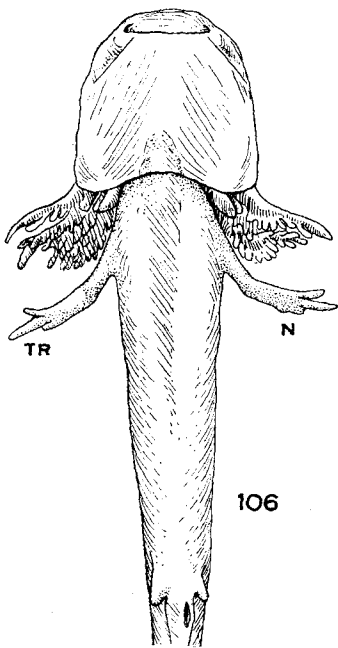
In the preceding study of transplanted limbs certain experiments were described, which showed that the mesoderm from two limb buds, when fused together, would develop into a single normal limb. At first larger than normal, the size of such a limb is soon regulated. In the former communication only those experiments were considered in which the orientation of the superposed bud was normal (*hom.dd*). The effect of the orientation of the graft will now be taken up.

TABLE 3
Superposed limbs. Summary of results

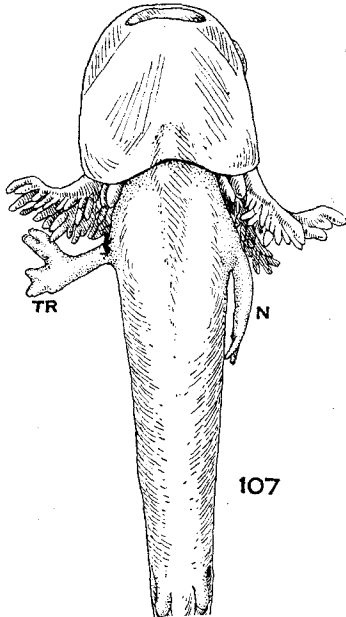
OPERATION	NUMBER OF EXPERIMENTS		NORMAL SINGLE LIMBS		NORMAL SINGLE WITH REDUCED REDUPLICATION		REDUPLICATED	
	Total	Positive	Number	Per cent	Number	Per cent	Number	Per cent
Hom. dd.	5	5	5	100	0	00	0	00
Hom. dv.	5	5	1	20	0	00	4	80
Het. dd.	6	5	0	00	1	20	4	80
Het. dv.	9	5	5	100	0	00	0	00
Total.	25	20	11	55	1	5	8	40

The experiments are summarized in table 3. There were twenty-five operations, of which twenty are available for the analysis. Two of the combinations, the ones which the ordinary transplantations have shown to be harmonic (homopleural dorsodorsal and heteropleural dorsoventral) yielded only normal appendages (ten cases). The two disharmonic combinations (homopleural dorsoventral and heteropleural dorsodorsal) yielded reduplications in nine cases out of ten. One case, in which one member of the duplicate limb was reduced to a spur, is included among the reduplications.

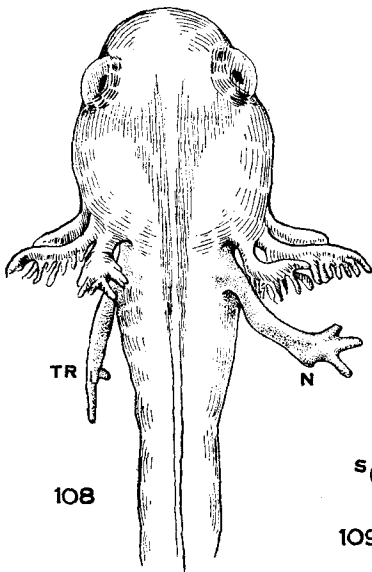
13. *Homopleural transplantations, dorsodorsal orientation.* In this group development went forward with a minimum of disturbance. The only abnormal feature to note is the large size of the double bud in certain individuals. In several of the cases



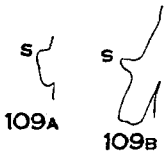
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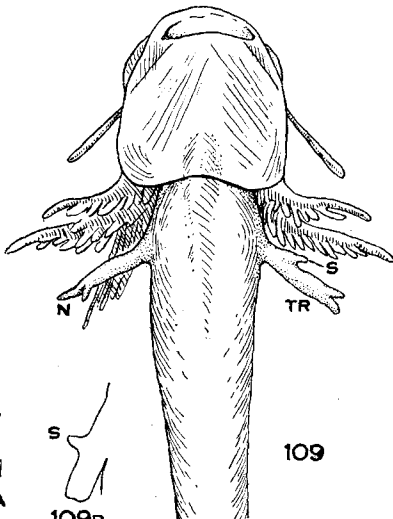


108



109A

109B



109

the difference from normal size persisted for twelve or more days,⁶⁸ gradually diminishing during that period (fig. 106). In others the difference was less marked, though in all some difference in favor of the limb on the operated side was noted.

14. *Homopleural transplantations, dorsoventral orientation.* Four out of the five cases in this group gave rise to reduplications in the grafted limb.

The reduplications vary. One⁶⁹ is a typical case of radial mirroring of the lower part of the forearm and hand (fig. 107). Another⁷⁰ is similar, except that the anterior member is itself reduplicated, the hand being a nearly symmetrical complex with four digits. These two individuals are in every respect like those cases of reduplication resulting from simple inverted limb buds, in which the primary member is reversed and is accompanied by a non-reversed twin which takes up the normal position. In two other cases⁷¹ the reduplication is less and is of a character not necessarily attributable to disharmonic combination, though there is nothing to indicate that it is not due to such a cause. Principally the digits are involved (fig. 108).

In the remaining case⁷² a normal limb developed. This one and possibly also the two foregoing are analogous to those cases of simple transplantation in which the inverted limb bud develops into a normal limb without reversal by means of rotation (p. 40).

Fig. 106 Superposed limb bud; right limb bud to right side, normal position (*hom.dd.*). Exp. S. E. 3. Normal limb (*TR*) on operated side. *N*, normal left limb. Preserved specimen, ventral view, eighteen days after operation. $\times 10$.

Fig. 107 Superposed limb bud; right limb to right side inverted (*hom.dv.*). Exp. S. E. 18. Reduplicated appendage (*TR*) on operated side. Preserved specimen, ventral view, eighteen days after operation. $\times 10$.

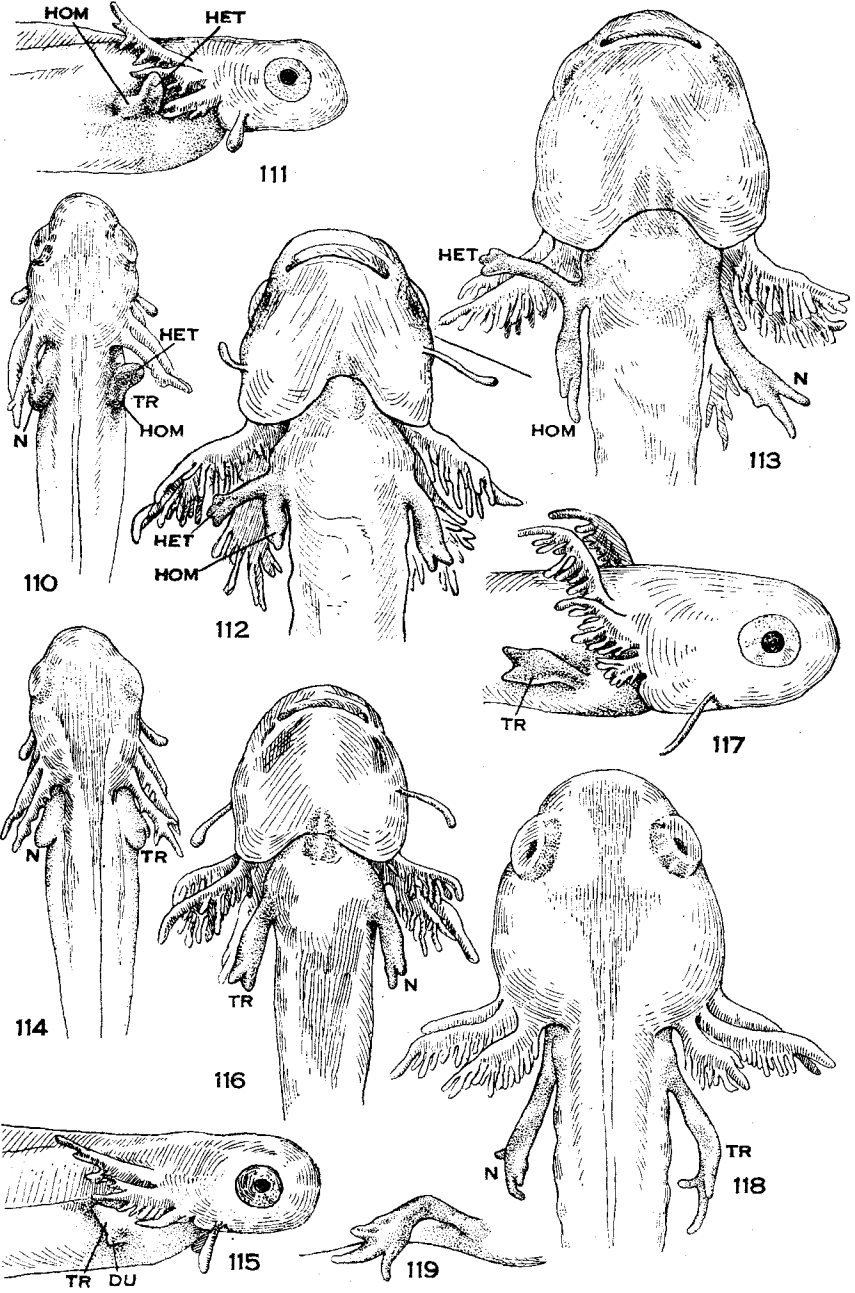
Fig. 108 Superposed limb bud; right limb bud to right side inverted (*hom.dv.*) Exp. S. E. 9. Operated limb (*TR*) normal except for reduplication of second digit. $\times 10$.

Fig. 109 Superposed limb bud; right limb bud to left side (*het.dd.*). Exp. S. E. 6. Reduplication with heteropleural member reduced to spur (*S*). $\times 10$.

Fig. 109A Outline of limb bud from above. Five days after operation (free-hand sketch).

Fig. 109B Same, eleven days after (free-hand sketch).

⁶⁸ S. E. 3. ⁶⁹ S. E. 18. ⁷⁰ S. E. 2. ⁷¹ S. E. 9 and 14. ⁷² S. E. 10.



15. *Heteropleural transplantations, dorsodorsal orientation.* In this group the results of five experiments all fall clearly within the same category. A normal appendage in approximately normal position developed, and this had a reduplicating member attached to its radial border. The differences between the cases consist in the extent of reduplication and the degree of development attained by the disharmonic (anterior) member. The most extreme cases are one⁷³ in which the heteropleural member is reduced to a spur (fig. 109), and one⁷⁴ in which there is almost complete doubling from just below the shoulder down, the heteropleural member being, however, atrophic (figs. 110 to 113). Two of the three remaining cases⁷⁵ are very similar to one another, the anterior member with two digits arising from near the elbow. In the third individual the hand is reduplicated⁷⁶ externally, and the whole arm is somewhat shorter and thicker, indicating some degree of internal reduplication.

16. *Heteropleural transplantations, dorsoventral orientation.* The five cases in this class all gave normal limbs (figs. 117 and 118). Three of them showed slight indication of doubling (figs. 114 and 115) in the early stages of development (four or five days after operation), but the more ventral-lying prominence had disappeared at the time of the next observation in each individual

Figs. 110 to 113 Superposed limb bud; left to right side (*het.dd.*). Exp. S. E. 12. Reduplication. *N*, normal left limb; *TR*, operated limb; *HET*, heteropleural member; *HOM*, homopleural member.

Fig. 110 Dorsal view, five days after operation.

Fig. 111 Lateral view, same age.

Fig. 112 Ventral view, ten days after operation.

Fig. 113 Ventral view, seventeen days after operation.

Figs. 114 to 119 Superposed limb bud; left to right side (*het.dv.*). Exp. S. E. 11. Operated limb (*TR*) of large size; *N*, normal unoperated limb. $\times 10$.

Fig. 114 Dorsal view, five days after operation.

Fig. 115 Lateral view, same age. A distinct nodule or bud (*DU*) on ventral border of limb was soon afterward resorbed.

Fig. 116 Ventral view, ten days after operation.

Fig. 117 Lateral view, same age.

Fig. 118 Dorsal view, seventeen days after operation.

Fig. 119 Lateral view of limb, same age.

⁷³ S. E. 6.

⁷⁴ S. E. 12.

⁷⁵ S. E. 8 and 21.

⁷⁶ S. E. 15.

(figs. 116 and 117). A sixth case⁷⁷ is inconclusive owing to general weakness of embryo, but it is not inconsistent with the results in the other cases. A typical history is given in the appendix (p. 131).

Though more than the normal amount of material is present in the composite limb bud, in two of the cases of this group the developing limb is recorded at first as slightly smaller than the normal. In one case no difference in size was noted, while in two the limb on the operated side is noted as larger. In the other harmonic combination (homopleural dorsodorsal) all cases showed the limb on the operated side to be somewhat larger in size.

17. *Discussion of experiments with superposed limb buds.* The principal differences between these experiments and those of simple transplantation are that in the former the tissue available for the formation of the limb is approximately double in amount, and there is a mixture of tissues having two different orientations except in the one group, homopleural dorsodorsal. In the harmonic combinations the amount of tissue is so regulated that after a time size-differences disappear. The amount of tissue is, moreover, never quite double that of the normal limb because of the material lost by the operation and of the general retardation of growth due to the same cause. In the case of the heteropleural dorsoventral grafts, which are classed as harmonic, some readjustment must be necessary, as shown by the amount of retardation.

In all of the disharmonic combinations there is a mixture of tissues differently oriented and with different prospective meaning as regards the particular asymmetry of the future limb. The twin limbs that arise are, therefore, not necessarily due to reduplication by budding, as they must be in the simple transplantations, but probably in part at least to the circumstance that one of the pair develops out of the original limb bud, while the other is from the transplanted tissue.

⁷⁷ S. E. 16.

D. Transplantation of half buds

Partly as a further test of the question of equipotentiality and partly to study more thoroughly the effect of harmonic and disharmonic combinations, a series of experiments with half limb buds was instituted. Instead of removing the whole circular disc comprising the limb rudiment, a semicircular piece was cut out, the wound bed carefully cleaned, and the removed portion replaced by a piece of similar size and shape from another limb bud. Considering only vertical and horizontal halves and replacing vertical only with vertical and horizontal only with horizontal, there are sixteen different experiments possible, which have been numbered in the diagram (fig. 120) from 1 to 16.

There are five different pairs of attributes, which appear as alternatives in the operations. Thus the transplanted half bud is either—1) homopleural (*hom.*) or heteropleural (*het.*); 2) upright (*dd.*) or inverted (*dv.*); 3) homogeneous (*homogen.*) or heterogeneous (*heterogen.*); 4) vertical (*vert.*) or horizontal (*horiz.*); 5) anterior (*ant.*), dorsal (*dors.*) or posterior (*post.*), ventral (*vent.*).

This aggregation would consist of 2^5 or thirty-two classes, were it possible to combine the attributes of operation independently without restriction, as would be the case were the pieces rectangular. Since, however, they are semicircular they fit in only half the cases, and the total is therefore reduced to sixteen. All of the possible experiments have been performed.

If both halves of the disc are considered movable, further possibilities open up. There would then be thirty-two different combinations, which, however, in eight cases would be practically identical with the experiments where the whole disc is transplanted. None of these experiments have been performed, since the technical difficulties would be at least doubled, and, as far as the study of either of the questions at issue is concerned, they would offer no advantage over those in which only one half of the bud is transplanted. Again, were the limb disc homogeneous, either or both halves could be turned inside out and then one hundred and twenty-eight different combinations would be possible. These are precluded, as in the case of the whole discs, by the

impossibility of grafting successfully pieces with the mesoderm turned toward the outside and by the difficulty of handling pieces of mesoderm free from ectoderm without disturbing their arrangement. Perhaps we may consider ourselves fortunate in being subject to such restrictions.

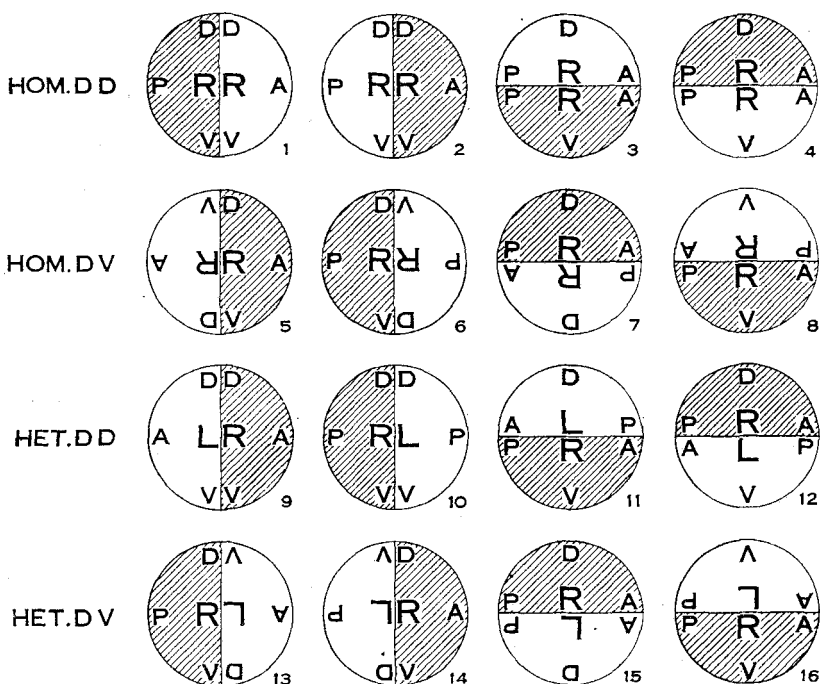


Fig. 120 Diagram showing the sixteen possible combinations (1 to 16) obtainable by transplanting half limb buds. The shaded area signifies the stationary half, the clear area the transplanted half. *R*, right; *L*, left; *D*, Dorsal; *V*, ventral; *A*, anterior; *P*, posterior. The operations are represented as on the right side of the embryo.

Returning to the experiments actually carried out (fig. 120), we find that four of them consist merely in replacing the excised piece with another of exactly the same kind in normal orientation. These serve, therefore, as controls for testing the effect of the operation as such on the further course of development. It is also seen that half of the combinations are harmonic and half disharmonic (p. 8). Half are of course homogeneous or com-

posed of two similar halves, while the other half are heterogeneous. Of the former, six belong to the disharmonic group and only two to the harmonic, while of the latter the reverse is the case, a circumstance that affects the proportionate results of the experiments.

The effect of removal of the various halves of the limb rudiment has already been described (Harrison, '18). As shown by such experiments, any half of the limb bud can give rise to a whole limb, though quantitatively the material is eccentrically distributed, there being more limb-forming tissue in the dorsal and anterior halves than in the ventral and posterior halves, respectively. Accordingly, four of the homogeneous combinations would have somewhat less than the normal amount of tissue, while four would have a little more. In the later experiments an attempt was made to compensate for this by not cutting the area exactly in half.

Owing to the large number of combinations in the experiments, it has not been possible to perform a sufficient number of each, for accurate statistical treatment. The number is sufficient, however, to compare the more comprehensive groups; for instance the homogeneous with the heterogeneous and the harmonic with the disharmonic.

Seventy-nine operations were done, sixty-eight healing successfully. Badly defective limbs developed in but four cases, so that sixty-four remain for the purpose of the analysis. These experiments are summarized in table 4.

From the results of transplanted whole limbs we should expect the following to take place; the harmonic combinations should give rise to simple normal limbs, the disharmonic to reduplications. The homogeneity or heterogeneity of the combination should not be expected to make any difference in view of the other tests of the equipotentiality of the system. These expectations were in the main realized, probably in fifty-five out of the sixty-four cases (85.9 per cent) (table 7). There are, however, sources of confusion, which in certain cases make several interpretations possible, and which for this and other reasons must not be overlooked. For example, it is known from experiments with whole

limb buds, that a normal limb may arise from a disharmonic combination by the suppression of the original bud or by its reduction to a mere excrescence on the reduplicating member, which latter may develop into a normal limb. Eight of the ten normal cases which would otherwise appear anomalous may certainly be thus explained, and possibly the remaining two. It has been found

TABLE 4
Transplantation of half limb buds. Summary of results of actual experiments

OPERATION						RESULTING LIMB				
Number	Side of origin of graft	Orien-tation	Composition	Direction of halving	Designa-tion of trans-planted half	Normal	Normal by re-sorp-tion	Re-duplicated	Abor-tive	Dead
1	hom.	dd	heterogen.	vertical	ant.	2	0	0	0	1
2	hom.	dd	heterogen.	vertical	post.	2	0	0	0	1
3	hom.	dd	heterogen.	horiz.	dors.	2	0	0	0	0
4	hom.	dd	heterogen.	horiz.	vent.	2	0	0	0	0
5	hom.	dv	homogen..	vertical	ant.	0	2	2	0	1
6	hom.	dv	homogen.	vertical	post.	0	0	5 ²	0	2
7	hom.	dv	homogen.	horiz.	dors.	0	2	2	0	0
8	hom.	dv	homogen.	horiz.	vent.	0	0	4	1	0
9	het.	dd	homogen.	vertical	ant.	2	2	0	3	1
10	het.	dd	homogen.	vertical	post.	0	1	3 ¹	0	0
11	het.	dd	heterogen.	horiz.	dors.	0	0	5 ¹	0	1
12	het.	dd	heterogen.	horiz.	vent.	0	0	4	0	1
13	het.	dv	heterogen.	vertical	ant.	4	0	1	0	1
14	het.	dv	heterogen.	vertical	post.	4	0	0	0	2
15	het.	dv	homogen.	horiz.	dors.	5	0	2	0	0
16	het.	dv	homogen.	horiz.	vent.	6	0	0	0	1
Total number of cases, 79; positive cases, 64 . . .						29	7	28	4	11

¹ Includes one case of anomalous reduplication.

² Includes two cases of anomalous reduplication.

also that almost any transplantation or even simple defect experiment may sometimes bring about reduplication. The three anomalous reduplications, being slight, are probably of this class. A further source of error might arise from the circumstance, that either the grafted or the stationary half may in certain cases be solely responsible for the limb that develops; for it is known, on the one hand, that any graft may be resorbed and, on the other,

that when half the disc is excised, complete suppression of development may sometimes result, probably through accidental injury to the remaining part. The result of the former contingency would be confusing, owing to the development of a normal limb in place of a reduplication.

In connection with these questions it must also be borne in mind that the cases of union of two disharmonic halves differ, with respect to the disharmony of the combination, from those in which the limb-bud has been transplanted as a whole. In the former only one half of the rudiment is involved, the other being in all cases harmonic with the surrounding tissues. We are, therefore, dealing with a rudiment that is disharmonic in itself, while in the case of the whole limb the transplanted bud is harmonic in itself, though disharmonic with respect to the organism as a whole. This might possibly give rise to some differences in the results in the two classes of experiments.

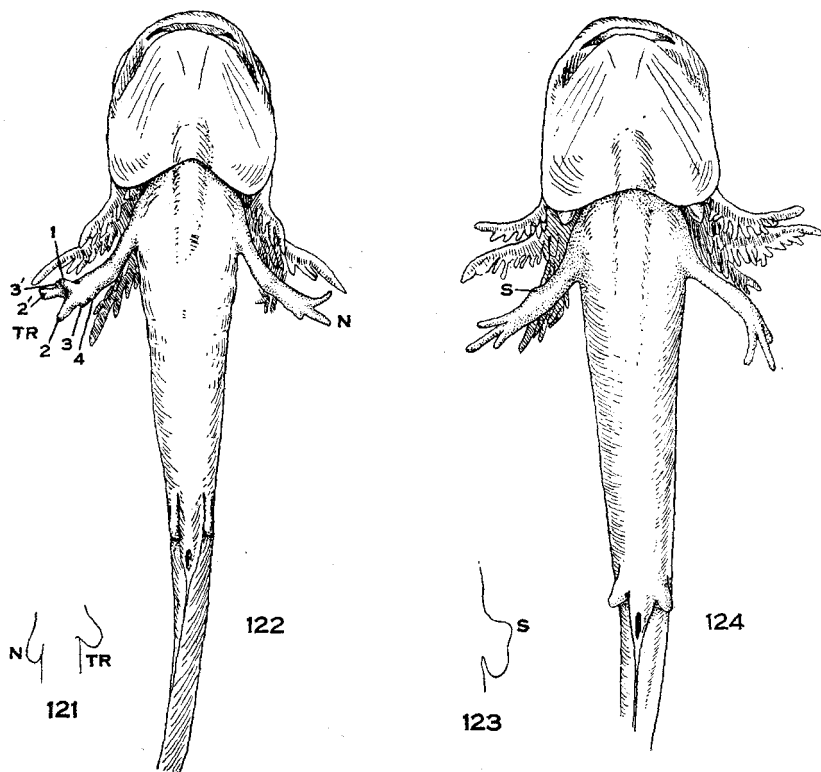
In view of these considerations, we should not expect the transplantation of half buds to give such clear-cut results as the experiments with whole ones. On the other hand, it must not be overlooked that the sources of confusion above enumerated, while accounting for nearly all of the anomalies, also render less cogent the cases which conform to the rules. Nevertheless, after taking all circumstances into consideration, it can scarcely be doubted, that the experiments with half discs do afford a valuable confirmation of the results obtained from the other experiments.

18. *Homopleural transplantations, dorsodorsal orientation.* The eight cases of homopleural grafts in upright orientation (*hom.dd*), two involving each half of the bud, all resulted in normal limbs, as was to have been expected, for this operation is nothing more than replacing an excised portion with one exactly similar. Only slight retardation of development is recorded in some of the cases.

19. *Homopleural transplantations, dorsoventral orientation.* The nineteen experiments with homopleural grafts in inverted position (*hom.dv*) resulted, in accordance with expectation, in a large number (fifteen) of duplicities⁷⁸ (figs. 121 and 122). The

⁷⁸ H. E. 29 and 31.

remaining four cases were normal. In the early stages, however, all of the latter gave evidence of reduplication (fig. 123). The limb bud, when it first appeared, showed two distinct nodules or prominences, one of which developed into a normal limb. In



Figs. 121 and 122 Transplantation of half limb bud (comb. 6, fig. 120); posterior right to anterior right (*hom.dv.*). Exp. H. E. 31. Partial reduplication of hand, mirror plane being radiodorsal. Arm and medial hand homopleural. 1 to 4, numbers of digits of main hand; 2', 3', digits of reduplicating member.

Fig. 121 Outline of normal (N) and operated (TR) buds from above, nine days after operation (free-hand sketch).

Fig. 122 Ventral view, preserved specimen twenty-one days old. $\times 10$.

Figs. 123 and 124 Transplantation of half limb bud (comb. 7, fig. 120); dorsal right to ventral right (*hom.dv.*). Exp. H. E. 2. Operated limb slightly thicker, reduplicating member reduced to a nodule (S).

Fig. 123 Outline of operated limb, dorsal view eight days after operation (free-hand sketch).

Fig. 124 Ventral view preserved specimen, twenty days old. $\times 10$.

three cases the other prominence persisted also, at the elbow in one⁷⁹ in the form of a spur (fig. 124), and as a nodule at the shoulder in two others.⁸⁰ In the remaining case⁸¹ all external traces of reduplication disappeared.

On the other hand, two of the cases of reduplication are of an anomalous nature and cannot be regarded as conforming to the rule. Both of these were experiments in which the anterior half of the limb bud was replaced by a posterior half. We should expect in such a case to find posteriorly a homopleural member developed out of the stationary portion of the bud, while anteriorly there should be a reversed limb which might itself be reduplicated. The opposite is, however, true. In both cases the anterior member is not reversed. The posterior member is reversed (a left) in one case⁸² (fig. 126) and in the other⁸³ it is itself double, the anterior portion being reversed and the posterior homopleural (fig. 125).

The operated limb in all of these cases was composed of two homogeneous halves.

Histories of typical cases are given in the appendix (p. 132).

20. *Heteropleural transplantations, dorsodorsal orientation.* This combination, being disharmonic, yielded out of seventeen cases twelve duplicities (figs. 127 and 129) and three limbs that became normal by reduction of the reduplicating member (fig. 130). In two individuals⁸⁴ normal limbs resulted without external evidence of incipient doubling, and two of the reduplications, in one of which both members are of the same side in linear series, are of an anomalous nature. This makes four cases out of seventeen that do not follow the rule. Two of the combinations are heterogeneous; all of these conform to the rule except one of the anomalous reduplications. The other three non-conforming cases belong in the homogeneous class, and it is interesting that all of the normal cases in this group resulted from the combination of two like halves.

⁷⁹ H. E. 2.

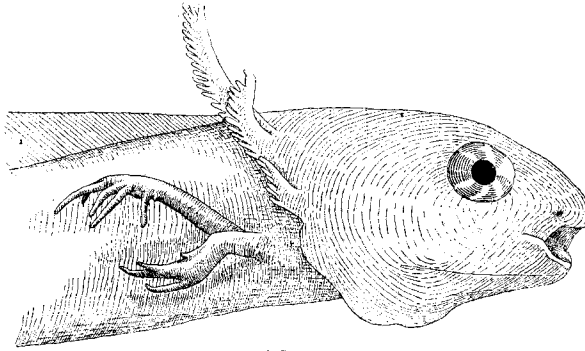
⁸⁰ H. E. 18 and 21.

⁸¹ H. E. 4.

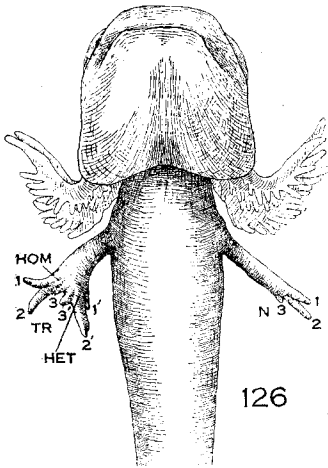
⁸² H. E. 13.

⁸³ H. E. 5.

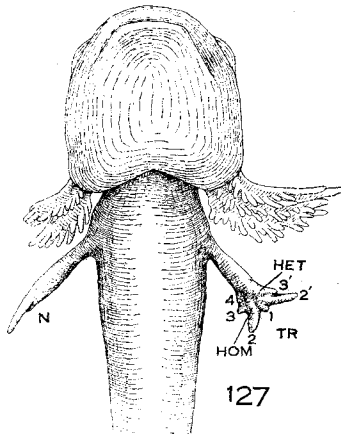
⁸⁴ H. R. E. 43 and 44.



125



126



127

Fig. 125 Transplantation of half limb bud (comb. 6, fig. 120); posterior half right to anterior right (*hom.dv.*). Exp. H. E. 5. Two limbs, the posterior of which has a double hand. Anterior member an almost normal right; of the two parts of the posterior member, the anterior one is a left hand and the posterior, a right. $\times 10$.

Fig. 126 Same operation (comb. 6, fig. 120) (*hom.dv.*). Exp. H. E. 13. Ulnar reduplication! *HOM*, homopleural hand; 1 to 3, digits of same; *HET*, heteropleural hand; 1' to 3', digits of same; *N*, normal (unoperated) left limb $\times 10$.

Fig. 127 Transplantation of half limb bud (comb. 10, fig. 120); posterior half right to anterior left (*het.dd.*). Exp. H. R. E. 1. Double hand. *HOM*, homopleural hand with digits (1 to 4); *HET*, heteropleural hand with digits (2' to 3'). $\times 10$.

The double limbs are of various degree and kind. The least involved is one in which only the first digit is doubled.⁸⁵ In this individual the ventral half of the limb was replaced by a ventral half of the opposite side, and in all probability very little limb material was actually transplanted, since, in the embryo from which the graft was taken, the operated limb developed almost as rapidly as the normal. In five other cases⁸⁶ (fig. 127) the whole hand is involved, with indications that in three of these at least⁸⁷ the internal reduplication extends farther proximally. In two cases the fore arm and hand⁸⁸ (figs. 128 and 129) are externally double, and in one,⁸⁹ which was not fully developed when preserved, doubling would probably have shown from near the shoulder down. In two of the individuals⁹⁰ there are secondary reduplications. In the two anomalous cases⁹¹ (figs. 131 and 132) there are two entirely separate limbs.

Histories are given on page 134. Unfortunately, external observation does not always reveal the relations of each of the two halves of the bud to the developing members.

21. *Heteropleural transplantations, dorsoventral orientation.* Out of twenty-two successful experiments in this group nineteen resulted in the development of normal limbs (fig. 133), which is according to rule, and only three gave rise to reduplications (fig. 134). Two of the latter⁹² involved only the radial digits, in which palmar reduplication was present, the limbs being otherwise normal. In the remaining one a bifurcated appendage arose, but the dorsal branch remained merely as a spur attached to the main limb, which was normal though undersized and with slight syndactyly.

In one case,⁹³ which has been classed as normal, a filamentous appendage probably not a limb, developed a short distance ventral to the main limb, which was normal though slightly shorter.

⁸⁵ H. R. E. 30.

⁸⁶ H. R. E. 1, 15, 46, 47, and 48.

⁸⁷ H. R. E. 15, 46, and 47.

⁸⁸ H. R. E. 5 and 21.

⁸⁹ H. R. E. 28.

⁹⁰ H. R. E. 21 and 47.

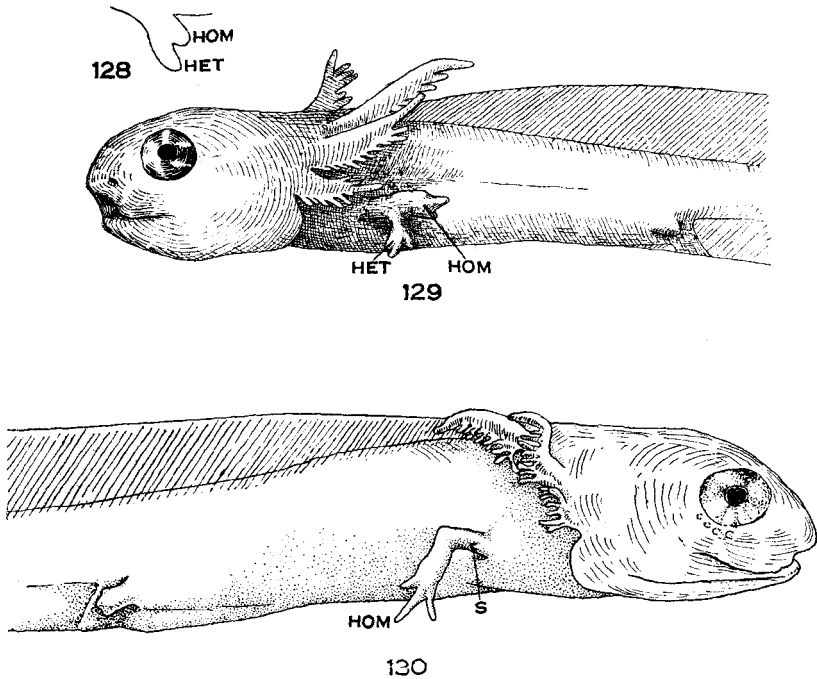
⁹¹ H. R. E. 9 and 20.

⁹² H. R. E. 10 and 16.

⁹³ H. R. E. 12.

This group of experiments is interesting because two of the combinations (ventral half in place of dorsal and dorsal in place of ventral) are homogeneous. Out of thirteen such cases, normal limbs developed in eleven.

For histories of representative cases see appendix (p. 136).



Figs. 128 and 129 Transplantation of half limb bud (comb. 11, fig. 120); dorsal half right to dorsal left (*het.dd.*). Exp. H. R. E. 5. *HOM*, homopleural member; *HET*, heteropleural member.

Fig. 128 Outline of limb from above, ten days after operation (free-hand sketch).

Fig. 129 Preserved specimen lateral view, nineteen days old. $\times 10$.

Fig. 130 Transplantation of half limb bud (comb. 9, fig. 120); anterior half left limb bud to posterior right (*het.dd.*). Exp. H. R. E. 11. Normal limb with small spur (*S*). $\times 10$.

22. *Discussion of experiments with half buds.* In order to establish the conclusion stated in the introduction to this section, that it is the harmony or disharmony of the half-and-half combi-

nation and not one of the particular qualities of the operation that determines whether normal or reduplicated limbs arise, it will be necessary to examine the numerical results of the experiments more carefully.

If we take the actual figures of the experiments and examine the qualities in pairs, we find the actual number of each class and

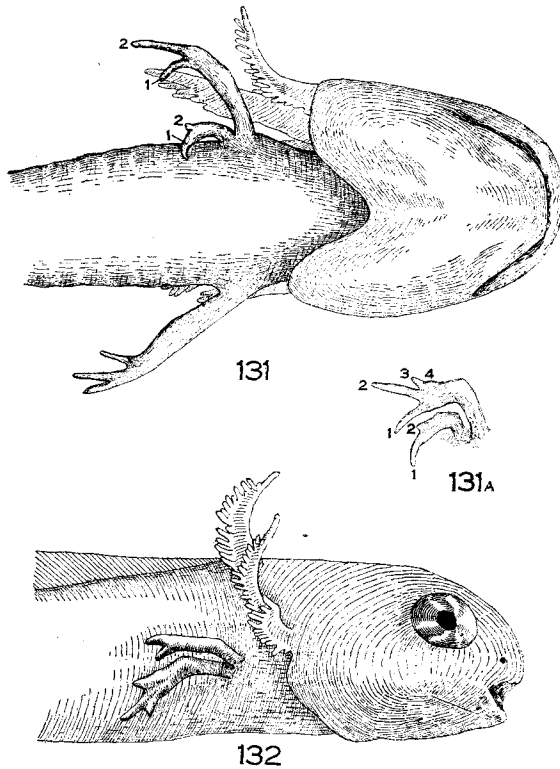
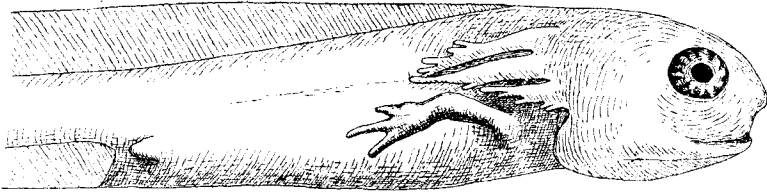


Fig. 131 Transplantation of half limb bud (comb. 10, fig. 120); posterior half left side to anterior right (*het.dd.*). Exp. H. R. E. 9. Anterior member a right (homopleural), the posterior one a left (heteropleural), but imperfect. Ventral view of specimen preserved forty days after operation. $\times 10$.

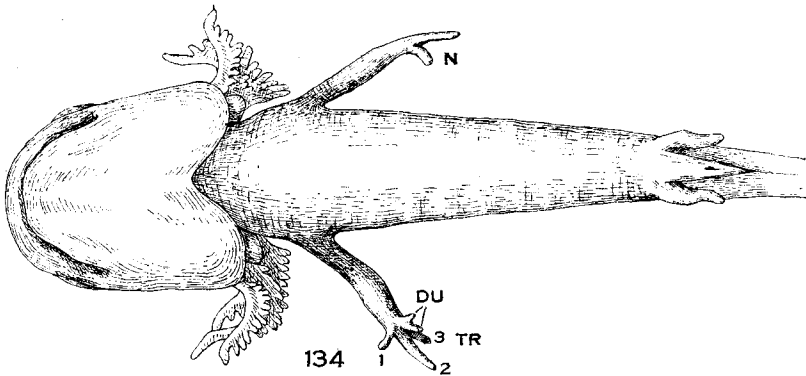
Fig. 131A Lateral view of limbs of same.

Fig. 132 Transplantation of half limb bud (comb. 11, fig. 120); dorsal half left side to dorsal right (*het.dd.*). Exp. H. R. E. 20. Two right limbs, the anterior one imperfect. $\times 10$.

the proportion of normal results to be as given in table 5, column 6. 'Normal by resorption,' being fundamentally the same as reduplication, is classed as such.



133



134

Fig. 133 Transplantations of half limb bud (comb. 16, fig. 120); ventral half left side to dorsal right (*het.dv.*). Exp. H. R. E. 36. Normal limb.

Fig. 134 Transplantation of half limb bud (comb. 13, fig. 120); anterior half left side to anterior right (*het.dv.*). Exp. H. R. E. 10. *N*, normal left arm; *TR*, grafted arm with palmar reduplication of two digits (*DU*). $\times 10$.

The one thing that stands out is the great difference between the results of the harmonic and those of the disharmonic combinations. In the case of none of the other attributes of operation is there anything like the same difference between those of a pair, though in the case of homogeneity vs. heterogeneity the difference is considerable (37.1 vs. 64 per cent).

However, the comparisons cannot be accurately made without the same number of experiments in each class, unless made by means of percentages. This is quite obvious, for instance, in the case of the homopleural transplantations with dorsodorsal orientation, which all result in normal limbs. The relatively

TABLE 5
Transplantation of half buds. Comparison of pairs of qualities of operation

PAIRS OF QUALITIES COMPARED	NUMBER OF CASES				PER CENT NOR- MAL	PER CENT NORMAL AFTER CORRECTION FOR INEQUAL- ITY IN NUMBER OF EXPERI- MENTS
	Normal	Normal by resorp- tion	Re- duplica- tion ¹	Total		
Homopleural.....	8	4	11	23	34.8	50.0
Heteropleural.....	21	3	13	37	56.8	50.2
Dorsodorsal.....	10	3	10	23	43.5	56.25
Dorsoventral.....	19	4	14	37	51.4	43.9
Homogeneous.....	13	7	15	35	37.1	27.7
Heterogeneous.....	16	0	9	25	64.0	72.5
Vertical.....	14	5	8	27	51.9	53.75
Horizontal.....	15	2	16	33	45.5	46.8
Anterior or dorsal half trans- planted.....	15	6	11	32	45.45	50.2
Posterior or ventral half trans- planted.....	14	1	13	28	50.0	50.0
Harmonic (hom. dd and het. dv)	27	0	3	30	90.0	93.9
Disharmonic (hom. dv and het. dd).....	2	7	21	30	6.7	6.25

¹ The four cases of anomalous reduplication have been omitted from this tabulation.

small number of cases in this group affects the record for homopleural transplantations by reducing considerably the number of cases that would have developed normally, thereby giving undue weight to the larger number of dorsoventral cases which result in reduplications. Likewise the dorsodorsal vs. dorsoventral record is influenced by the relatively small number of homopleural cases.

On this account the operations in each class have been reduced to a common basis. While the probable error of these figures is in most cases large, the comparisons resulting therefrom are no doubt much more reliable than those resulting from the figures of the actual experiments. They are given in the last column of table 5.

In examining this table we find that there is little or no association between the experimental results and the following qualities of operation: homopleural vs. heteropleural, dorsodorsal vs. dorsoventral, vertical vs. horizontal, anterior and dorsal vs. posterior and ventral, the deviation from total lack of association (50 per cent) being in the most extreme case but 6.1 per cent. When we examine the figures with reference to the pair, homogeneity vs. heterogeneity, we find that there is a much wider difference (27.7 per cent as compared with 72.5).. This would have to be regarded as a significant difference but, as will be seen below it is only secondarily so. The marked association between the harmonic combinations and normal development (93.9 per cent) and the very small proportion of normal development (6.25 per cent) in the disharmonic group, show that it is largely this pair of attributes that determines whether development will be normal or not. This quality of harmony or disharmony, however, is not like the simple qualities of side of origin, orientation, or direction of the incision, but is itself a combination of two of them. Those that are harmonic are the homopleural dorsodorsal and the heteropleural dorsoventral combinations, the other two being disharmonic, as in the experiments with whole limb buds.

When we consider the homogeneous and heterogeneous combinations, we find them unevenly distributed with respect to the harmonic and disharmonic. This is on account of the restriction of operation due to the semicircular shape of the transplanted pieces, which makes half of the combinations impossible of execution. Were these all possible, there would be complete symmetry in the aggregation as a whole. In reality, it will be recalled, six of the homogeneous combinations are disharmonic, while only two are harmonic. On the hypothesis that it is the harmony of the combination that determines normal develop-

ment, and with an equal number of experiments in all of the sixteen possible classes, the expectation would be that only 25 per cent of the homogeneous and 75 per cent of the heterogeneous would be normal. This corresponds closely to the figures 27.7 and 72.9, respectively, found in table 5.

As regards the question of equipotentiality, the results of these experiments are equally striking. The two homogeneous combinations which, according to expectation, should yield normal limbs did so. Thus two ventral halves yielded normal limbs in all six experiments, as did two dorsal halves in five out of seven. In three cases of disharmonic homogeneous combination normal limbs developed by resorption of the reduplicating bud; two of these were from two anterior halves and one from two posterior. Two further cases of normal limbs from two anterior halves developed without external evidence of resorption. While the last five, if interpreted according to the rules, can only be accepted as evidence of equipotentiality in so far as they show that a whole limb can develop out of a single half bud, the others show that two half buds which are alike except that they are from opposite sides of the body may give rise, when harmonic, to a single normal limb.

GENERAL DISCUSSION

In this section the following questions will be considered: 1) the foundation of the rules of symmetry; 2) the mode of representation of symmetric relations in the limb rudiment; 3) the formation of reduplications; and 4) form regulation and function in transplanted limbs.

E. The rules of symmetry.

The validity of the rules of symmetry which have already been stated in the introduction (p. 4) will best be realized by considering the results of the several experiments in tabular form (table 6). Conformity is shown most strikingly in the heterotopic group, where there is only a single apparent exception in forty-five cases; and this exception, as already pointed out, is probably due to an error in recording the operation.

In the orthotopic group the lowest percentage of conformity (65.8) is found in the inverted homopleural buds (*hom.dv*), where the exceptions are due entirely to adjustment by rotation of the limb as a whole. In the superposed buds the sole exception is due probably to the same cause. The exceptional

TABLE 6
Showing conformity to rules in the several experiments

OPERATION				RESULTING LIMB					
Type of experiment	Side of origin of graft	Orientation	Harmonic or disharmonic	Conforming to rules		Exceptions		Total	
				Number	Per cent	Number	Per cent		
Whole bud heterotopic..	hom.	dd	harm.	7	100.0	0		7	
Whole bud heterotopic..	hom.	dv	disharm.	12	100.0	0		12	
Whole bud heterotopic..	het.	dd	disharm.	10	100.0	0		10	
Whole bud heterotopic..	het.	dv	harm.	15	93.8	1(?)	6.3(?)	16	
Whole bud orthotopic...	hom.	dd	harm.	9	100.0	0		9	
Whole bud orthotopic...	hom.	dv	disharm.	25	65.8	13	34.2	38	
Whole bud orthotopic...	het.	dd	disharm.	31	100.0	0		31	
Whole bud orthotopic...	het.	dv	harm.	16	100.0	0		16	
Superposed buds.....	hom.	dd	harm.	5	100.0	0		5	
Superposed buds.....	hom.	dv	disharm.	4	80.0	1	20.0	5	
Superposed buds.....	het.	dd	disharm.	5	100.0	0		5	
Superposed buds.....	het.	dv	harm.	5	100.0	0		5	
Half buds.....	hom.	dd	harm.	8	100.0	0		8	
Half buds.....	hom.	dv	disharm.	15	100.0	0 ¹		15	
Half buds.....	het.	dd	disharm.	13	86.7	2 ¹	13.3	15	
Half buds.....	het.	dv	harm.	19	86.4	3	13.6	22	
Total.....				199	90.9	20	9.1	219	
Average of percentages.....					94.5		5.5		

¹ Four cases of anomalous reduplication have been omitted from this tabulation, inasmuch as they cannot be classified either as conforming or as exceptional.

cases arising after transplantation of half buds have already been discussed, and, as pointed out above, there are in this group of experiments obvious disturbing factors which might readily account for the exceptions. Taking the experiments as a whole, 90.9 per cent conform and 9.1 per cent are exceptional, but if allowance is made for the difference in the number of experiments in each class, assuming that each is a fair sample of what would

occur in a large number of cases, then but 5.5 per cent are exceptional.

The behavior of transplanted limb buds in accordance with the above rules indicates that the posture and the asymmetry of the limb is determined neither by the limb itself nor by its surroundings exclusively, but by an interaction between the two. This is best described by the assumption, that in the stages experimented upon the anteroposterior axial differentiation is already determined within the limb bud, while the ventrodorsal axis (probably radio-ulnar of the grown limb) is determined by its orientation with reference to the surrounding tissues of the host (fig. 135). In a given place a right limb bud upside down thus behaves like a left limb bud right side up and vice versa (fig. 2). It is scarcely necessary to point out that this is not a gravity effect, for the embryo lies on its side during the period when the dorsoventral axis of the limb is determined, 'upside down' being used here merely with reference to the cardinal points of the embryo itself.

What the nature of the influence exerted by the organic environment may be, has not been determined. Whether it acts upon the intimate structure of the limb bud or directly upon the differentiating systems contained therein, without affecting the intimate structure as a whole, cannot be answered from the present data (p. 101). The influence is not sharply localized, for it is the same both in the limb region itself and elsewhere along the flank of the embryo, so that it is probably an effect of the axial differentiation of the tissue elements themselves. It is possible that light may be thrown upon this question by transplanting the limb bud to the dorsal or to the ventral midline of the embryo.

F. The mode of representation of symmetric relations in the limb rudiment

The question whether the adult parts are localized in the germ, forming a mosaic, must be answered in the negative for the limb bud, as used in the experiments, i.e., if we consider as such a disc of tissue, three and a half somites in diameter, centering ventral to the fourth myotome, and leave out of account the outlying regions from which certain portions of the shoulder-

girdle develop. This conclusion is based upon the following evidence derived from the experiments: 1) After extirpation of any half of the limb bud, a complete normal limb may develop from the remaining half; 2) fusion of two limb buds by superposition is followed, if the combination is harmonic, by the development of a single normal limb, which at first is usually larger than normal, but in which there is rapid regulation of size; 3)

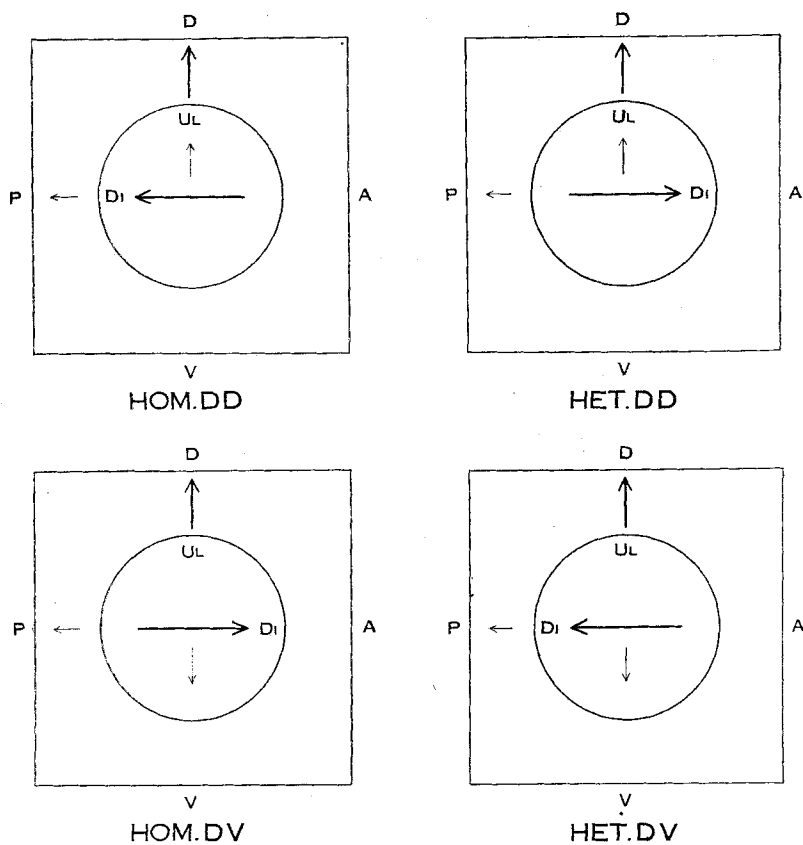


Fig. 135 Diagram to show determination of asymmetry of limb. The circles represent the limb bud, the squares the surrounding part of the embryo. A, D, P, V, the cardinal points of the embryo—anterior, dorsal, posterior, and ventral, respectively. The heavy arrows represent the determining axes, i.e., the antero-posterior axis of the bud and the dorsoventral axis of the surrounding parts; UL, future ulnar border; DI, approximate direction of outgrowth. The smaller arrows show the other axes of bud and surroundings, respectively, which are not effective in determining the axes of the definitive limb.

a normal limb usually develops out of two like halves, i.e., two dorsal, or two ventral halves, if properly oriented, when the opposite half is entirely missing; 4) after inversion of the limb bud the material that normally would have formed the radial half of the limb gives rise to the ulnar half and vice versa, so that practically no part of the bud has the same fate that it would have had if it had been left in place; 5) the inoculation of mesoderm from the limb bud under the skin of the flank of another embryo may result in the formation of a normal limb, although the inoculated tissue is badly disarranged by the operation. According to all tests that have been applied, the embryonic limb rudiment constitutes, therefore, an harmonic equipotential system, though, as a whole, it is self-differentiating except for the determination of its dorsoventral axis. The term 'harmonic equipotential system' is employed here, as defined by Driesch, in the sense that the potencies of all parts of the system are the same, the constituent cells being totipotent.⁹⁴ Its use does not imply that the writer attaches to the existence of such systems the same significance as Driesch, who considers them as constituting a proof of the 'autonomy of life.' Even without this, however, and even though the actual system may not reach the abstract perfection demanded by its definition, it remains as a useful conception in experimental morphogenesis. The existence of the equipotential system necessitates, in fact, the assumption of some sort of molecular hypothesis for the representation of adult form in the germ, and herein lies its importance in connection with the present study.⁹⁵ In particular, we must look to the constitution of

⁹⁴ The concept 'harmonic equipotential system' is defined by Driesch ('05, p. 679) as follows: "Bekanntlich nenne ich harmonisch-äquipotentielle Systeme solche Formganze, bei denen eine Differenzierungs- oder Wachstumsgesamtleistung in ihren Einzelheiten jeweils einzelnen Elementen des Ausgangsganzen zufällt, derart, dass jedes Einzelne dieses Ganzen jedes Einzelne jener Leistung vermag, alles Einzelne aber derart in Harmonie steht, dass die Leistung selbst ein Ganzes ist." The bearing on the question of vitalism is discussed in various papers, especially: '99, p. 99; '01, p. 170; '08 b, p. 138.

⁹⁵ Child has expressed skepticism as to the very existence of equipotential systems; for instance: "I think we may say that there is at present no valid evidence for the belief that any living system which is undergoing regulation or development in nature is at any given time an equipotential system" ('11, p. 306). Cf. also Child, '08.

the elementary units of the limb bud, rather than to their arrangement, for the representation of those relations of symmetry that the experiments here described have revealed.⁹⁶ In other words, it is the intimate protoplasmic structure that underlies symmetry.

In an equipotential system without axial differentiation, it is most natural to assume that the elements themselves are isotropic.⁹⁷ Axial differentiation would then result from the gradual modification of these units by reaction with other elements of the system or through external influences. These differentiations with reference to directions in space may be referred arbitrarily to three axes crossing one another at right angles. They are geometrically of four grades, according to the number of axes along which polarization has taken place.

Taking the models used in stereochemistry to show the spatial relations of the atom groups in certain carbon compounds, we may represent the above four conditions of the elements of the organism or system by four figures (fig. 136) in which the groups that determine the axial relations are situated at the four angles of a tetrahedron. At the center of each tetrahedron we might by analogy assume a carbon atom linked to the four groups occupying the angles of the figure, though this is not necessary for the present purpose. By hypothesis the groups at the angles are supposed to be at first all alike (fig. 136, 1). If one of them should be changed by some reaction, the structure of the molecule would become polarized (fig. 136, 2), and if all the molecules should assume approximately the same orientation, the system which they constitute would show a similar polarity. If two of

⁹⁶ The question whether relations of symmetry of the organism are to be based upon symmetrical relations of the intimate protoplasmic structure is answered in the affirmative by Driesch ('08 a, p. 144): "Wir müssen also alle Symmetrie und auch alle Wirkungen, die von äusseren Faktoren ausgehen und sich auf Symmetrie beziehen, auf präformierte, gerichtete Elemente des 'Protoplasmas' beziehen und können in jenen Wirkungen nur richtende und umordnende Geschehnisse sehen."

⁹⁷ To avoid misunderstanding, it should be stated that when we speak of equipotentiality and isotropy, we do not lose sight of the fact that the system in its entirety is heterogeneous.

the groups become differently modified, then the structure becomes bilaterally symmetrical (fig. 136, 3). And, finally, if three become modified, so that all four are different, then the arrangement becomes asymmetrical (fig. 136, 4 and 5) as in the case of optically active substances with an asymmetric carbon atom. In the last phase there are two kinds of individuals, which are exactly alike in every respect, except that they are the mirror images of

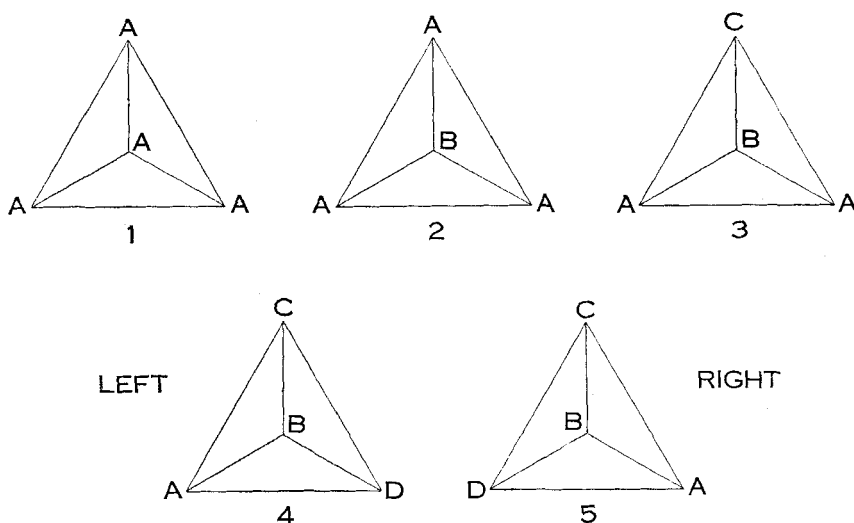


Fig. 136 Diagram to show hypothetical progressive differentiation of the structural units. 1) condition of isotropy; 2) polarization with reference to one axis; 3) bilateral symmetry (two axes differentiated); 4 and 5) condition of complete asymmetry (three axes differentiated) giving right and left enantiomorphs.

one another—in other words, rights and lefts. This is expressed in aggregate form in the right- and left-handed crystals corresponding, respectively, to the dextro- and laevo-rotatory forms of otherwise identical substances.

The experiments with limbs show that the bud at the time of transplantation is in either the second or the third phase, probably the former. There must be a differentiation along the antero-posterior axis, because if this is reversed the limb shows it by growing in a direction nearly opposite the normal. The medio-

lateral axis is probably not differentiated, though in the absence of sufficient experiments in reversal of this axis, it is better to make no definite assumption regarding the intimate structure in relation to it. The dorsoventral axis is at most but slightly differentiated, and if it is at all, then the differentiation is reversible.⁹⁸ As already pointed out (p. 55), there is some ground for the latter assumption, for it has been observed that after transplantations in the heteropleural dorsoventral position (the harmonic combination in which the dorsoventral but not the anteroposterior axis is reversed) the adjustment of the tissues of the limb bud is apparently not immediate, but involves a time factor, probably not entirely accounted for by the effect of the operation as such. Whatever the character of this dorsoventral differentiation may be, it is nevertheless very slight in comparison with the anteroposterior differentiation, which has become irreversible by the time the stage in question is reached.

If we could experiment over a wide enough range of stages, it should be possible to determine the time limits of the above phases of axial differentiation of the limb rudiment. At present, however, there are no data bearing upon the question, for in the earliest stages in which the transplantation of limbs has been carried out (embryo with wide open medullary folds), as shown by Detwiler ('18), the limb bud follows in its development the same rules as here formulated.

Lest the foregoing scheme seem too formal, it may be pointed out that the model has been chosen to explain solely the relatively simple characters of polarity and symmetry. Upon this as a basis, further experimentation may yield facts from which the mode of representation of more specific form features may be determined. There is nothing in such a scheme inconsistent with the fact that the cell itself is not a homogeneous system, for the model is supposed to represent only that constituent of the system which determines the adult character in question.

⁹⁸ This is perhaps odd in view of the facts brought out by Przibram ('10 b), showing that dorsoventral differentiation is very marked in the animal organization, more so, for instance, than the anteroposterior differentiation.

The point which it is desired to emphasize is that in an organic 'equipotential system' there must be some intimate structural basis for adult characters in the units that make up the embryonic rudiment.⁹⁹ It cannot be in the arrangement of these units; for in that case marked disturbances of development would be produced by such operations as removing half of the rudiment, fusing two buds together, combining two like halves, inverting the dorsoventral axis, or inoculating masses of mesoderm cells from the limb rudiment under the skin of the flank; and yet normal development may follow any of these procedures.

These experiments yield, of course, no information concerning the localization in the cell of the representatives of the adult form characters in question. The system here dealt with is a pluricellular one, but it is interesting to find that in the most thorough and careful studies of polarity and symmetry in the egg, the basis of these properties is found to be in the cytoplasm of the egg cell. Lillie ('06, '09) shows that the polarity of the *Chaetopterus* egg must be located in the ground-substance, because any amount of shifting of visible granulations in the egg, such as yolk, oil droplets, pigment, etc., has no effect on the polarity of the resulting embryo. With this conclusion the work of Morgan and his collaborators ('08 b, '09, '10) on the centrifuged eggs of various animals, more particularly *Arbacia* and *Cumingia*, is in substantial accord. Conklin ('16, '17), in his study of *Crepidula*, concludes that it is the spongioplasmic framework of the egg-cell that determines its polarity, though he does not consider how this quality is determined in relation to the intimate structure of the spongioplasm. To the extent that Conklin places the seat of polarity in the more viscid rather than in the more fluid constituent of the cytoplasm, he takes issue with Lillie, but in the main, there is agreement between these two investigators. Lillie, however, goes a step further when he says ('09, p. 77): "The existence of polarity and bilaterality in an optically homogeneous medium, and the persistence of both as to orientation under experimental conditions that seriously modify the quantitative relations of the oriented medium in different regions (as, for instance, when

⁹⁹ Cf. Driesch, l. c.

the yolk granules are packed closely into the small cell of the two-celled stage of *Chaetopterus*) seem to me to argue for a molecular basis of the fundamental principle of vital organization." Morgan, likewise, takes this view when he says ('09, p. 114), "These considerations incline us to the view that there exist in the molecular constitution of the egg the potential factors of symmetry." The scheme outlined above is in harmony with this concept.

On the other hand, Child ('13, '15), as also Della Valle ('13) rejects all such hypotheses, basing the phenomena of axial differentiation upon the occurrence of gradients, which, according to Child, are primarily of a functional (metabolic) nature. It seems to the present writer that such gradients may well be an expression of the polarity rather than its cause.

G. Reduplication and the problem of polarity and heteromorphosis

The reduplications which have been observed in the various experiments have already been described sufficiently for the present discussion (pp. 35, 45, 65, 73). The salient facts are: 1) that the duplicate is the mirror image of the original limb; 2) that more than one secondary member may arise by budding from the same primary bud, in which case both of the former stand in some relation of symmetry to the original; 3) that the secondary appendages themselves may be doubled, forming a more or less symmetrical pair. There are a few exceptional cases, where two members of the same side stand in linear series, but probably these have arisen only where the two rudiments are far enough apart not to influence one another.¹⁰⁰

¹⁰⁰ Several cases are to be considered here. One (H. R. E. 10-) is a case in which the anterior half of the limb bud was removed. Two limbs developed, one clearly from the remaining posterior half and the other probably from the anterior border of the wound (cf. Harrison '18, p. 441). The operation was done on the left side and both limbs were lefts, the posterior one being somewhat defective. In another case, which had an early history similar to the above, the posterior member was very defective and it was impossible to determine whether it was a right or a left. A third case (H. R. E. 20) is the one figured on page 79. This is not a case of regeneration, but one in which the anterior member probably developed from the grafted half, while the posterior member may have developed from the stationary ventral half. Both are attached to the same shoulder-girdle, but there are two separate glenoid cavities.

Bateson, in his "Materials for the Study of Variation" ('94), has given an exhaustive review of the literature relating to supernumerary parts, in which the limbs are fully considered. In this treatise he has made a masterly analysis of the available material, particularly with reference to the appendages of arthropods. The phase of the problem which is especially relevant to the present discussion is that concerning what Bateson calls minor symmetries, in which the supernumeraries are in some way symmetrical with respect to themselves or to the normal appendages with which they are associated. The other class of supernumeraries, in which two identical appendages stand in simple succession to one another are, according to Bateson, practically unknown, and even those that have been described are considered by him to be of somewhat doubtful nature, though many cases of simple hyperdaetyly would seem to belong in this category.

The symmetrical extra appendages fall into two groups: 1) those in which there is a pair of extra members symmetrical with themselves, arising from the normal appendage with which one of the supernumeraries appears to have a definite relation of symmetry, and, 2) those in which the single supernumerary is symmetrical with respect to the normal. The former condition Bateson considers to be the more usual, and, in fact, he accepts the existence of the latter with a certain skepticism which seems unnecessary.¹⁰¹ It is true that many cases that apparently fall within the latter group may upon closer examination be found to belong in the former, but the converse is also true, as will be shown below (p. 97). Bateson has devoted special attention to the first group, and, on the basis of about one hundred and twenty cases in insects and a considerable number in the Crustacea, he has formulated the following rules,¹⁰² showing the relation of the supernumerary appendages to each other and to the original member:

I. The long axes of the normal appendage and of the two extra appendages are in one plane: of the two extra appendages one is therefore nearer to the axis of the normal appendage and the other is remoter from it.

¹⁰¹ Op. cit. p. 539 and 553. Consider, however, in this connection, the clear case described by Bender ('06).

¹⁰² Op. cit. p. 479.

II. The nearer of the two extra appendages is in structure and position formed as the image of the normal appendage in a plane mirror placed between the normal appendage and the nearer one, at right angles to the plane of the three axes; and the remoter appendage is the image of the nearer in a plane mirror similarly placed between the two extra appendages.

Transverse sections of the three appendages taken at homologous points are thus images of each other in parallel mirrors.

In the vertebrates Bateson marshals a large amount of material, of which about fifty cases are in amphibians.¹⁰³ At the time Bateson's book was written, however, little or nothing was known regarding the origin of supernumerary appendages in either the arthropods or the vertebrates. Since then a large amount of experimental evidence has accumulated to show that they may be formed by superregeneration, especially by regeneration from complex or irregular wound surfaces.¹⁰⁴ The evidence all corroborates Bateson's main generalization regarding the relation of symmetry of supernumerary limbs, and there are practically no exceptions.¹⁰⁵

The importance of double supernumeraries (*Bruchdreifachbildung*, *la doppia rigenerazione inversa*, see p. 95) is emphasized in the papers by Emmel ('07) and Della Valle ('13), and this conception is given prominence in Przibram's more general discussion of the question ('09, p. 234).

¹⁰³ See Bateson (pp. 554-5) for a discussion of the older literature.

¹⁰⁴ In the amphibians the investigations of Barfurth ('94), Giard ('95), Tornier ('97, '00, '05, '06), Lissitzky ('10), Fritsch ('11), Kurz ('12), Della Valle ('13), and others have added much to our knowledge of the subject. In the crustaceans Przibram ('02), Reed ('04), Zeleny ('05), and Emmel ('07) have reported experiments which, though not so numerous, are none the less important. The more recent literature is fully discussed in many of these papers, especially in those of Lissitzky, Fritsch, and Della Valle, to which the reader is referred for details.

¹⁰⁵ A remarkable exceptional case has recently been described by Dawson ('20) in a lobster, in which there is an extra pair of chelipeds attached to the normal. The two extra chelae are mirror images of one another, but the one nearer the primary claw is not mirrored from the latter, but is of the same side. Furthermore, the primary claw is a 'nipper,' while the supernumeraries are both of the 'crusher' type, so that the case proves to be likewise an exception to Przibram's rule ('11), according to which, in heterochelous forms, the extra appendages are of the same type as the primary. The case described by Cole ('10), also in a lobster, is an almost diagrammatic example of Bateson's rule, if allowance is made for the effects of torsion.

Likewise, the well-known experiments of Tornier ('05) upon tadpoles of *Pelobates*, in which the hind-limb bud was divided in an early stage, some of the cases of Lissitzky ('10), and Della Valle's case of reversed regeneration conform closely to Bateson's rules. Although the end results of the experiments of Tornier and of Della Valle are analogous, there is, however a sharp difference of opinion regarding the exact mode of origin of Tornier's double supernumerary hind legs, Tornier maintaining that they both arise from the dorsal part of the pelvis, which was split off by the operation, while Della Valle holds them to be analogous to his own case.

Della Valle has laid particular stress upon the supposed identity of change of asymmetry and reversal of polarity, and has sought to make the various cases of superregeneration which have been reported fit into his scheme of '*doppia rigenerazione inversa*.' The case on which Della Valle bases his discussion of these questions is that of a newt (*Triton*) in which the left anterior limb was fractured in the region of the brachium and cicatrization prevented by tying a silk thread around the limb at that level. Twenty days later the same limb was amputated a short distance below the point of fracture. There regenerated three perfect limbs, one from the distal end of the stump and two from the region of the fracture. Of the latter, one was from the proximal end of the small portion beyond the ligature and the other was apparently a continuation of the stump proximal to the fracture. The first and last of the three were left limbs, i.e., of the same side as the original, while the one which regenerated in a distoproximal direction was reversed. The end result was a triple appendage in which the three members were placed in accordance with Bateson's rule.

Della Valle seeks to make the cases of Tornier ('05) and Lissitzky ('10) conform to this scheme, and falls into line with Przibram ('09) who had previously given a schematic representation of the same phenomena, which he termed '*Bruchdreifachbildung*.' He also interprets in the same way the reduplications obtained by Braus ('04, '09) and myself ('07) in the transplantation of embryonic limb buds. He suggests that when the limb

bud is implanted in normal location, triplicate appendages could be accounted for in the following way: one member derived directly from the grafted bud; one member, of mirror symmetry, by an inverse regeneration from the base of the bud, and the third member, of original asymmetry, from the wound surface of the host. This view is, however, not borne out by the present experiments.¹⁰⁶

When the whole of the evidence bearing on the question is taken into consideration, one cannot but think that too much weight has been placed by Bateson and his followers on the double supernumerary. The other class of cases, where the single supernumerary is symmetrical with the normal appendage with which it is associated, while neither so numerous nor so spectacular, is nevertheless of wide occurrence. Cases reported by Tornier ('97), Przibram ('02), Reed ('04), Zeleny ('05), and Megušar ('07) show that truly double appendages in mirror symmetry with respect to each other may be formed by constrict-

¹⁰⁶ Se dunque noi considerassimo uno di questi innesti praticati invece che in una regione lontana (come p. es. nell'orbita), nell'immediata vicinanza della regione donde fu tolto l'innesto, noi osserveremmo l'uno presso dell'altro lo sviluppo oltre che dell'arto normale, anche dell'arto rigenerato dalla superficie di sezione della regione prossimale del corpo, nonchè dell'arto sviluppatosi dalla superficie di sezione della regione periferica, identico all'arto che lo ha prodotto, ma con simmetria speculare. La identità anche di questo fenomeno con la doppia rigenerazione inversa dalle due superficie di una ferita risulta in questo modo evidente. Della Valle: op. cit. p. 125.

There is opportunity to test this hypothesis by comparing the experiments in which the wound-bed was cleaned with those where it was not. In the former, regeneration from the host is precluded (p. 6), and triplicate limbs could only arise by a second reduplication from the base of the graft; whereas in the latter, regeneration from the host should occur in a large number of cases, if at all, and thus yield a large proportion of triplicate appendages. An examination of the results shows that this is not the case. In the first place, as shown in table 2, the total number of reduplications in the series with cleaned wounds is fifty-three, which is 56 per cent of the total number of positive experiments, while there are but sixteen cases (33.3 per cent) in the group with non-cleaned wounds. The disproportion is much greater when the number of triplicate appendages in each group is compared. Out of a total of eighty-seven cases old enough to be determined, there are twenty-five triplicate limbs (28.7 per cent) in the clean-wound experiments and only three in forty-eight cases (6.25 per cent) in the others. It is quite clear, then, that leaving in the wound-bed cells that are capable of giving rise to a new limb reduces greatly, instead of increasing, the chance of formation of supernumerary limbs, so that Della Valle's suggestion is untenable.

tion of a simple regenerating bud. This harmonizes with Driesch's ('06) observations on double *Echinus* embryos.

In the present work, the reduplicated extremities are nearly all found to be in minor symmetry, and many of those in which three members are present, if seen only in the fully developed condition, would appear to be cases of paired supernumeraries, conforming, though with some aberration, to Bateson's rules. The individual histories show, however, that they are mostly simple duplicities in which the supernumerary mirrors the original, and this seems to be the case in Braus's experiments, too. Two reduplicating limbs often do develop, but usually each grows as a bud from the original instead of the two arising as a pair in themselves. Each of them mirrors the original limb, so that the two supernumeraries are both of the same side. In other cases the supernumeraries are themselves double, in which event there is strict conformity to Bateson's rule, but the former constitute a large majority, and conformity there is only superficial, for the original limb is the middle member and not one of the extremes.

In view of these facts, there is probably no very fundamental difference between the two classes of reduplications, i.e., between the double supernumeraries symmetrical with each other and the single supernumerary symmetrical with the original; had Bateson had the developmental stages at his disposal, he himself might not have drawn so sharp a distinction.

In accordance with the above, Bateson's rules might be stated in more general form, so as to include both simple duplicities and symmetrical pairs, as follows:

1. The long axes of duplex or multiplex appendages lie in one plane.
2. Two adjacent members form in structure and position the image of each other, as reflected from a plane mirror bisecting the angle between the respective axes and perpendicular to the common plane of the two axes (figs. 3 and 4).

The present experiments show (tables 2, 3, 5, and 8) that, excepting heterotopic grafts, it is in the disharmonic combinations that reduplications are most frequent. What, now, is the nature of the disturbance that causes the doubling of transplanted

limb buds and of regenerating limbs, which, when it occurs, is always combined with reversal of one member? The first visible sign of reduplication both in the embryonic limbs and in the regenerating blastema is the presence of two growth centers for the limb in place of one; each becomes an apex of growth, with a resulting bifurcation of the appendage as a whole. The question arises whether the doubling of the growth center is antecedent to or resultant from the reversal of the asymmetry. From the fact that mere mechanical division of a simple regenerating center¹⁰⁷ may bring about doubling, it would seem to be more probable, if not certain, that the existence of two growth centers within spheres of mutual influence is the factor that produces the reversal in one—the one that is less advantageously placed, or in which differentiation is less advanced.

The problem before us thus resolves itself into two phases: that of division or repetition of parts and that of symmetry. This was clearly seen by Bateson, who has emphasized the fundamental nature of the power to divide.¹⁰⁸ No attempt will be made here to analyze this phase of the question. The symmetric relations of the repeated parts are, however, so definite and of such general recurrence that they, too, are beyond question of a fundamental nature.

The phenomenon of reversal of asymmetry has been treated by many investigators as one with that of axial heteromorphosis, and yet this is not strictly correct, for the reversal of asymmetry may be brought about by the interchange of the poles of any one of the three axes to which the object is referred, and not necessarily the one along which regeneration and differentiation is taking place. This is true not only when regeneration occurs in a proximodistal direction, as in the cases of Tornier, Zeleny, and others, cited above, but also when it takes place distoproximally, as shown in the two experiments reported by Kurz ('12).¹⁰⁹

¹⁰⁷ Cf. Tornier ('97), Przibram ('02), Reed ('04), Zeleny ('05), Megušar ('07).

¹⁰⁸ "This power to divide is a fundamental attribute of life and of that power cell division is a special example." (Problems of Genetics, p. 38.)

¹⁰⁹ In somewhat similar experiments by Morgan ('08 a) only the bone, not the soft parts, was reversed. Nothing is said regarding the exact character of the limbs regenerated.

The fundamental phenomenon, therefore, is not that a particular axis is reversed, but that reversal occurs at all, and how it is brought about.

Organic polarity, in general, has been based either on the supposed polarization of the organic units themselves or upon a supposed gradient of a functional (Child) or material (Morgan, '05) nature, running from one end of the organism to the other. There is evidence for the occurrence of both factors, and what seems most likely is that both are at play. Under certain circumstances they act in the same direction; under different conditions one may antagonize and retard, or even overcome, the other, as seems likely in heteromorphic regeneration where polarity is reversed (earthworm, planarians, amphibian tail, etc.). Przibram ('13), who advocates a theory combining the two factors, which he calls 'Richtungspolarität' and 'Schichtungspolarität,' respectively, nevertheless regards the reversal of polarity as due to actual rotation of the cells. He ('06, '10 a) cites unpublished work by Hadži in support of this view, and adopts it in his diagrams ('09) illustrating the five fundamental varieties of operation leading to regeneration (Biotechnik).

The figures are not convincing, however, for just as much rotation of the cells is shown at the end where polarity is not reversed as at the other end where it is reversed (Przibram, '09, pl. XV, 1h-3h), and in fact, as expressed in these diagrams, what turning is shown is nothing more than a wound-healing process. Until it is demonstrated that rotation of the cells as a whole takes place solely in heteromorphic regeneration, it cannot be used to explain reversal of polarity.

So long as the elementary units of the limb bud have one plane of symmetry left, and the final asymmetry of the limb remains to be determined by its relation to certain axes of the embryo,¹¹⁰ it

¹¹⁰ In the case of asymmetric organisms, the elementary units, if representing the form of the organism at all, must be postulated as asymmetric themselves. In the case of paired organs, each asymmetric in itself, but symmetrical with respect to its opposite, polarization on the transverse axis may be assumed as due to the position of the parts with respect to the other two axes (cf. Przibram, '13, p. 38) and not as necessarily due to actual differentiation of the elements in the transverse direction.

will of course be possible to account for its reversal by rotation of the elements about the proper axis. As an alternative to the rotation hypothesis, we might, however, consider reversal as due to an interchange in position of two of the determining groups in the elementary units (p. 89, fig. 136). In case of differentiation on all three of the axes, i.e., if the units themselves are asymmetric, then reversal could take place only in the latter way, unless it occurs altogether independently of the intimate structure.

There is an analogy for reversal of this kind in the change of the asymmetry of organic molecules of known composition, as, for instance, in the Walden inversion by means of successive substitutions, or in the conversion of dextrotartaric acid into racemic acid, by which transformation half of the dextrorotating groups are changed into the laevo form. Of course, these examples are mere analogies.

Such questions have been touched upon by many of those who have studied twins and double monsters, but, unfortunately, the evidence both as to the cause and as to the occurrence of reversal of asymmetry is conflicting. In the case of human duplicate twins, it is certain that there is no *situs inversus viscerum*, except very rarely, and apparently even in double monsters the degree of fusion of the two individuals must be considerable for the asymmetry of the internal organs—heart and alimentary canal—to be reversed. On the other hand, it has been shown by Wilder ('04) that in duplicate twins the friction-skin patterns of the two mates may show mirror imaging, particularly those on the index fingers. A similar condition has been found by Newman ('16) in his study of variation of the scutes in armadillo quadruplets, except that here the matter is further complicated by the relation, in pairs, of the four individuals of a litter.¹¹¹

¹¹¹ "Now in the armadillo there are many definite evidences of a system of symmetry common to all of the quadruplets, upon which has been superimposed a secondary symmetry system between twins. This in turn is more or less completely obliterated later by a tertiary symmetry between the antimeric halves of the single individuals. In some sets evident traces of the primary system of symmetry persist as mirror-image relations between individuals of opposite pairs, but it is more usual to find no trace of the primary system. The secondary mirror-imaging between pairs is far more commonly in evidence, but is frequently

The evidence which Morrill ('19) has collected from the study of double monsters in fish embryos shows that situs inversus does occur, but that it is the exception, not the rule, and that there is no "very precise relation between the amount of separation of the two components and the occurrence of mirror imaging."¹¹² This would seem to oppose the view expressed above, that it is the proximity of two growth centers that causes the reversal of one. Still, in the absence of statistical data regarding the correlation of the two events, it is unsafe to draw a definite conclusion.

In this connection the recent work of Spemann and Falkenburg ('19) is of the greatest importance. By extension and modification of the earlier methods of the former, these investigators obtained a large number of twins in Triton by constricting the eggs in segmentation stages or in the early blastula. They found that in a large number of the cases one individual of a pair (the right-hand member in all cases but one) has complete situs inversus viscerum. Spemann, after an admirable critical analysis of the question, reaches the conclusion, that while some asymmetric intimate structure must be postulated to account for the normal asymmetry of the vertebrate body, there is no proof from these

obliterated by the tertiary mirror-imaging between antimeric halves of the same individual, which latter is the prevailing symmetry system. . . . In general, mirror-imaging between individuals of opposite pairs is interpreted as an evidence of the early system of symmetry present in the embryonic vesicle before polyembryonic budding began. When the primary buds are formed they are the product of the antimeric halves of the undivided embryo and therefore should have mirror-image relations, but a partial physiological isolation of the two buds permits a certain degree of reorganization or regulation in the symmetry relations, that tends partially to obliterate the original symmetry relations of the undivided embryo. Similarly, when each primary bud subdivides to form the secondary buds that are the primordia of the definitive individuals, a certain residuum of the primary bud symmetry system is carried over, manifesting itself in mirror-imaging between the twins derived from the same primary bud. But here again a certain amount of regulation occurs so that a third system of symmetry, the bilateral symmetry of each individual, tends to obliterate former systems of symmetry." Newmann: *op. cit.*, pp. 200-201.

¹¹² Tannreuther ('19, p. 359) has recently figured a double chick embryo in which the two individuals are united only by the posterior tip of the primitive streak. Although trunk and head are entirely separate, the heart of the embryo on the left shows situs inversus. No mention is made of this fact in the text.

experiments that it has been completely reversed by the operation. The evidence points, on the contrary, to the introduction of another factor which is manifested also in the tendency of the individuals to show defects on the inner side (i.e., the side turned toward the partner). In the case of the left-hand member, this acts in the same sense as the innate tendency to asymmetry of the viscera, while in the case of the right-hand member, it antagonizes, and in many cases, overcomes the latter.¹¹³ Spemann suggests many different experiments to throw light upon this question. The interesting point in Spemann's discussion, in connection with the present work, is that the necessity for assuming some sort of intimate structure to account for external symmetry relations is recognized. In the case of the reduplicated limbs it is not clear whether the reversal of the secondary bud is a result of direct action upon the individual processes of development going on within it, or whether the influence of the primary bud actually reverses the intimate structure. If it should be found that the reaction takes place before the cells of the limb blastema lose their totipotency, then the latter is undoubtedly true. Otherwise it may be that the differentiation in the limb blastema takes place directly under the influence of the tissues of the environment.

H. Form regulation and function in transplanted limbs

That the limb bud after transplantation becomes adapted in a measure to the new conditions is obvious from a casual consideration of the experimental results. There are different types of adaptation, however, for although regulation of form and function go largely hand in hand, in some cases there may be very complete functional regulation without form regulation,¹¹⁴ and, particularly in the heterotopic grafts, form regulation without function. By form regulation is meant, in the present connection, the process by which a limb bud that is implanted abnor-

¹¹³ Cf. also Pressler ('11).

¹¹⁴ For instance, in Experiment I. E. 64, the single disharmonic limb functioned very actively and effectively.

mally in relation to the embryo as a whole becomes adjusted so as to give rise to a limb in harmony with its new surroundings. Perfect adaptation is attained, however, only in cases where there is functional adaptation as well, i.e., only when both form and function are adjusted to the organism as a whole, and this occurs only in the orthotopic position or in positions very close to it. Functional regulation is here largely a question of innervation, and, inasmuch as this has been made a subject of special investigation by Detwiler ('19, '20), it will not be considered at present further than is necessary in its relation to form regulation.

Considering only the orthotopic grafts, there is no *a priori* ground for expecting any of the combinations to yield normally functional limbs except the homopleural dorsodorsal group, in which the axial relations of the transplanted bud remain normal with respect to the cardinal points of the embryo. Nevertheless, it has been found that inverted limb buds from the opposite side of the body yield almost as high a proportion of normally formed and normally functional limbs as the first-named group. This phenomenon has been interpreted as due not so much to a secondary regulation as to the structure of the elementary units of the limb bud, which are supposed to be symmetrical or reversibly differentiated (p. 89) along their dorsoventral axis. With an equal number of experiments in each of the four groups and without any disturbing factors, the primary processes of regulation should therefore lead, in accordance with the fundamental rules of symmetry, just as often to adaptive (harmonic) as to non-adaptive (disharmonic) end results, but not more often. This proportion is, however, exceeded by nearly 20 per cent of the expectancy, so that, taking the orthotopic experiments as a whole, 59.6 per cent yielded normally functioning limbs in normal posture, while 40.4, for the most part reduplications, were non-adaptive or only imperfectly adaptive (table 7). The proportion is almost the same in the three classes of experiments, whether with whole buds after extirpation of the normal bud, with superposed buds, or with half-buds, though, as more fully discussed below (p. 107), it is quite different in the heterotopic grafts.

TABLE 7
*Showing the adaptive or non-adaptive character of the resulting limbs after
 orthotopic transplantation*

TYPE OF EXPERIMENT	TOTAL NUMBER	ADAPTIVE (SINGLE HARMONIC LIMBS)				NON-ADAPTIVE (REDUPLICATION OR SINGLE DISHARMONIC LIMBS)			
		Primarily	By secondary regulation	Total	Per cent	Primarily	By secondary regulation	Total	Per cent
Whole buds									
hom. dd.	9	9	0	9	100.0	0	0	0	0.0
hom. dv.	38	0	10	10	26.3	25	3	28	73.7
het. dd.	31	0	5	5	16.1	26	0	26	83.9
het. dv.	16	15	0	15	93.8	0	1	1	6.3
Total.	94	24	15	39	41.5	51	4	55	58.5
Average of percentages.					59.0				41.0
Superposed buds									
hom. dd.	5	5	0	5	100.0	0	0	0	0.0
hom. dv.	5	0	1	1	20.0	4	0	4	80.0
het. dd.	5	0	1	1	20.0	4	0	4	80.0
het. dv.	5	5	0	5	100.0	0	0	0	0.0
Total.	20	10	2	12	60.0	8	0	8	40.0
Average of percentages.					60.0				40.0
Half-buds									
hom. dd.	8	8	0	8	100.0	0	0	0	0.0
hom. dv.	17	0	4	4	23.5	13	0	13	76.5
het. dd.	17	2	3	5	29.4	12	0	12	70.6
het. dv.	22	19	0	19	86.4	0	3	3	13.6
Total.	64	29	7	36	56.3		3	28	43.8
Average of percentages.					59.8				40.2
Total harmonic.	65	61	0	61	93.9	0	4	4	6.1
Total disharmonic.	113	2	24	26	23.0	87	0	87	77.0
Grand total.	178	63	24	87	48.9	87	4	91	51.1
Average of percentages ¹					59.6				40.4

¹ As given in the three main groups in the upper part of the table.

The secondary regulation of form may be brought about in one of two ways: either by rotation of the limb as a whole during development, whereby it is gradually brought into normal posture, or by a process of reduplication, which is more complicated. In the latter the original grafted limb bud gives rise to a secondary or reduplicating bud of mirror symmetry, which outstrips the former in development, reducing it to a spur or even practically suppressing it. The process of reduplication more often yields, however, actual double appendages, and there is no hard and fast line between the latter and the single limbs with the original bud reduced to a spur. The figures given in the table are therefore somewhat arbitrary in this respect.

Regulation by rotation has been noted only in the inverted limb buds from the same side of the body (*hom.dv*), and it has been further shown that this mode of adjustment probably occurs when the limb bud at the time of operation is rotated anteriorly along the dorsal semicircumference somewhat less than 180° (p. 41). The mechanics of this process is not yet understood. In the other disharmonic group single limbs are very rare (only one case), unless reversal by reduplication and reduction occurs, and no cases of rotation have been observed.

Regulation by reduplication and reduction, which is attended by reversal of asymmetry, has been observed in five cases out of thirty-one in the heteropleural dorsodorsal group, but no perfect cases of single limbs so produced have been found in the other disharmonic combination (*hom.dv.*). In the latter group, however, reduplication is frequent, and the reduplicating member is often normally attached and assumes the same posture as the normal limb, but it has never been found to begin its development early enough to bring about the suppression of the original bud.

In a measure offsetting the regulative cases, there are four others in which the same process, reduplication, has resulted not in regulation, but rather in its prevention. In three cases of inverted buds¹¹⁵ (*hom.dv.*), where there was tendency to regulate by rotation, this regulative process was rendered futile by secondary budding, and also in one case of a heteropleural dorso-

¹¹⁵ I. E. 86, 88, 90 and possibly two other cases. See p. 37.

ventral graft, the usual primary regulative result was vitiated by reduplication. The same was true in three cases of half-buds inverted on the opposite side of the body (*het.dv.*). Reduplication, therefore, is not in itself a regulatory process, but leads merely to a condition where regulation may take place through the reduction of the disharmonic member.

The question now arises, whether the experiments give any ground for assuming that there is anything teleological in these regulatory processes. While there is no proof that regulation by rotation is not of this nature, it would be a mistake to draw any conclusion regarding it until the details of the process are better understood. With regard to regulation through reduplication and reduction, it is clear that mechanical factors are sufficient to account for the process. It is in the disharmonic combinations that secondary regulation is necessary to produce normal results. When this is not accomplished by rotation, reduplications almost always arise, and there are only two cases of single disharmonic limbs among the orthotopic grafts. The disharmonic relation, which is brought about by the inversion of the anteroposterior axis of the limb bud, is thus seen to be a factor of prime importance in the production of reduplications. It is not the only factor, however, for some reduplications have occurred in harmonic combinations, due probably to other disturbances at the time of operation. But quite aside from the latter, it would be wholly unjustifiable to assume that there is any causal relation between the possible utility of the process of reduplication for regulatory purposes and its frequent, or even almost universal, occurrence where it might lead to this result. What seems to be the condition that brings about regulation after the reduplicating bud has arisen is the chance placement of the latter in a position corresponding to that of the normal single limb. This relation is attended by a more advantageous situation with respect to blood and nerve supply than that occupied by the original bud, and in extreme cases this leads to the resorption or suppression of the latter. It is only in such cases that complete regulation takes place and these constitute but about 15 per cent of the total number of reduplications.

In this connection it is important to compare the orthotopic with the heterotopic operations in respect to the regulatory processes just considered. When the limb bud is implanted in abnormal location, functional adaptation does not occur at all, the limbs rarely showing any motor function whatever, unless placed close to the normal position of the limb.¹¹⁶

Now the records show (table 8) that the two harmonic combinations in the heterotopic series produced ten single harmonic limbs and twelve reduplications (44.4 and 55.6 per cent, respectively).

TABLE 8

Comparison of heterotopic and orthotopic transplantations with reference to the relative number of single limbs and duplicities in the harmonic and disharmonic combinations

CHARACTER OF COMBINATION	HETEROTOPIC				ORTHOTOPIC			
	Single		Reduplicated		Single		Reduplicated	
	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent
Harmonic	10 ¹	44.4	12	55.6	24	96.0	1	4.0
Disharmonic	19	86.4	3	13.6	2 ²	3.4	57 ³	96.6

¹ Excluding one anomalous case in which an error of record is probable.

² Excluding five cases which became normal single limbs by development of the duplicate and resorption of the original bud, and ten cases which became normal by rotation.

³ Including five cases in which the original member was resorbed and the single normal limb arose from the reduplicating bud.

In many of the latter, however, the doubling was but slight, involving only the digits. The two disharmonic combinations, on the other hand, produced nineteen single limbs (86.4 per cent) and three (13.6 per cent) reduplications. The results obtained by Detwiler ('18), using much younger limb buds from embryos with open medullary folds, sustain the above results, as far as the harmonic group is concerned. There are eight cases of single limbs and ten reduplications. In the disharmonic group, how-

¹¹⁶ This has been subjected to a careful analysis by Detwiler ('19, '20), who has shown that the failure to function is not due to lack of peripheral innervation so much as to the insufficient connections within the central nervous system.

ever, there are four normal limbs and three reduplications. If combined with the present results, this would reduce the disproportion somewhat, though it would still leave it very large. There are no cases of complete regulation over to the harmonic position either by rotation or reduplication. Moreover, reduplication in over half the harmonic grafts disturbs the possible harmonic end result.

As pointed out above, the case is very different in the orthotopic operations. Here the harmonic group produced twenty-four out of twenty-five (96 per cent) single limbs, while the disharmonic group produced but twelve single limbs (17.4 per cent), ten of which became harmonic by rotation and are therefore omitted from the tabulation, and fifty-seven reduplications (82.6 per cent), in three of which, however, the primary bud became harmonic by rotation and a possible adaptive result was prevented by the process of reduplication. Leaving out of consideration the cases in which single normal limbs were produced by rotation, there are but two cases left (3.4 per cent) in which the disharmonic relation failed to be followed by reduplication.

In a word, with orthotopic grafts the almost completely dominant factor in producing single limbs or reduplications is the harmony or disharmony of the combination, whereas the case is quite different in the heterotopic grafts, where the harmonic group produced a slight preponderance of reduplications and the disharmonic group a great preponderance of single limbs.

At first sight the above figures might be advanced in support of some hypothesis of an end purpose in development, inasmuch as reduplications are produced in overwhelmingly great number only where they may be taken advantage of to produce an adaptive end result. This, however, would be a hasty conclusion to draw. The orthotopic experiments may be explained as above (p. 106). There the tendency to reduplicate is due first to the disturbance of operation, which, being very slight, is almost always suppressed in the harmonic combinations by the advantage the primary bud has in connecting normally with the surroundings; while in the disharmonic combinations the tendency to reduplicate is not only greatly increased by the reversal of the axis

of the bud, but the primary bud also gains a headway in development that usually cannot be overcome even by the more advantageous position of the reduplicating bud. In the heterotopic operations the frequency of reduplication in the harmonic group may be ascribed to the disturbance due to the operation, together with lack of any special anatomical relations at the seat of implantation that would overcome the tendency to bud by giving the harmonic member a special advantage. All that remains to be accounted for is, therefore, the small proportion of reduplications in the disharmonic group. This should be subjected to further investigation. Standing alone, it can hardly be advanced as an argument for a teleological theory of development.

GENERAL SUMMARY

1. The results given below are based upon the following experiments with the fore-limb bud of the embryo of *Amblystoma punctatum*:

a. Transplantation to the flank of another embryo posterior to the normal position of the fore limb (heterotopic transplantation), the grafted buds being taken either from the same side of the body (homopleural) or from the opposite (heteropleural), and implanted with the dorsoventral axis upright (dorsodorsal) or inverted (dorsoventral).

b. Transplantation to the normal location of the fore limb after extirpation of the original fore-limb rudiment (orthotopic transplantation), with the same variations as in the heterotopic group.

c. Superposition of one limb bud upon another after removal of the ectodermal covering of the latter, also with the same variations as in the previous groups.

d. Transplantation of half of the circular disc constituting the limb bud, after extirpation of one-half the rudiment. Sixteen different combinations of this experiment (all possible within the limitations imposed) were tried (p. 70).

2. In the early stages of development in any position the transplanted buds give evidence of their constitution by growing out.

(‘pointing’) in the direction of what was originally the posterior pole of the anteroposterior axis. Thus, in two of the combinations (homopleural dorsoventral and heteropleural dorsodorsal) they point anteriorly or dorsoanteriorly, and in the two others (homopleural dorsodorsal and heteropleural dorsoventral) they point posteriorly or dorsoposteriorly like the normal. In the latter case the subsequent development is usually normal, barring reduplication; in the latter there is a tendency for the limb to stick out to the side and to rotate more or less from the position in which it would be found, were the position determined entirely by the orientation of the bud itself.

3. The palmar surface of the limb tends to develop on the side turned toward the body of the animal, and the ulnar border is dorsal, although the rotation mentioned in the previous paragraph tends to change these positions.

4. The above circumstances determine the asymmetry of the limb as follows: when the dorsoventral axis is not inverted, the original prospective asymmetry persists; when the axis is inverted, the asymmetry is reversed (rules 1 and 2, p. 4). In more general terms: the asymmetry of the limb is determined by two factors, the polarization of the anteroposterior axis of the limb bud and the orientation of the limb bud with respect to the dorsoventral polarization of its organic environment (figs. 2 and 135).

5. In two of the combinations (homopleural dorsodorsal and heteropleural dorsoventral) the asymmetry of the limb which develops corresponds to that of the side of the body on which it is placed (harmonic); in the other two (homopleural dorsoventral and heteropleural dorsodorsal) it corresponds to that of the opposite side (disharmonic).

6. Duplex and multiplex limbs arise frequently from the transplanted buds. They are of all grades and kinds and occur in different proportions in the several experiments. In the heterotopic grafts they are more frequent in the harmonic combinations, while in the orthotopic position they are much more frequent in the disharmonic combinations.

7. In nearly all cases one member of a pair or group can be distinguished as the original (primary) and the other one or ones

as buds. The reduplicating bud is in each case the mirror image of the original, and, when the reduplicating bud is itself doubled, then the one next to the original is the mirror image of the latter, while the one further away is mirrored, with respect to its mate, approximately in accordance with Bateson's rule.

8. Limbs placed in abnormal location, where the specific blood and nerve supply is lacking, are frequently resorbed, and when they do develop, are usually stiff and functionless, or at best show imperfect function. The shoulder-girdle in such limbs is reduced in size and the more outlying elements are lacking.

9. Limb buds placed in normal location (orthotopic) are rarely resorbed and nearly always become functional.

10. Limb buds from the same side of the body normally oriented in orthotopic position develop normally with but slight retardation.

11. When the limb bud from the same side is rotated 180° in its normal location, the results vary considerably, and in the majority of cases reduplications occur. The single limbs are of two kinds, reversed and normal. The former develop in accordance with rule 2 (p. 4), but only one case of this kind has been observed, the others that conform to the rules being reduplicated (rule 3). In the other cases the normal position was reached by the rotation of the limb as a whole about the shoulder-joint. These cases are exceptions to the rules.

12. Of the twenty-seven cases of reduplications in the above group, the original bud grew anteriorly and was reversed. In the three remaining cases the primary limb righted itself by rotation and the reduplicating member was reversed.

13. Regulation by rotation usually takes place when at the operation the limb bud has been rotated anteriorly over the dorsal semicircumference not quite 180° .

14. Limb buds from the opposite side of the body, with the dorsoventral axis normally oriented, produced but one unreversed single limb, in accordance with rule 1 (p. 4), the rest being reduplications (rule 3). In one-sixth of the latter the original bud, which was disharmonic, was resorbed and remained as a small nodule or spur on the reduplicating appendage. The dupli-

cate bud in these cases, having its asymmetry reversed and occupying the right position, became a normally functioning fore limb, perfectly adjusted both functionally and structurally to its organic environment.

15. Limb buds, taken from the opposite side of the body and implanted with the dorsoventral axis inverted, so as to leave the anteroposterior axis in normal relation, formed, with the exception of one reduplication, single limbs, all of which were reversed. These limbs were often considerably retarded in development, but, as regards both function and form, they became perfectly adjusted to their new surroundings (rule 2).

16. In the superposed grafts two limb buds are fused into one. In the two harmonic combinations normal single limbs arise. Though at first usually above normal in size, they soon become regulated in this respect. In the disharmonic combinations duplex appendages were formed in a large majority of cases. One case of adjustment by rotation and one case of regulation by reduction of one member of a pair were found.

17. In experiments with half buds there are sixteen combinations possible with the restrictions imposed by the character of the experiment. In addition to the two pairs of attributes of operation common to all of the experiments (*hom.* or *het.*, *dd* or *dv*) there are three others: the bud may be halved vertically or horizontally; the anterior or the dorsal half, or the posterior or the ventral half may be transplanted, the other remaining intact; two like halves or two unlike halves may be united. An analysis of the results shows that no one of these qualities in itself determines the result, but that it is the harmonic or disharmonic character of the combination that determines whether normal or reduplicated appendages arise. Thus, allowing for differences in the number of experiments in each class, 93.4 per cent of the harmonic combinations produced normal limbs, while in the disharmonic groups about that same proportion produced reduplications, of which, however, a considerable number were regulated secondarily through resorption of the disharmonic member.

18. That the limb bud is an equipotential system is shown by the fact that a normal limb may develop after the following oper-

ations, provided the combination is harmonic: 1) extirpation of any half of the bud; 2) fusion of two whole buds; 3) combination of two like halves, the other half being entirely missing; 4) inversion of the limb bud; 5) inoculation of mesoderm cells from the limb under the skin in some other region of the embryo.

19. Except for the circumstance that the dorsoventral differentiation of the limb bud is a function of the orientation of the bud with respect to its organic environment, the limb bud is a highly specific self-differentiating system. Its definitive form must, therefore, be represented in the organic elements (intimate structure) of the limb rudiment.

20. One quality of these elements is their polarization, as shown by the definite relation to the direction of out-growth, assumed by the anteroposterior axis of the limb bud. It is suggested that the asymmetry of the limb rudiment and of other similar systems may be gradually brought about by the change in constitution of the structural elements in a manner similar to the building up of asymmetric molecules in organic compounds.

21. Reduplications are produced as a result of that fundamental attribute of living matter, the power to divide (Bateson). They are induced, in the case of the limb bud especially, by a disharmonic relation between graft and host.

22. There is no fundamental distinction between double supernumerary limbs constituting a symmetrical pair and the single supernumerary symmetrical with the normal one with which it is associated. Bateson's rules may be stated in simplified form in accordance with this conclusion (p. 97).

23. Exceptions to Bateson's rule regarding symmetry relations of supernumerary parts are very rare. Those found in the present study, where two limbs of the same side occurred in linear series, are probably due to the appendages having been far enough apart not to influence one another in development, and at the same time having been under the influence of the same organic environment.

24. Review of the data on regeneration of supernumerary appendages shows that the reversal of asymmetry in one of the members of an enantiomorphic pair is not dependent upon the

reversal of direction of growth, regeneration, or differentiation. The reversed member may grow and differentiate in the same direction as the original, another axis than that on which growth is taking place being the one that is reversed. Reversal may thus occur without axial heteromorphosis and vice versa.

25. In any system, like that of the limb bud at the time of transplantation, in which at least one axis is left undifferentiated, rotation of the elements of which the system is made up might account for reversal. The rotation of cells observed by Hadži and Przibram is, however, concerned primarily with wound healing, and heret is no evidence that it is correlated with the occurrence of axial heteromorphosis or reversal of asymmetry.

26. As an alternative to the hypothesis of rotation, we might consider reversal as due to reversal of molecular asymmetry according to analogy with the behavior of optically active compounds.

27. There is an analogy between the production of enantiomorphic limbs and the production of situs inversus viscerum, as effected by Spemann. Either the reversal may be due to reversal of the intimate structure, or it may take place in spite of the intimate structure through the direct action of mechanical factors on the individual parts of the differentiating system.

28. The transplanted limbs show both regulation of form and functional adaptation. The two often go hand in hand, but not necessarily, for some cases show regulation of form without function, and others functional regulation without form regulation.

29. Functional regulation is largely a matter of innervation, and it occurs only in orthotopic grafts or in those approximately in that position (Detwiler).

30. Form regulation is either primary, as in the case of harmonic combinations, or secondary, as in the disharmonic. In the latter it takes place in one of two ways, either by rotation of the developing limb or by means of reduplication and reduction of the disharmonic member.

31. Form regulation by rotation has been observed to occur only in orthotopic grafts; reduplications in disharmonic combinations are more frequent in orthotopic than in heterotopic

transplantations. While these circumstances lead to an harmonic end-result more frequently where there is functional adaptation as well, this cannot be used as a cogent argument for a teleological theory of development.

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APPENDIX

HISTORIES OF SELECTED INDIVIDUAL CASES

A. Heterotopic transplantations

1. Homopleural, dorsodorsal

Experiment Tr. E. 148. May 4, 1915. Right limb bud to right flank; orientation normal.

May 5. Healed except for two small oval areas.

May 8. Transplanted bud points caudally like normal limb.

May 12. Sketch (fig. 5).

May 15. Transplanted limb slightly bidigitate—not so long or as far differentiated as normal.

May 21. Transplanted limb has grown considerably, normal posture; two digits well marked, third appearing.

May 26. Third and fourth digits show more plainly than in sketch made on May 24 (fig. 6).

June 1. Limb a normal right (homopleural) limb in normal posture (fig. 7).

Experiment Tr. E. 182. April 13, 1916. Right limb bud to right flank; orientation normal.

April 14. Perfectly healed.

April 16. Transplanted bud as large as normal, points caudally.

April 25. Not so large as normal, points dorsocaudally.

May 1. Much shorter than normal. Points caudally, arising from a ridge-like prominence on side of body.

May 7. Two distinct digits and trace of third. A new bud is growing from base anterolaterally.

May 15. Original limb (the posterior one in fig. 9) has three distinct digits and beginning of fourth, shows elbow bend. It is a normal right (homopleural) in nearly normal posture, though it sticks out stiffly to side. The two limbs are entirely separate from their origin. The anterior member, which is the reduplicating one, shows only a faint trace of the third digit on the anterior border.

Experiment Tr. E. 210. May 10, 1916. Right limb bud to right flank; orientation normal.

May 11. Healed except for small area of uncovered yolk at caudal border of wound.

May 15. Transplanted bud somewhat smaller than normal, points in same direction.

May 18. Posture as of normal right limb. At base a rounded prominence which points headward.

May 22. The prominence has become a reduplicating limb, showing first trace of digits.

May 28. Both limbs well developed. Original is longer and is a right (homopleural) with three digits and faint trace of fourth. Reduplicating member branches from other at elbow and is a mirror image of latter, except that it is less advanced in development.

2. Homopleural, dorsoventral

Experiment Tr. E. 219. May 17, 1916. Right limb bud to right flank, inverted.

May 18. Healing fair; a considerable area anterior to grafted bud not covered with ectoderm.

May 21. Bud looks nearly normal, but points dorso-anteriorly. See sketches made on May 22 (figs. 10 and 11).

May 25. Transplanted bud growing as rapidly as normal. The two point toward each other (figs. 12 and 13).

May 29. Transplanted limb growing well; two distinct digits; points dorso-anteriorly and is apparently a mirror image of normal limb on same side (figs. 14 and 15).

June 5. Rapid growth has continued; limb reaches gills; third digit well marked, fourth beginning to show, elbow bend is slight; hand in an almost vertical plane, palm being anteromedial. Limb is an undoubted left, i.e., reversed (figs. 16 and 17).

Experiment Tr. E. 139. April 28, 1915. Right limb bud to right flank, inverted.

April 29. Healing perfect, transplanted tissue rather prominent.

May 5. Limb bud points slightly dorso-anteriorly.

May 8. Points distinctly dorso-anteriorly.

May 17. Limb points laterally at angle of a little less than 60° to axis of body. Third digit developing.

May 26. Four digits present (fig. 18). Limb does not look atrophic, but gives no evidence of motility or sensitivity. Position as when last observed. Specimen preserved.

Examination of serial sections shows that the anteromedial surface of the hand is the palm. There can be no doubt, consequently, that the limb is a normal left, having been reversed.

Experiment Tr. E. 140. April 28, 1915. Right limb bud to right flank, inverted.

April 29. Healing of wound good; slight areas still uncovered by ectoderm at ventral border of wound.

May 5. Transplanted tissue growing; but points anterodorsally.

May 11. Digitations plain; limb points as before.

May 17. Limb now points almost transversely, with ulnar digits on anterior border.

May 26. Perfect limb with extensor surface of elbow and ulnar digits dorso-anterior. No motility (fig. 19). Sections of preserved specimen show that ventral surface is palm, and that there can be no question about its being a left limb, i.e., reversed.

Experiment Tr. E. 220. May 17, 1916. Right limb bud to right flank inverted.

May 18. Wound perfectly healed.

May 21. Transplanted bud very prominent. Sticks out to side and slightly anteriorly.

May 25. Transplanted limb points dorso-anteriorly and not much to side. A small reduplicating bud points ventroanteriorly from near base.

May 29. Limb points as before. Two well-marked digits which are slightly irregular. Probably no reduplication.

June 5. Limb sticks out to side almost horizontally; first two digits short, third distinct, fourth barely indicated. Reduplicating hand attached to radial border of the same limb and pointing ventrally has two digits.

N. B. It is uncertain whether this member developed out of the bud noted on May 25.

3. Heteropleural, dorsodorsal

Experiment Tr. E. 227. May 19, 1916. Right limb bud to left side, dorsodorsal.

May 20. Well healed.

May 23. Transplanted bud points dorso-anteriorly and is more massive than normal.

May 26. Grafted limb about same length as normal; points anteriorly and laterally, scarcely dorsally (figs. 20 and 21 drawn one day later).

June 2. Grafted limb now reaches to the axilla of the normal limb; trace of third digit (fig. 22, made day before).

June 5. Limb a normal right (is not reversed), pointing antero-laterally, crossing the normal limb on the medial surface (fig. 23).

Experiment Tr. E. 107. April 9, 1913. Right fore limb to left side, dorsodorsal orientation. Diameter of transplanted bud 3 somites (3 to 5).

April 10. Crescentic uncovered area of yolk ventral to bud, which otherwise is well healed in.

April 14. Grafted bud shows indications of pointing headward.

April 21. Limb growing, pointing anteriorly exactly in reversed position. No digitations yet.

April 28. Limb is clearly a right; third digit now plain, with slight nodule to indicate fourth.

May 5. Possibly slight motility of grafted limb. It is now seen to be a perfect right, sticking out sharply to side, upper arm is inclined dorso-anteriorly, fore arm slightly posteriorly; palmar surface of hand ventro-anterior (figs. 24 and 25).

Experiment Tr. E. 127. May 12, 1914. Right limb bud to left side, dorsodorsal orientation; diameter of bud 3 somites.

May 13. Healing perfect.

May 16. Transplanted bud growing well with slight indication of pointing anteriorly.

May 18. Limb points distinctly anteriorly.

May 22. Two digits show; a reduplicating bud is growing out from base of graft.

June 1. The primary limb now shows fourth digit and is a perfect right (fig. 26, *PR*). The reduplicating member (*DU*) is a left, though not quite so far along in its development. The primary limb now points laterally and posteriorly with its ulnar border headward.

4. Heteropleural, dorsoventral

Experimental Tr. E. 193. April 14, 1916. Right limb to left flank, dorsoventral orientation; diameter of transplanted bud $3\frac{1}{4}$ somites.

April 15. Wound perfectly healed.

April 17. Transplanted bud, rather larger than normal, points posteriorly.

April 21. Transplanted bud has large base, but free portion is slender; points distinctly posteriorly.

April 28. Transplanted bud growing, though much smaller than normal. Points dorsoposteriorly and has two digits.

May 1. Digits more distinct. Slight bending at elbow. Has position of normal left limb.

May 7. Three digits and trace of fourth. A normal left limb in almost normal posture (fig. 27).

Experiment Tr. 167. February 24, 1916. Right limb bud to left flank, dorsoventral orientation.

February 25. Wound completely healed. Transplanted bud prominent.

Feb. 28. Transplanted bud a large blunt prominence which points posteriorly.

March 3. Transplanted bud is very small; may be resorbed.

March 7. Grafted limb much smaller than normal, but growing.

March 11. Considerable growth, but still smaller than normal. Slight indication of elbow bend and digits.

March 15. Digits marked. Limb looks like a normal left reversed, though it sticks out to side more than the normal limb.

March 24. Third digit now plain. Arm points to side. Hand transverse and vertical with respect to body.

March 29. Preserved. Transplanted limb a normal left in nearly normal posture. Fourth digit indicated. Upper arm still sticks out more to side than normal, but otherwise little difference except in size.

Experiment Tr. E. 129. May 13, 1914. Right limb to left side, dorsoventral orientation. Transplanted disc 3 somites in diameter.

May 14. Wound perfectly healed.

May 18. Transplanted bud has grown considerably, points posteriorly.

May 25. Grafted limb points laterally and about 30° caudally, is bidigitate. Hand and perhaps forearm are being reduplicated on anterolateral border.

June 6. Specimen preserved. Imperfectly symmetried double hand, mirrored from radial plane. Arm stretches out to side with elbow bending ventrally. The posterior (original) hand is a left (reversed), as is the arm as a whole; it has four digits normally placed; the anterior (reduplicating) hand is less perfect with only three digits, one long one in the middle and a short one on each side, the first digit being imperfect.

Experiment Tr. E. 163. February 24, 1916. Right limb bud to left flank, dorsoventral orientation.

February 25. Well healed. Very small uncovered area ventral to bud.

February 28. Transplanted bud larger than normal and a little further ventral.

March 3. Bud is growing ventroposteriorly.

March 7. Bud long and slender, growing posteriorly almost on ventral surface of body; probably defective.

March 11. Same.

March 15. Original limb has not changed essentially. A second outgrowth of considerable length arises some distance dorsal to former and projects laterally. Judging by its size, it must have been present and overlooked at last observation.

March 20. Original limb a long bent appendage tapering gradually to a point. New bud has made considerable growth and is bidigitate, the digits spreading more than normally. Indication of elbow bend.

March 28. Secondary appendage has developed a third digit, which, with elbow bend, shows that it is probably a left (reversed).

April 4. Specimen preserved (figs. 33 and 34). Transverse sections show that limb is undoubtedly a left, since it is the dorsoposterior surface of the hand that is the palm—a very unusual posture. The two limbs have separate girdles which articulate with one another, but are not fused.

Experiment Tr. E. 217. May 17, 1916. Right limb bud to left flank, dorsoventral.

May 18. Perfectly healed.

May 21. Grafted bud almost exactly like normal, points dorso-posteriorly.

May 29. Limb short, sticks out more sharply to side. Digitations will probably be abnormal.

May 31. Possibly symmetrical reduplication of digits (fig. 31).

June 5. Uncertainty about reduplication. One distinct digit (third) on posterior (ulnar) border and also a slight nodule (fourth digit). Prominence on opposite border may not be digits (fig. 32).

June 8. One distinct digit and trace of another formed out of hump on radial side so that hand is now practically symmetrical.

Limb amputated; afterward regenerated; form much like original.

B. Orthotopic transplantations

7. Homopleural, dorsodorsal

Experiment N. E. 3. May 19, 1918. Right limb bud to right side—normal orientation. Pronephros intact.

May 21. Perfectly healed; limb bud normal.

May 25. Both limbs same except that transplanted one is slightly shorter.

May 28. No difference between the two limbs.

June 7. Same. Specimen preserved.

8. Homopleural, dorsoventral

Experiment I. E. 64. May 13, 1916. Left limb bud to left side, inverted. Pronephros removed.

May 15. Small uncovered area ventral to bud, which is larger than normal and slightly posterior to it.

May 18. Limb bud points dorso-anteriorly (fig. 35).

May 24. Transplanted limb points anteriorly into gills. Slight trace of digits; no reduplicating bud (fig. 36, drawn one day earlier).

May 28. Limb points anterolaterally below gills.

May 29. Limb is undoubtedly a right (reversed) and is perfectly normal except as to posture. Ulnar digits appearing on the antero-dorsal border of hand (figs. 37 and 38).

June 1. Transplanted limb sticks out more to side than before and can be brought back further toward normal position. Motility was first observed two days ago.

June 4. Larva has grown rapidly. Transplanted limb functions well; elbow bends toward tail instead of head. Limb a perfect right (figs. 39 to 41, drawn one day later).

Experiment I. E. 60. May 11, 1916. Left limb to left side, inverted. Pronephros removed.

May 12. Well healed, normal-looking bud.

May 15. Grafted bud points dorso-anteriorly.

May 16. Sketches (figs. 42 and 43).

May 18. Beginning of bud like outgrowth (reduplication) posteriorly (fig. 44, *DU*).

May 24. Main limb (*PR*) points more sharply to side and rather more dorsally than normal. Posterior (reduplicating) bud (*DU*) has grown considerably (figs. 45 and 46, drawn May 23).

May 29. Specimen preserved. Limb double from lower part of fore arm. The primary member, which is anterolateral, is a right (reversed), while the other, produced from the posterior bud, is a left (reversed back). Former has short first digit and well-marked third. On the reduplicating hand a trace of third digit present (figs. 47 and 48). Mirror plane is radiodorsal.

Frontal sections show the radii of the two arms are fused at the proximal end, the two ulnae being entirely separate. The humerus is single, i.e., fused throughout and is much more massive than normal. Glenoid cavity is also much larger, but normally oriented. Barring slight abnormalities, the shoulder-girdle seems to be a normal left (not reversed).

Experiment I. E. 63. May 12, 1916. Left limb to left side, inverted. Pronephros removed.

May 13. Fairly well healed.

May 15. Transplanted bud smaller than normal; points dorsally, sharply laterally, and slightly anteriorly (figs. 49 and 50, drawn May 16).

May 19. Points dorso-anteriorly. Slight indication of posterior reduplicating bud at base (figs. 51 and 52).

May 24. Main limb points much more dorsally and laterally than normal (figs. 53 and 54, drawn May 22); no digits. Two reduplicating buds; posterior one (*P.DU*) points as in I. E. 60 (figs. 44 and 45); anterior one (*A.DU*) smaller and spur-like.

May 29. Specimen preserved (fig. 55). Main limb is a right (reversed) with two long digits and third digit on anterodorsal (ulnar) border. The posterior reduplicating member with three digits is free and is in approximately normal position for left limb (reversed back). It is mirrored in a radiodorsal plane. The anterior hand is bidigitate, and is mirrored in an ulnopalmar plane. Arm and fore arm of complex is short and thick.

Experiment I. E. 49. May 14, 1915. Left limb bud to left side of same individual turned 180° . Pronephros removed.

May 15. Fairly well healed; irregular band of uncovered yolk at posterior border of wound.

May 21. Limb bud developing well, points dorsally with very slight anterior inclination.

May 25. Limb points dorsally (fig. 56); two digitations—hand flattened in vertical plane, making angle of 45° with body axis.

May 29. Limb points dorsoposteriorly, as if approaching normal position.

May 30. Limb has rotated still further toward normal position.

June 3. Limb in nearly normal position (fig. 57, drawn June 4).

June 11. Limb tends to lie partially adducted with dorsal surface of manus on bottom.

June 17. Limb now perfectly normal in every respect—form, size, posture, motility (fig. 58, drawn June 21, and fig. 59, drawn from specimen preserved thirty-nine days after operation).

Experiment I. E. 55. May 23, 1915. Left limb bud removed and replaced inverted. Pronephros removed.

May 24. Very well healed. Only a very narrow uncovered strip at posterior border.

May 27. Limb bud smaller and more pointed than normal.

May 30. Limb bud increasing, but not so large as normal. Sticks out more to side.

June 2. Limb bud points dorsoposteriorly, ventral part of bud prominent.

June 7. Limb has grown considerably, points more posteriorly than before. Two digits; manus flattened in transverse plane.

June 11. Posture of limb still abnormal. It points dorsoposterolaterally at about 45° to median and horizontal planes. Trace of third digit.

June 17. Limb normal in every respect—form, size, posture, motility.

Experiment I. E. 85. April 10, 1917. Left limb bud to left side, rotated $180^\circ +$ (fig. 60). Pronephros removed; pronephros grafted along with limb bud.

April 11. Well healed.

April 14. Grafted bud about as distinct as normal, points dorsally and slightly anteriorly.

April 16. Bud points dorso-anteriorly ca. 60° to horizontal; attachment slightly posterior to normal. Distinct pronephric swelling ventral to limb.

April 19. Original bud points as before. Posterior reduplicating bud in approximately normal position. Anteriorly, at base of original bud, is a third bud pointing anterolaterally.

April 24. Middle (primary) member points dorsally and has two long digits; anterior member is attached along anterior border of middle; posterior member, in normal posture shows beginning of digits.

May 7. Specimen preserved (fig. 61). Posterior member, an essentially normal left limb from its origin above elbow down, has four digits; it is mirrored from the middle member in a radiodorsal plane. Anterior and middle limbs fused except for distal portion of manus; mirror plane, palmar. The middle member has two long digits and nodules on ulnar border, and is probably a right (reversed). Anterior member has three long digits and ulnar nodule, the third being partly fused with the second of the middle hand. Anterior hand possibly hyperdactylous.

Experiment I. E. 86. April 10, 1917. Left limb bud to left side, rotated 180° — (fig. 60). Pronephros removed; pronephros transplanted with limb tissue.

April 11. Well healed.

April 14. Limb bud prominent, placed a little further posteriorly than normal; points slightly dorsally.

April 16. Transplanted bud points dorsoposteriorly, but more dorsally and slightly more laterally than normal. Pronephric swelling ventroanterior to bud.

April 19. Limb points more dorsally than normal. Small nodule at base is part of pronephros.

April 24. Limb in normal posture not quite so long as normal. Third digit beginning. Reduplicating digit comes off palmar side between first and second digits.

April 29. Second and third digits reduplicated (palmar); limb otherwise very nearly normal. Motility apparently not so good as normal.

May 5. Limb clearly a left (not reversed); arm a little shorter and thicker. Motility now better.

May 7. Specimen preserved (fig. 62).

9. Heteropleural, dorsodorsal

Experiment R. E. 87. May 19, 1915. Right limb to left side, dorso-dorsal orientation. Pronephros left intact.

May 21. Small round uncovered area posterior to bud.

May 25. Transplanted bud points anteriorly and laterally (ca. 45°). and is nearly as large as normal (fig. 63, drawn May 26).

May 28. Limb points as before and is growing into gills, one of which is caught in notch between limb and neck and is bent ventrally. Limb itself bent slightly at tip. Digitations faintly indicated.

June 2. Limb thinner than normal and somewhat limp. Bends backward at tip. Two digits only.

June 4. Specimen preserved (fig. 64). The arm sticks out horizontally to side; elbow bends posteriorly; two digits in same horizontal plane with very faint indication of ulnar digits. Limb clearly a right (not reversed), though it is distinctly smaller than normal and is otherwise defective.

Experiment R. E. 70. May 12, 1915. Operation: right limb bud to left side, dorsodorsal orientation. Pronephros left in.

May 13. Still a crescentic area of uncovered yolk at ventral border of bud.

May 15. Transplanted bud more prominent than normal; indications of pointing anteriorly (fig. 67, drawn May 17, at which time the posterior bud could just be made out).

May 21. Points anterolaterally. Attached to base is an almost equally large reduplicating bud, pointing posteriorly and normally located (fig. 68, drawn May 22).

May 27. Both buds growing. The original (anterior) one is still a little further advanced than the other. Figure 69, drawn May 28, shows beginning of digits.

June 7. The original limb is still the larger one, the reduplicated one has two digits and trace of third. The complex has some motility.

June 11. Specimen preserved (fig. 70). The arm is double from the elbow down; the posterior or reduplicating member is not so well developed as the other. The anterior member is a right (not reversed) while the other is mirrored in a radiodorsal plane and occupies a position approximately normal for the left limb.

Experiment R. E. 133. May 25, 1916. Right limb bud to left side dorsodorsal orientation. Pronephros removed.

May 26. Fairly well healed. Small uncovered area ventral to bud.

May 29. Transplanted bud well developed, points dorsoanteriorly about 45°. It is in proper position and about same size as normal.

June 2. Limb points dorso-anteriorly, but more sharply dorsally, extending into notch behind last gill. Two digits show distinctly as in normal limb. Reduplicating bud from posterior border of base has position of normal left.

June 5. Original limb (fig. 75, *PR*) points dorsolaterally toward gills and looks nearly normal. Posterior (reduplicating) bud (*P.DU*) is rather short and arises further tailward than normal limb; digitations beginning. A third small limb (*A.DU*) anterior to the original, projects dorsolaterally, parallel to it.

June 7. Original limb a normal right (not reversed) having two long digits with trace of third and fourth. The posterior bud has grown considerably and is in normal position. Anterior bud shows traces of digits.

June 12. All three limbs have grown and differentiated. Posterior has nodule for third digit and is a normal left (reversed). It is mirrored in a radiodorsal plane while the anterior limb, which is more

nearly parallel to the original, is mirrored in an ulnopalmar plane (fig. 76, drawn June 13).

Experiment R. E. 134. May 25, 1916. Right limb bud to left side, dorsodorsal. Pronephros removed.

May 26. Perfectly healed.

May 29. Transplanted bud, normal size and position, points antero-dorsally ca. 45° .

June 2. Limb points dorsally and somewhat laterally. Reduplicating bud on anterior border near base also points dorsally and slightly anteriorly.

June 5. Limb points anteriorly into gills, is short and massive. Three distinct digits, irregular.

June 12. Specimen preserved (fig. 77). Arm short and thick, internal reduplication, certainly from elbow down, mirrored in ulnopalmar plane. The two hands nearly separate; each has two long digits and nodular third. The main limb is a right (not reversed) and the other a left. No posterior reduplication in this case.

Experiment R. E. 69. May 12, 1915. Right limb to left side, dorso-dorsal orientation. Pronephros left in.

May 13. Wound well healed. Only a small area at ventroposterior border of wound uncovered.

May 15. Transplanted and normal buds equally prominent; former points anteriorly.

May 21. Transplanted limb double. Smaller (originally main) part (fig. 86, *PR*) points anteriorly and is borne upon the posterior portion, *DU*, which is an almost normal looking limb, though smaller than the normal on the opposite side.

May 24. The posterior bud has grown materially and is normal looking with two digits. The anterior bud is relatively much less developed (fig. 87).

May 27. The original bud is reduced to a small spur (*PR*) on the lateral surface of the reduplicating bud, which is almost as large as the normal limb on the opposite side (fig. 88, drawn one day later).

June 3. Transplanted limb perfectly normal. Spur much reduced.

June 7. Spur reduced to a nodule (fig. 89, *PR*).

10. Heteropleural, dorsoventral

Experiment R. E. 80. May 18, 1915. Right limb bud to left side, dorsoventral orientation. Pronephros removed.

May 19. Crescentic band ventroposterior to bud still uncovered.

May 21. Limb bud almost normal in size points posteriorly.

May 24. Limb bud points posteriorly, but more sharply laterally than normal (fig. 93, *TR*).

May 30. Limb points dorsoposteriorly angle of 45° .

June 2. Limb stiff and projects rather more to side than normal; has two digits and slight indication of third on dorsal border (fig. 94, drawn June 1).

June 11. Limb mobile, in normal posture. Brachium shorter than normal. Third digit distinct, fourth indicated.

June 21. Four digits all plain. Upper arm still short (fig. 95 *TR*).
Experiment R. E. 107. May 10, 1916. Right limb bud to left side, dorsoventral orientation. Pronephros out.

May 11. Rather large area of yolk, posterior to graft, still uncovered.

May 15. Transplanted bud points a little more dorsally and more sharply to side than normal.

May 19. Grafted limb (*TR*) about size of normal, though more pointed and projecting more dorsally and laterally (fig. 96, drawn May 18); divided at end (digits or reduplication?).

May 24. Limb on side of operation still projects a little to side. Normal limb seems to move and transplanted one still stiff (figs. 97 and 98, drawn May 23).

May 28. Motility of operated limb still seems deficient. Third digit distinct (fig. 99, drawn May 29).

June 2. Motility better. Specimen preserved.

Experiment R. E. 116. May 16, 1916. Right limb bud to left side, dorsoventral orientation; pronephros removed.

May 17. Perfectly healed.

May 20. Transplanted bud rather small, points slightly dorso-posteriorly.

May 24. Transplanted bud slightly smaller than normal and points a little more to side. Figure 100, drawn on May 23, shows scarcely any difference between the two buds.

May 28. Transplanted limb (*TR*) practically exact counterpart of normal (figs. 101 and 102, drawn May 29).

May 31. Transplanted limb has third and trace of fourth digit. Function normal.

Experiment R. E. 93. May 19, 1915. Right limb bud to left side, dorsoventral orientation; pronephros mostly removed.

May 21. Well healed; bud smooth and mound-like.

May 25. Transplanted bud points dorsoposteriorly with a secondary bud at anterior border of base.

May 28. Posterior bud (*PR*) is normal in position and orientation, and is even a little longer than the normal limb on the opposite side. The other (anterior) nodule (*DU*) is growing into a limb and also points posteriorly (figs. 103 and 104).

May 30. Posterior bud growing more rapidly than other; digitations plain. Anterior bud sticks out more to side, shows trace of digitations.

June 4. Two hands, each with two digits; some excrescences. Arm a little shorter than normal. The posterior bud is (probably) a left, and its position is approximately normal. The other is mirrored from its dorsal surface. Both hands have faint trace of third digit. Excrescences which seem to involve mainly the skin make an exact interpretation of this case difficult (fig. 105).

C. Superposed transplantations

13. Homopleural, dorsodorsal

Experiment S. E. 3. April 20, 1916. Right limb bud to right side, normal orientation; mesoderm of host torn in posterior part of wound, otherwise intact.

April 21. Well healed; limb bud considerably larger than normal.

April 24. On operated side limb bud larger than normal.

April 26. Operated limb slightly larger, otherwise no difference.

May 2. Operated limb still slightly larger.

May 8. The two limbs exactly alike (fig. 106).

14. Homopleural, dorsoventral

Experiment S. E. 18. April 13, 1917. Right limb bud to right side, inverted; mesoderm all left in.

April 14. Perfectly healed.

April 17. Operated limb bud sticks out more to the side, and is more massive than normal.

April 21. Operated limb double; posterior component more massive, occupies normal position; other is attached to radial border and points dorsoposteriorly; neither has digits.

April 25. Reduplication of hand and part of fore arm; one component is in approximately normal position, the other sticks out to side.

April 30. Limb has grown; relations essentially the same as before.

May 1. Specimen preserved. The main limb projects horizontally to side and is inclined about 30° to the transverse plane; palm is ventral, ulnar border posterior. The reduplicating member with two digits is attached to the radial border of the wrist, pointing anteriorly and laterally (fig. 107).

Experiment S. E. 10. May 26, 1916. Right limb bud to right side inverted; mesoderm of host torn along posterior border of wound and a little tissue lost.

May 27. Perfectly healed.

May 30. Limb on operated side points a little more dorsally than normal. Ventro-anterior to main bud another fairly prominent mass of tissue (like S. E. 9, in which an extra digit developed).

June 4. Limb on operated side considerably smaller than normal, and still points more dorsally; digits just beginning.

June 12. Operated limb still less developed than the unoperated; third and fourth digits beginning to show.

June 15. Practically no difference between the two limbs; the operated one is possibly a little thicker.

15. Heteropleural, dorsodorsal

Experiment S. E. 12. May 26, 1916. Left limb bud to right side, dorsodorsal orientation; mesoderm of host not injured at all.

May 27. Perfectly healed.

May 30. Limb on operated side (*TR*) more massive than normal and points dorsally; on posterior border at base there is a small rounded prominence or bud (figs. 110 and 111, *HOM*, drawn May 31).

June 4. Main limb (*HET*) is spindling and points laterally, though inclined slightly dorsally and posteriorly; there are three imperfectly marked digits at the tip. From the bud at the posterior border there has developed a second limb (*HOM*) which has normal posture; it is of considerable size and shows beginning of digitations (fig. 112, drawn June 5).

June 7. The posterior member is much more massive than the other, which still sticks out to side and shows a very imperfect hand.

June 19. The posterior member (*HOM*) is practically normal with four digits; it is mobile, though probably there is some extensor weakness of hand. The anterior member (*HET*), a left (not reversed), is thin and atrophic, the imperfect hand having three digits. It arises from near the shoulder of the other, the reduplicating plane being approximately radial (fig. 113, drawn June 12).

Experiment S. E. 6. April 21, 1916. Right limb bud to left side, dorsodorsal orientation.

April 22. Perfectly healed.

April 26. On operated side there are two projections in limb region (fig. 109 A).

May 2. Operated limb not quite so advanced as the normal; distinct spur (fig. 109 B, S) on radial border, probably from the anterior of the two prominences.

May 8. Specimen preserved (fig. 109). Operated limb (*TR*) not so advanced as normal, digits not so well developed. The spur (*S*) is attached to the antero-lateral border of arm above elbow and is as large as one of the primary digits. It is a radial reduplication which has remained abortive.

16. Heteropleural, dorsoventral

Experiment S. E. 11. May 26, 1916. Left limb bud to right side, dorsoventral orientation; mesoderm torn along posterior border of wound; no tissue lost.

May 27. Perfectly healed.

May 30. On side of operation a large limb bud points dorsoposteriorly like the normal. Ventrally another distinct but smaller bud (*DU*) points ventroposteriorly (figs. 114 and 115 drawn May 31).

June 4. Operated limb (*TR*) longer and further advanced (digits) than normal (*N*). Otherwise no abnormality (fig. 116, drawn June 5). No trace of ventral bud noted last time.

June 7. Limb on operated side still a little larger than the other.

June 12. Still some difference in size (fig. 118); limb normal in form; motility not so good as normal, extensors of hand weak.

June 19. Function of operated limb very much better, almost, if not quite, normal. Specimen preserved. No difference in size of limbs.

D. Transplantation of half-buds

18. Homopleural, dorsodorsal

Experiment H. E. 6. April 12, 1917. Anterior half of limb bud to anterior half, normal orientation (fig. 120, 1). Operation on right side. Pronephros removed and transplanted.

April 13. Perfectly healed.

April 17. Limb bud on operated side normal.

April 20. Same.

April 30. Same.

19. Homopleural, dorsoventral

Experimental H. E. 31. April 9, 1918. Posterior half of right limb (inverted) in place of anterior right (fig. 120, 6).

April 10. Wound perfectly healed.

April 15. Operated limb bud smaller than normal, and stands out more sharply from body. It is more distinctly marked off (points) anteriorly.

April 18. Operated bud distinctly more massive (fig. 121, *TR*).

April 21. Hand double, but coalesced to end. Digitations indistinct.

April 30. Practically a normal right limb with an accessory hand growing from back of hand. The preserved specimen shows this to be a case of reduplication mirrored from a dorsal plane slightly inclined to the radial. The reduplicating member consists of second and third digits, (2' and 3') the former slightly bifurcated. The first (radial) digit is not doubled (fig. 122).

Experiment H. E. 29. April 9, 1918. Ventral half (inverted) of right limb bud in place of dorsal right (fig. 120, 8). Embryo from which graft was taken, stained in Nile-blue sulphate.

April 10. Healing good. Small round area still uncovered by ectoderm at ventroposterior border of wound.

April 15. Operated bud has grown considerably, pointing dorsally, and slightly anteriorly. Whole free portion of bud covered by stained (grafted) ectoderm.

April 18. Limb on operated side points dorsoposteriorly with reduplicating nodule on posterior border.

April 21. Limb short; double hand.

April 26. Well-developed double hand. Anterior member further developed, sticks out to side; is a left; palm anterior; other a right; dorsoradial reduplication.

May 4. Almost perfectly symmetrical double hand.

May 6. Specimen preserved. Arm as a whole a right, as is the posterior hand. This has three digits and a nodule for fourth, the first

two digits being partly syndactylous. Reduplication is radial. The anterior hand (which is a left) has two long digits and a well-developed third.

Experiment H. E. 2. April 18, 1916. Dorsal half of right limb bud (inverted) in place of ventral right (fig. 120, 7).

April 19. Healing good, but a groove dividing the limb region horizontally still indicates line of suture.

April 21. Two rather distinct humps on operated side; larger one ventro-anterior.

April 26. Operated limb bud nearly normal, but there is a bud-like projection on the anterolateral border (fig. 123, S).

May 1. Transplanted limb larger than normal. The anterior process is now a spur at elbow of the main limb, which is nearly normal.

May 8. Preserved. The operated limb, especially above the elbow is a little thicker, but is otherwise normal. The spur is a nodule just above elbow on radial border (fig. 124).

Experiment H. E. 5. April 12, 1910. Posterior half of right limb bud (inverted) in place of anterior right (fig. 120, 6).

April 13. Perfectly healed except for minute uncovered area at dorsal border.

April 17. Operated limb bud double; large posterior bud points normally, but is not so large as normal; anteroventral bud is much smaller; it is clear that former is developing out of the remaining half of the limb bud of the host, while the latter is from the graft.

April 20. Both buds have grown; posterior one points more dorso-laterally than normal and shows first beginnings of digitations; anterior bud prominent, rounded, no digitations; the two are separate to base.

April 25. Posterior member is fan-shaped, with three long digits, and beginning of ulnar digits on each margin; anterior member much shorter, with faint indication of digitations.

April 30. Posterior member has a symmetrical fan-shaped five-digitate hand; as a whole it is a right limb, as indicated by elbow bend. Anterior member much shorter and thicker.

May 21. Specimen preserved. The posterior member has a double hand, of which the posterior is a right and the anterior a left. The anterior member is a nearly normal right, with the hand a little twisted and syndactylous second and third digits (fig. 125). This is an anomalous case.

Experiment H. E. 13. May 3, 1917. Posterior half of right limb bud (inverted) in place of anterior half (fig. 120, 6).

May 4. Perfectly healed.

May 7. Two distinct though small limb buds, one ventro-anterior, the other dorsoposterior.

May 10. Two buds united at base; both project sharply laterally and slightly posteriorly.

May 14. Two limbs; bifurcation in Y-form about at elbow. Anterior member shows two digits; posterior, none.

May 21. Specimen preserved (fig. 126). Hand double; limb (*TR*) as a whole looks like a right, including the anterior hand (*L'OM*), which has two long digits and a distinct third. The posterior hand (*LET*) is a left, also with three digits, the first of which is truncated. Reduplication ulnar.

20. Heteropleural, dorsodorsal

Experiment H. R. E. 1. April 18, 1916. Posterior half of right limb bud in place of anterior left (fig. 120, 10).

April 19. Well healed.

April 21. Two distinct humps on operated side; dorsal one larger, ventro-anterior one quite small.

April 26. Limb on operated side about as large as normal, but projects laterally.

May 1. Reduplication of hand; three digits, fan-like.

May 8. Specimen preserved (fig. 127). Arm somewhat thicker than normal, projects posteriolaterally with hand flexed ventrally. Hand symmetrical, with five digits, of which middle one (*I*) is defective. The posterior half of the hand (*HOM*) is a left, the anterior (*HET*) a right.

Experiment H. R. E. 5. April 19, 1916. Dorsal half of right limb bud in place of dorsal left (fig. 120, 11).

April 20. Perfectly healed.

April 24. Operated limb bud not quite so large as normal, points laterally and slightly anteriorly.

April 29. Operated limb double (fig. 128).

May 8. Specimen preserved (fig. 129). The dorsal (posterior) member (*DOM*) is a nearly normal left arm; first digit short; third is small, and fourth not visible. The ventral (anterior) member (*HET*) arises just below elbow, and likewise shows three digits, of which first is short; plane of mirroring is radial.

Experiment H. R. E. 11. April 11, 1917. Anterior half of left limb bud in place of posterior right (fig. 120, 9). Wound perfectly healed same evening.

April 14. Operated limb bud very large and prominent, position normal.

April 16. Limb bud massive, points dorsolaterally; almost perfect anteroposterior symmetry.

April 23. Limb has now assumed normal position; first two digitations show; a distinct but small spur at elbow on radial border points laterally.

May 4. Operated limb normal; spur much reduced.

May 7. Specimen preserved. Arm normal; spur a mere nodule about middle of upper arm, radiodorsal border (fig. 130).

Experiment H. R. E. 43. April 5, 1918. Anterior half of left limb bud in place of posterior right (fig. 120, 9).

April 6. Perfectly healed.

- April 9. Operated limb a little more massive than normal.
- April 11. Limb more massive, rounded, not more distinctly marked off posteriorly than anteriorly when viewed from above. Points dorsally slightly posteriorly in lateral view.
- April 14. More massive, but otherwise normal.
- April 22. Not quite so long; syndactyly first two digits, otherwise normal.
- May 4. Normal except for syndactyly.
- Experiment H. R. E. 9.* April 11, 1917. Posterior half left limb bud in place of anterior right (fig. 120, 10). Wound perfectly healed same evening.
- April 14. Operated limb bud looks nearly normal. No definite pointing.
- April 16. Two rather distinct nodules, the posterior one considerably more prominent.
- April 18. Posterior bud smaller than normal, points dorsolaterally. Anterior one not so definite.
- April 23. Two entirely separate limbs; anterior one is shorter, points ventroposteriorly; posterior one points straight to side; no digitations.
- April 28. Two limbs point posterolaterally, parallel to one another; anterior one thicker, has two digits and beginning of third; apparently a normal, though much smaller, right limb in approximately normal position. Posterior limb thinner, rod-like, with no digits.
- May 4. Anterior limb a normal right; posterior, very imperfect, has one long digit and third digit nodule on upper border; elbow bend shows; limb probably also a right (?)
- May 12. Anterior limb has good function.
- May 21. Specimen preserved. The anterior member is a normal right, somewhat smaller than the unoperated limb. Posterior member has one long and one short digit, and has same general position as the anterior. Total view leaves in uncertainty which is palmar surface (fig. 131). Sections show clearly that the ventrolateral surface is the palm and that the limb is therefore a left.
- Experiment H. R. E. 20.* May 2, 1917. Dorsal half left limb bud in place of dorsal right (fig. 120, 11).
- May 3. Wound perfectly healed.
- May 7. Operated limb about full size, points anteriorly and slightly dorsally.
- May 10. Projects straight to side. Slight indication of posterior reduplicating bud from near attachment of limb.
- May 14. Main limb points dorsolaterally and slightly posteriorly. The reduplicating bud is attached near base and in part directly to body wall; it has grown considerably, but is still without digits.
- May 18. Main (anterior) member sticks out to side, but now points distinctly posteriorly as well, is slender and with two digits. Other limb shorter, but considerably stouter, with faint indication of two digits.

May 27. Anterior member has remained slender, and still has but two digits; distinct elbow bend; general shape indicates it to be a right. The posterior member, practically normal except slightly underdeveloped, is an undoubted right; first two digits partly syndactylous (first short); third is distinct and fourth indicated. Some function in this member.

May 28. Specimen preserved; form of limbs shown in figure 132. Sections show that there are two separate sockets in the shoulder-girdle for the two limbs. The medial surface of both is the palm. Hence both are rights.

21. Heteropleural, dorsoventral

Experiment H. R. E. 36. April 4, 1918. Ventral half left limb bud (inverted) in place of dorsal right (fig. 120, 16). Pronephros removed.

April 5. Perfectly healed.

April 8. Operated bud a little sharper; points normally (dorso-posteriorly).

April 10. Nearly normal; points slightly more dorsally.

April 13. Normal.

April 16. Normal limb; digitations show.

April 30. Specimen preserved (fig. 133).

Experiment H. R. E. 10. April 11, 1917. Anterior half left limb bud (inverted) in place of anterior right (fig. 120, 13). Wound completely healed same evening.

April 14. Limb bud operated side nearly normal looking.

April 16. Almost perfectly normal.

April 18. Operated limb normal except slightly larger.

April 23. Reduplication of digits radial side.

April 28. Operated limb a normal right with two digits arising from palmar surface near radial border; arm somewhat thicker than normal.

May 7. Specimen preserved (fig. 134). The first digit is somewhat small; the reduplicating digits (*DU*) are united at base.