

Resumen por el autor, Harry Lewis Wieman.

Los efectos de la transplatación de una porción del tubo neural de Amblystoma en una posición perpendicular a la normal.

I. Consideraciones generales.

Cuando se extirpa una pequeña sección del tubo neural al nivel del segundo al cuarto somita y se implanta en una posición perpendicular a su posición normal se obtienen los siguientes resultados: 1) El trozo transplantado se desarrolla y retiene su polaridad originaria; 2) Fibras aparecen creciendo posteriormente desde el extremo anterior hacia la pieza transplantada y desde esta hacia el extremo posterior; 3) Las fibras ascendentes crecen hacia delante desde el extremo posterior solamente después de haberse establecido conexiones por medio de las fibras descendentes; 4) La pieza transplantada es reabsorbida en el tubo neural reconstruido, pero hasta cierto punto retiene su polaridad estructural originaria la cual dura hasta cuarenta días después de la operación.

Translation by José F. Nonidez
Cornell Medical College, New York

THE EFFECT OF TRANSPLANTING A PORTION OF THE NEURAL TUBE OF AMBLYSTOMA TO A POSITION AT RIGHT ANGLES TO THE NORMAL

H. L. WIEMAN

Zoological Laboratory, University of Cincinnati

EIGHTEEN FIGURES

I

It has been known for a long time that when the amphibian spinal cord is severed, in early embryonic stages, restoration of anatomical and physiological continuity takes place (Born, '97; Harrison, '98; Hooker, '15). It is also known that when a section of the cord is excised and replaced in a reverse anteroposterior position, healing per primam occurs if the cut edges are carefully apposed (Harrison, '98, '03; Spemann, '12; Hooker, '15). According to Hooker, the reversed section retains its original morphological polarity, but its functional polarity becomes reversed through adaptation. The nerve fibers show a marked tendency to avoid entering the opposite wound surfaces. The experiments I am about to describe were designed to test further the regulative capacity of the amphibian neural tube.

Eggs of *Amblystoma punctatum* were collected shortly after deposition and allowed to develop in the laboratory. The stages used for operation extended from the period of the fused neural folds to a point just before the larva becomes sensitive, but most of the operations were made in the earlier stages. The operation consisted in excising a piece of the neural tube and somites, equal to the length of two somites, and replacing it, dorsal side up, at right angles to its original position. Thus the axis of the transplant formed an angle of 90° to the remainder of the neural tube. The length of the transplant was made somewhat longer than the width of the tube, in order to obtain a maximum length

in the transplant, so that reactions and development due to its original polarity could be studied to best advantage.

The operation was accomplished by two transverse incisions through the neural tube and somites, followed by a longitudinal cut on each side connecting the lower ends of the transverse incisions and extending to the midline in a frontal plane. In some cases the notochord was purposely cut and moved with the transplant; in other cases it was left intact. The piece thus freed was lifted out, swung around through 90°, and pressed into the wound. The purpose in removing somite with the neural tube was to preserve as nearly as possible normal conditions for any nerves that might later develop from the transplanted tube, and, at the same time, to provide a non-nervous block between the stumps of the neural tube and the transplant. The usual practice was followed in holding the transplant in place by means of thin glass rods bent to fit snugly over the embryo at the site of operation. From five to fifteen minutes sufficed for the transplant to become attached, after which the holders were removed. After an hour or so in 0.4 per cent salt solution, in which also the operations were performed, the embryos were removed to tap-water and allowed to develop at a temperature of 15° to 20°C.¹

Following the operation, the embryos were examined from time to time under the binocular, sketched, and tested for nervous conductivity through the transplant by means of delicate tactile stimuli. The material was fixed in corrosive-acetic, embedded in rubber-paraffin, cut into 10 μ sections, and stained on the slide. On the whole, the most satisfactory staining results were obtained with Delafield's haematoxylin and orange G.

The stages most frequently used for operations reported on at the present time are shown in figure 1, *A* and *B*; *C* and *D* are outlines of older stages also used. The sketches were made five minutes after the operation. These stages showed no

¹ During the spring of 1920, the author enjoyed the privilege of spending two weeks at the Osborn Zoological Laboratory of Yale University, which afforded opportunity to observe the operative technique as practiced and so highly perfected by Professor Harrison and his students. To Professor Harrison and the zoological staff at Yale the writer begs to express his deep sense of appreciation of the courtesy and privileges extended to him during his visit there.

marked difference in healing, and no constant differences were noted in subsequent changes. The present account is based upon a study of some fifty operations, the results of which were of a uniform character.

Figure 2 illustrates the gross changes in the form of the body typical of embryos operated at stage *D* (fig. 1). Similar changes are noted when the operation is made at earlier stages, except

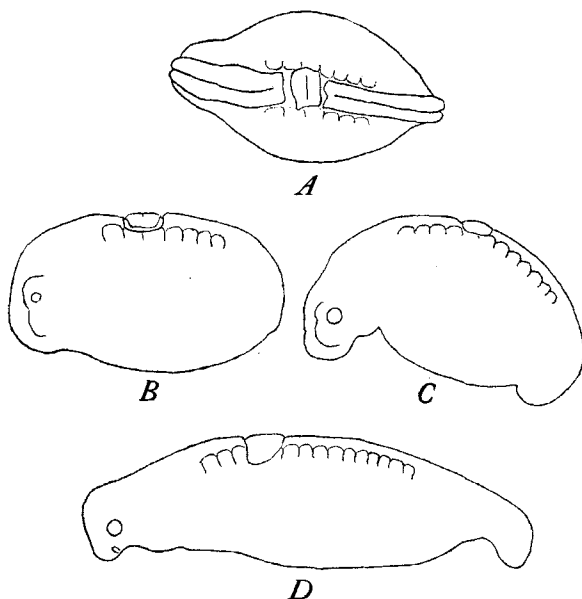


Fig. 1 *A* and *B*, dorsal and lateral views, T. R. and T. L. series; *C* and *D*, lateral views, F and G series, respectively. Five minutes after operation.

that the primary flexing of the head and tail toward each other dorsally is apt to be more marked when the operating is done in the earlier stages. This dorsal flexure is well shown in figure 3, *A*, and is probably due to contraction accompanying the closure of the wound. As may be noted in figure 2, this preliminary bending is followed by a straightening of the body axis, which in turn is succeeded by bending in a direction opposite to the first bringing the head and tail nearer to each other on the ventral side. It was found that in cases where the ventral bending was

least, or practically absent, the notochord had been cut and moved as a part of the transplant, whereas in all cases where the notochord was left intact marked ventral bending occurred. The principal agents in producing the bent axis are the transplanted neural tube and somites.

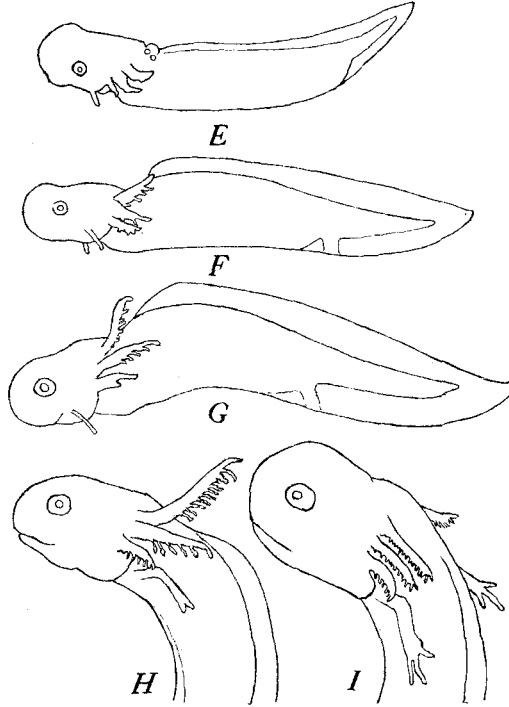


Fig. 2 Embryo G9. Operated April 16. *E*, April 30; *F*, May 4; *G*, May 7; *H*, May 17; *I*, May 26.

Another general effect of the operation was the underdevelopment of the gills, which never became as large and bushy as those of the controls. No special effect was produced on the development of the forelimbs, although many of the operations were made in the region of their nerve supply.

Figure 3, *A*, shows the appearance of embryo T. R. 1, eight days after the operation. In the sagittal section, *B*, the trans-

planted tube can be clearly seen, but as the section passes lateral (left) to the brain and cord of the host, none of the latter can be seen. It is evident that the transplant has progressed in its development, having closed completely and having grown in thickness and length. The section shown in *C* passes nearer to

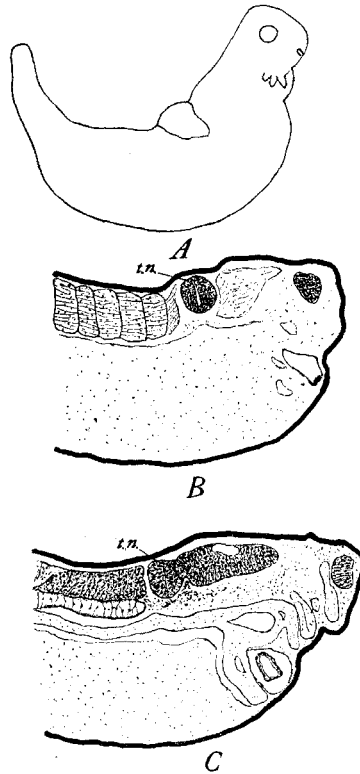


Fig. 3 *A*, embryo T.L.1; *B* and *C*, sagittal sections. *t.n.*, transplanted neural tube. Eight days after operation.

the midline. It shows the transplant and both stumps of the neural tube with the transplant in close contact with the anterior stump. At first glance, it might appear that these two regions had fused, but a closer examination under higher power (fig. 4) shows that this is not the case, and that the cells of the two regions are rather sharply marked off. The condition was brought

about by the growing brain's extending caudally and denting in the adjacent side of the transplant, without any positive tendency to fusion with the latter. Posteriorly (fig. 3, *C*), the transplant is entirely free of the neural stump; nor is there any evidence in other sections of the series of any connections whatsoever between the posterior stump and the transplant. The

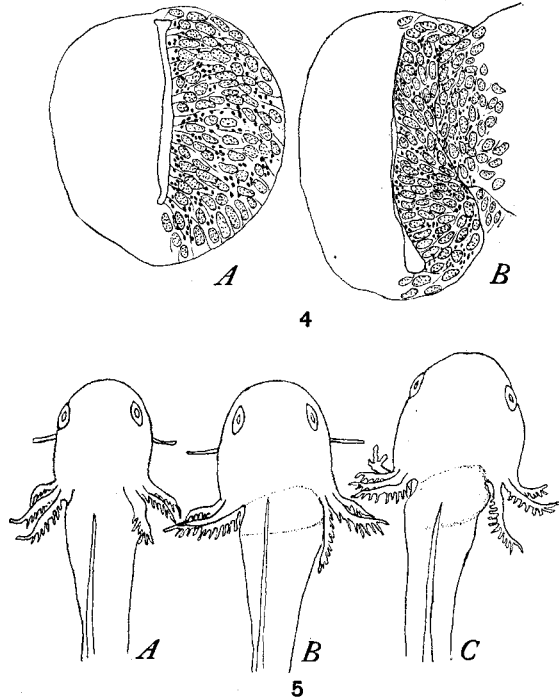


Fig. 4 *A* and *B*, enlargements of transplanted tube of figure 3, *A* and *B*, respectively.

Fig. 5 Embryo T.R.3, operated April 8. *A*, April 28; *B*, May 3; *C*, May 10.

relation shown in figure 4, *B*, may represent a first step in the restoration of anatomical continuity between the anterior stump and the transplant, but since it is also true that in other embryos of this series and age the transplant is entirely free of contact with either stump, it would seem that the condition found in T. R. 1 is more or less the result of a chance contact brought about by the rapid growth at this time of the brain stem anterior

to the transplant. There is much reason to believe that definitive union does not take place until actual nerve fibers develop.

Figure 5 and figure 6, *B*, show sketches of embryo T. R. 3, belonging to the same series as the preceding. Twenty days after the operation (*A*), there is scarcely any visible outward evidence of the operation except lessened size as compared with

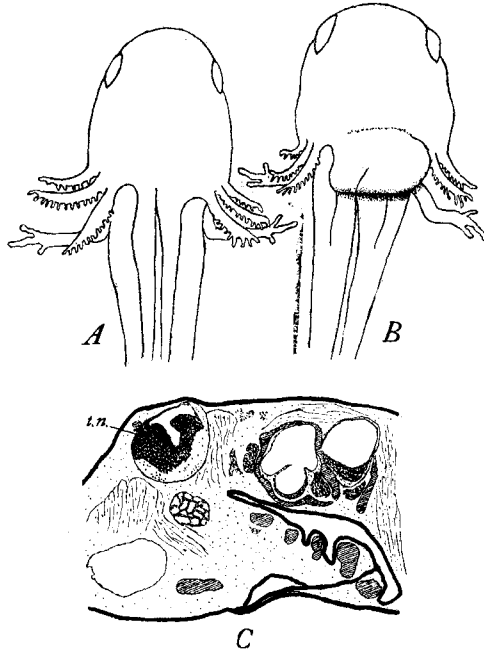


Fig. 6 Embryo T.R.3. *B*, May 17; *C*, sagittal section. *A*, control. *t.n.*, transplanted neural tube. In this and all subsequent drawings of sections the shaded parts represent cartilage.

the control. Five days later (*B*), a slight transverse swelling appeared at the site of operation, which became more marked a week later (*C*). Finally (fig. 6, *B*), thirty-seven days after the operation, the transverse ridge became well defined and projected slightly beyond the line of the body on the right. At this point the animal was killed and fixed. In this embryo there was practically no ventral bending of the body; the animal maintained an upright position and was very active. Its body length was

somewhat less than that of the control. The notochord was cut and the section moved with the transplant. Tactile stimulation in front of and behind the transplant gave no evidence of nervous conduction in an anteroposterior, or reverse, direction.

The production of a transverse ridge was not a constant feature of operated embryos. The fact is mentioned because it happened to be present in this particular embryo which was one of those selected for sectioning. Sections show that the position of the ridge does not coincide exactly with the position of the transplanted tube. The ridge was found to be due in part to the growth in length and breadth of the transplant, but principally to growth and differentiation of transplanted somites especially toward the right side (fig. 6, *B*). This larger projecting end lies near the original posterior end of the transplanted neural tube.

Figure 6, *C*, shows a section in a sagittal plane passing through the eye, otic vesicle, and the enlarged (original anterior) end of the transplant. The sections begin at the animal's left side. The appearance of the section shows that the transplant has not only increased in size, but has undergone differentiation typical of the hindbrain region from which it came.

A section 180μ nearer to the midline (fig. 7, *D*) and to the right shows a very broad connection between the transplant and the brain in front. The section shown in *E* passes 100μ to the right of *D*. The transplant is free, having no connections on either side. The posterior stump is seen in this figure with three thick nerve processes passing ventrally. It is separated from the transplant by muscle derived from transplanted somite.

Figure 8, *F*, 140μ to the right of *E*, passes approximately through the midline, and shows the transplant free and underdeveloped. The amount of development to be expected is indicated by the appearance of the posterior stump, which is large, showing every indication of differentiation. It terminates in a smooth, rounded surface.

The section seen at *G* is 120μ to the right of *F* and shows both stumps with the transplant underdeveloped and lying free between them.

The sections give an idea of the conditions at various levels as seen in selected sagittal sections. They show all of the important points that have come to light from the complete study of the entire series of sections of this embryo and others of the same age. These points may be summarized as follows: 1) Evidence of an active growth and differentiation of the original

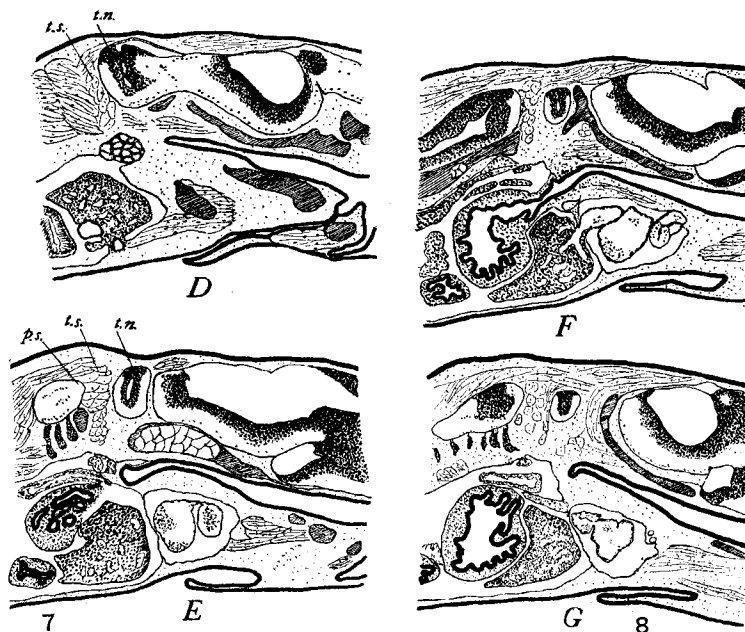


Fig. 7 Embryo T.R.3, sagittal sections. *t.n.*, transplanted neural tube; *t.s.*, transplanted somite; *p.s.*, posterior stump.

Fig. 8 Embryo T.R.3, sagittal sections.

anterior end of the transplanted neural tube; 2) the presence of a well-defined connection between the transplant and the anterior stump located to the left of the midline, that is, toward the original anterior end of the transplant; 3) the absence of any connection between the transplant and the posterior stump; 4) evidence of active growth and differentiation in the posterior stump; 5) evidence of progressive atrophy in the transplant to the right of its union with the brain, that is, in the direction of its original posterior end.

Embryo T. R. 5 (fig. 9) developed a marked ventral bend in the body which interfered with its maintaining an upright position. The notochord was not disturbed by the operation, which was performed April 8th. Tactile stimulation from time to time showed no evidence of conductivity anteroposteriorly, or reverse, through the transplant. Thus for May 10th my notes show the following record:

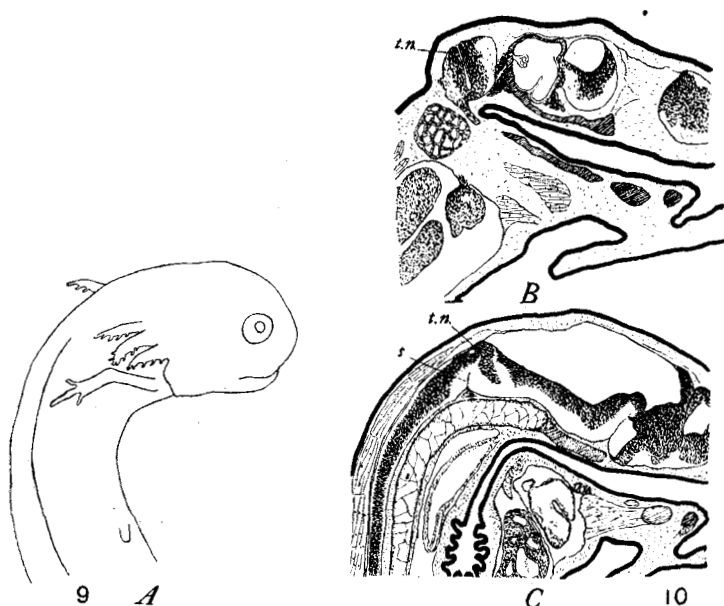


Fig. 9 Embryo T.R.5, May 17. Operated April 8.

Fig. 10 Embryo T.R.5, sagittal sections. s, connective-tissue septum.

Stimulation anterior to transplant: slight twitching of gills.

Stimulation posterior to transplant: active body movement; head quiet.

Stimulation at site of transplant: active twitching of gills.

This record points to a descending connection from the anterior stump to the transplant, but no posterior connection. In the course of a week the character of the responses changed. Thus on May 17th the record is as follows:

Stimulation anterior to the transplant: active movement of forelimbs, followed by quick body contractions.

Stimulation posterior to transplant: similar response.

Stimulation at site of transplant: similar response.

These results indicated that conduction was occurring back and forth through the transplant—that functional connections had been established between the transplant and the two stumps.

Figure 10, *B*, shows the original anterior end of the transplant in close contact with the ganglia of the ninth and tenth nerves, with which a union seems to have been formed. A large process passes ventrally from the transplant just in front of the pro-

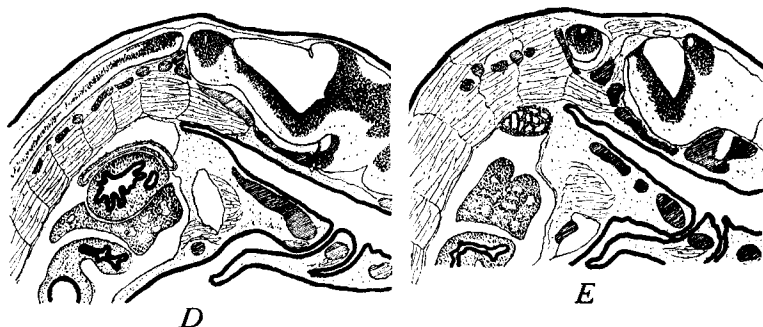


Fig. 11 Embryo T.R.5, sagittal sections

nephros. The section shown in *C*, passing through the midline, shows the transplant completely incorporated in the nervous system. The irregularity in the distribution of the nuclei and the connective tissue septum (*s*) indicate the position of the transplant. The fact that anatomical continuity is established leads to the belief that the results of stimulation noted above were due to nervous conductivity through the transplant rather than to direct muscular stimulation.

Figure 11, *D*, is a section passing to the right of the midline. It shows a well-defined union between the transplant and the brain in front. Section *E* passes still further to the right. The transplant shows signs of diminished vigor and growth, and is not connected with either stump.

Figure 12 is an outline of embryo T. R. 4, an animal belonging to the same series as the preceding, but not killed until May 27th. For some time preceding this date responses to stimulation indicated that conduction paths had been established through the transplanted tube.

In figure 13, *B*, is seen a sagittal section passing to the left of the midline. Complete anatomical continuity is evident, but

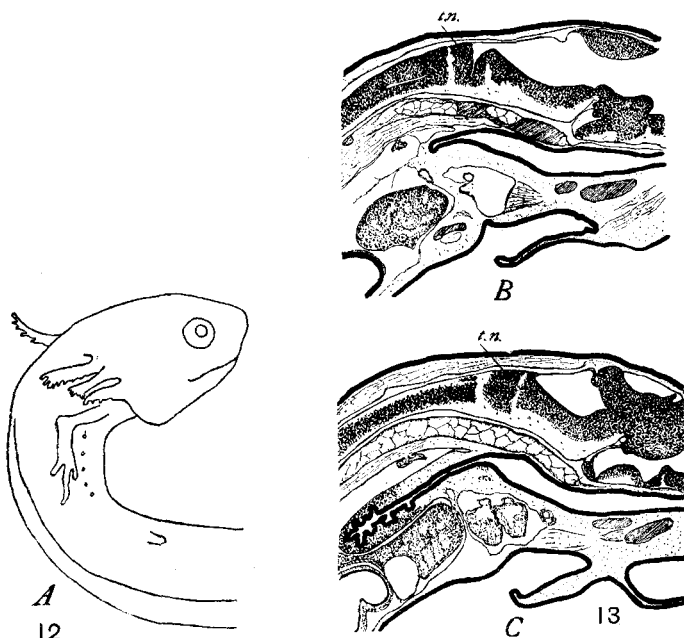


Fig. 12 Embryo T.R.4, May 27. Operated April 8

Fig. 13 Embryo T.R.4, sagittal sections

the V-shaped configuration of the nuclei reveals the location of the transplant. The section shown in *C* passes directly through the midline, where the transplant appears to be completely absorbed; but here again its boundaries are indicated by two breaks or gaps in the nuclear mass. Careful examination of all of the sections in the series shows that the fusion between the transplant and the nervous system is very intimate, none of the transplant extending beyond the lateral limits of the brain and cord.

A study of transverse sections was also made, but a description of such sections would be merely a repetition of what has already been described. For this reason but one transverse series will be considered here, and that because it throws some light on the completeness with which anatomical union is established through the transplant.

Embryo T. R. 7, shown in the figure with the control, had a history similar to that of T. R. 4. Like the latter it showed normal responses to stimuli twenty days after the operation, was very active and, save for a slight bend in the posterior part of its body and its slightly smaller size, behaved very much like

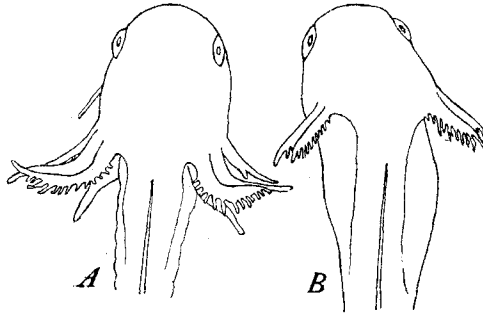


Fig. 14 B, Embryo T.R.7, May 5. Operated April 8. A, control.

the control. It was killed May 25th, twenty-five days after the operation, and by that time its body had straightened out considerably.

Transverse sections showed complete fusion of the transplant, but its location could be determined by the configuration of the cells and the irregularity in the shape and position of the canal as well as by the appearance of the regenerating notochord, which in this case had been cut. (It was left intact in T. R. 4.) Figure 15, C, shows a transverse section passing just in front of the transplant. D passes through the region of contact between the transplant and the anterior stump. It is at once evident that the cells are abnormally distributed and that there is no evidence of a canal. E passes through the center of the transplant. It shows a misplaced canal, and also the remains of the transplanted

notochord at *t. no.* Regeneration in the notochord is taking place in the stumps; the transplanted piece appears atrophied and seems to take no part in the process. *F*, passes through the posterior limit of the transplant. Here again the canal is missing. The section also shows the overlapping ends of the regenerating notochord. Posterior to this level the sections are normal.

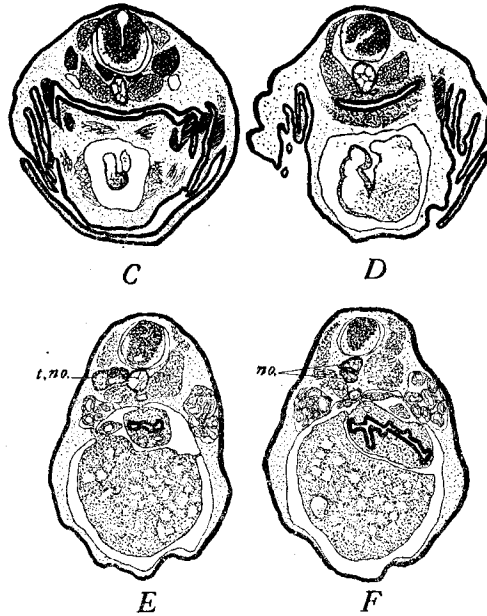


Fig. 15 Embryo T.R.7, transverse sections. *l.no.*, transplanted notochord; *no.*, regenerating ends of the notochord.

Therefore, the conditions found in the transverse series confirm what has already been shown in the sagittal series of T. R. 4.

Frontal sections afford a view of the process from still a different angle and supply further confirmation of the conclusions already reached. Embryo G9 was cut into frontal sections for this purpose. It is shown at the operating stage in figure 1, *D*. Figure 2 consists of drawings of the embryos at various intervals up to the time of killing, May 17th. Responses to tactile stimulation indicated that conduction pathways were established

through the transplant by May 7th (fig. 2, *G*), seven days after the operation.

The frontal section shows the transplant united with both stumps, but the readjustment is not as complete as in the two preceding cases, which was to have been expected from the shorter duration of time between operation and fixing in the case of G9. In the figure the original anterior end of the transplant is to the right. Owing to a shift in position, its axis cuts that of

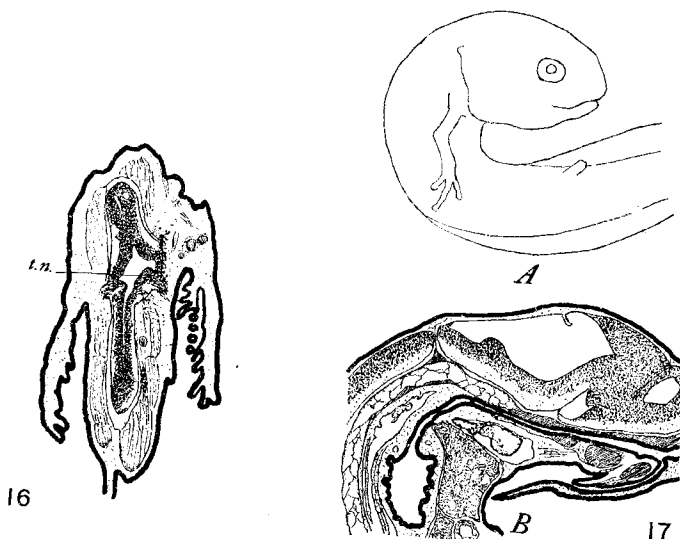


Fig. 16 Embryo G9, frontal section. Anterior end up.

Fig. 17 A, Embryo T.L.9, May 26. Operated April 8. B, sagittal section.

the embryo diagonally, the anterior end of the transplant lying cephalad to its posterior end. The shift forward of the anterior end of the transplant might have been due to factors connected with the process of bridging the stumps, or it might have been due to lack of success in implanting the excised tube at right angles to the embryonic axis at the time of operating. However, the fact that a similar shift in position of the transplanted tube has been noted in many other operated embryos points to the conclusion that it is not the result of accident in technique, but rather an indication of a definite orientation of the transplant.

It is also to be noted in figure 16 that the original anterior end of the transplant (to the right) is larger than the opposite end, and that the axis of the brain stem (above) points toward this larger end. Thus it would seem that the junction at this point with the transplant was brought about by descending fibers growing back from the brain not in a straight line, but toward the original anterior end of the transplant. This same fact appears from an examination of sagittal sections, where it may be recalled that the connection between the anterior stump and the transplant lies for the most part to one side of the midline, the side nearer to the original anterior end of the transplant.

Figure 16 also shows that the connection with the posterior stump leaves the transplant from its posterior end (to the left), which would be expected on the assumption that the transplant had retained its original anteroposterior polarization. As a result there is a zig-zag in the restored neural axis, which, evidence from older stages (figs. 12 to 14) shows, may be later partially straightened out.

II

The disturbance caused by the operation to which these embryos were subjected must be profound, and it seems remarkable that reestablishment of morphological and functional continuity to the extent noted occurs with such frequency in the relatively small number of cases that I have studied. However, the success of the operation depends to some extent upon the region in which the transplantation is made. Thus in the experiments considered up to this point the transplantations were made in the region of the second to the fourth somites approximately. Among these experiments only one case occurred from which recovery seemed impossible or at least unlikely.

Embryo T. L. 9, operated April 8th, showed by its reactions to stimulation that connections through the transplant were established by April 28th. At this time the embryo showed a slight bend (ventral) in the body axis. As time went on, the bend became more pronounced and response to stimuli more and more sluggish, until by May 26th, when it was fixed, no evidence

could be obtained of conduction through the transplant. By this time the bend in the axis had become extremely acute (fig. 17, *A*)

Sections showed that the loss in conductivity was due to the formation of a cartilaginous partition cutting through the neural axis just behind the transplant. Figure 17, *B*, a section in the medial plane, passes through the thinnest part of the partition and shows the transplant firmly united in front with the brain. In all probability the transplant completed its anterior connection, but was unsuccessful in developing a posterior connection of sufficient stability to resist the pressure of the growing posterior wall of the brain capsule, with the result that a foramen magnum failed to develop, and the brain became completely hemmed off anteriorly.

III

In the F series of embryos the operation was made in the region of the fifth and sixth somites, and the results of these experiments are somewhat different from those of the other series. All were operated the same day, April 16th, and none of them during thirty days following the operation showed any evidence, by the method of tactile stimulation, of nervous continuity through the transplant. The operating stage is shown in figure 1, *C*.

Let us consider a few examples. F7, killed May 7th, twenty-one days after the operation, showed in sections that the transplanted tube was widely separated from the stumps of the brain and cord by muscle that had developed from transplanted somites. The transplanted tube had, however, undergone a certain amount of growth and differentiation, and showed no signs of atrophy. Practically the same conditions were found in F1, killed May 17th, ten days later than F7. F3, killed May 18th, one day after F1, displayed in sections the same wide separation between the transplant and the stumps, but in this case the transplant gave every appearance of atrophy in its reduced size and ragged form. Had the embryo been allowed to live, the possibility of its establishing connections between the

neural stumps would seem to have been very remote indeed. Finally, F4, killed May 17th, was the only one of the entire series that in sections showed any connection between transplant and nervous system, and here the connection extended only between the anterior side of the transplant and the brain. The appearance of the sections does not exclude the possibility of a posterior connection through the transplant being established

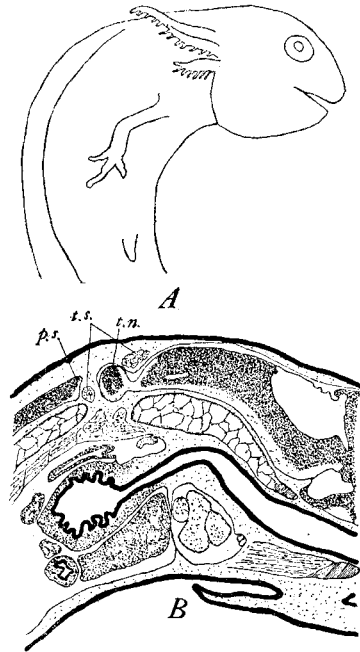


Fig. 18 A, Embryo F4, May 17. Operated April 16. B, sagittal section.

later had the embryo been allowed to live. However, the fact that this had not occurred by the end of thirty-one days speaks against it, since in the T. R. series both anterior and posterior connections were established as early as twenty days after the operation, and in the G series even earlier—seven days in the case of G9—but the latter series were much older at the time of operation (fig. 1). Further, F4 was the only one of the six embryos of the F series chosen at random for sectioning in which

there was any indication of any connection between the transplant and the stumps.

Only twelve operations were made in the F series, and this number is of course too small to serve as the basis for far-reaching conclusions; nevertheless, the fact remains that the results in this series differed consistently from the others in that restoration of anatomical and functional continuity was delayed or even excluded. At the time the operations were performed it was not foreseen that a matter of a few somites difference in level would make a material difference in the reaction of the neural tube to the operation; but it can be pointed out now that the results obtained are what one should expect if the initial step in the restoration process is a posterior growth of fibers from the brain to the transplanted tube, since the longer time required for fibers to reach the more posterior level would allow somites intervening between transplanted tube and the neural stumps to develop to such an extent as to delay materially, if not actually to prevent, the formation of a nervous connection. Therefore the nearer to the brain the transplantation is made the better the chances for connections being established. This point will be subjected to further experimental test, the results of which will be reported upon later.

IV

We may now review in their proper order the various steps involved in the process of restoring anatomical and functional continuity between the transplanted tube and the rest of the nervous system. In the first place, at the time of the operation in the T. R, T. L., and F series, no tracts were established, so that there was nothing to interfere with localized development taking its natural course; and as a result neuroblasts develop in all parts of the neural tube, including the transplanted portion. Since a stage just before appearance of sensitivity was used for operation in the G series, the first steps in the formation of the primitive somatic sensory and motor tracts had already started in this series. In the second place, whenever in the sectioned embryo a single connection was found between the transplant

and the neural stumps, the connection always occurred between the anterior stump and the transplant. Since an outgrowth fails to appear at this time from the posterior side of the transplanted tube, there is little reason to suppose that such an outgrowth takes place from its anterior side either; for which reason it would seem that the anterior connection is initiated by descending fibers from the anterior stump.

It is not known to what extent, if any, nerves developing from the transplant participate in this process; but since a certain amount of axial mesoderm was left attached to either side of the transplanted tube, it would seem that such nerves would develop their usual relations with somite and skin (Coghill, '14), rather than deviate from their ordinary path of development, which would still be open to them, to develop unusual relations with the anterior stump.

The rapid growth of the brain pushing the anterior stump against the transplant may be an incidental factor in bringing about a union, but complete fusion does not occur until fibers develop. This is illustrated by the results of the G series, in which it was found that connections between transplant and stumps developed in a much shorter time after operation than in the other series. In other words, the formation of a connection between the stump and transplant depends upon the state of development of nerve processes. The location of the connection between the anterior stump and transplant, as seen in figure 18, *B*, clearly indicates its motor character.

As regards the connection between the transplant and the posterior stump, everything points to its being initiated by the continued growth backward, through and beyond the transplant of descending fibers from the anterior stump, augmented perhaps by similar fibers from the transplant itself. In the first place, the participation in this process of nerves arising from the transplant may be ruled out for reasons already given. In the second place, there is no indication of processes growing forward from the posterior stump even after well developed anterior connections are formed (fig. 7). While the posterior stump at this time shows every indication of growth and development, its surface

is smooth and rounded without the slightest trace of outgrowth forward. In fact, the appearance of sections points to what amounts to a repulsive effect, or negative chemotaxis, between the posterior stump and transplant, or at least the complete absence of any attraction between the two regions. The significance, therefore, of the time interval between the formation of the anterior and posterior connections would seem to be that it represents the time required for the descending fibers from the anterior stump to make their way through the transplant; which they do in the direction of the original anteroposterior axis of the transplant. After traversing the transplant a short distance, the descending fibers again change their direction and leave the transplant to bridge the gap between it and the posterior stump. All evidence of repulsion between the two regions would then disappear just as soon as the descending fibers have opportunity to penetrate the posterior stump, which they do, presumably, because the posterior stump is territory that they traverse in normal development.

Incidentally, if the above interpretation be the correct one, it indicates that the first long tracts connecting the brain and cord are motor. After the motor connections are established through the transplant, there is every reason to believe that sensory tracts develop, with the result that there is restored a condition that is almost normal, but not completely so, for the period during which the embryos were under observation. To what extent the restoration approaches normal conditions remains to be seen.

Hooker ('17), in his study of the effect of reversing a section of the cord in the frog, found a marked tendency on the part of the nerve fibers to avoid entering the opposite wound surfaces. The conditions of his experiments excluded any opportunity for the dissipation of this state of mutual repulsion, with the result that nerve connections failed to develop between the ends of the reversed cord and the stumps. In my experiments the conditions are different, and the 'avoiding reaction' is more likely a phenomenon of the posterior stump only—the transplant being neutral, so to speak. The question as to why ascending fibers do not arise from the posterior stump would seem to have its answer

in the results of the experiment, namely, the development of ascending tracts from the cord to the brain awaits and therefore depends upon the formation of motor tracts. Once the stumps have been bridged by descending fibers, all semblance of repulsion between the posterior stump and the transplant disappears, and the way is prepared for ascending tracts to grow forward.

Hooker ('15), after severing the cord of the larval frog in the cervical region, found that when the wound surfaces are not apposed the first steps leading to a reunion and return to nearly normal form and structure are brought about by, 1) the development of nerve fibers from the motor cells of each segment of the cord and, 2) the growth of sensory axones from the cut surface of the posterior stump. In other words, the motor connections precede the sensory. Coghill ('13), in his study of the primary ventral roots and the somatic motor column of *Amblystoma*, has shown that the ventral root fibers occur in their full relation between the spinal cord and muscle some time before the muscle can be stimulated through the sensory field. The physiological properties can in no sense be determined through stimulation in the sensory field, for they may be actually functional for some time before they come under the influence of the sensory nerves. Thus Coghill's work indicates that in the formation of the early reflex arcs in the cord the motor connections are established before the sensory. My results similarly point to the formation of the long motor tracts from the brain to the cord before the sensory tracts.

It is also to be noted that the restoration of nervous connections through the transplant takes place in the direction of the normal metabolic gradient of the embryo. Such a gradient is also exhibited in the transplanted tube, the original anterior end invariably exhibiting evidence of more vigorous growth and developmental energy than the posterior end. This is true even after a connection has formed between the anterior stump and the transplant. The fact that this connection is always made at a point nearer to the anterior end of the transplant might explain the difference in behavior of the two ends of the transplant, were it not for the fact that a similar difference in the two

ends exists in cases where the stumps have failed to form unions with the transplant (F series). At the same time it seems doubtful that the transplant can for an indefinite period maintain its form and structure without establishing connections with the stumps of the brain and cord. This is indicated by the results of the F series, in which the operation was made at the level of the fifth and sixth somites, and in which in only one case out of four selected for sectioning was even a single connection developed between the stumps and the transplant within a period of thirty days. In the other three cases the transplant was separated from the neural stumps by muscle tissue to such an extent that nervous union seemed unlikely, if not impossible. In two of these the transplant showed evidence of growth and development, the original anterior end being larger, but in the third (F3) the transplant was disintegrating. Presumably, the degeneration of the transplant was due to its failure to form a connection with the nervous system.

As has already been mentioned, the results of the F series supply additional evidence for the conclusion reached regarding the manner in which the nervous stumps become reunited through the transplant. In the T. R, T. L., and G series the operation was performed in the region of the second to fourth somites, while in the F series the operation was behind the fifth somite. If the restoration of anatomical connection depends upon the down-growth of descending fibers from the brain, then a longer time would be required for such fibers to reach the site of operation in the F series. The results obtained in the F series would seem, then, to be due to the fact that the greater amount of time required for the descending fibers to reach the level of operation allowed transplanted somite to develop to such an extent on either side of the transplanted tube as to form a barrier on either side of it. That this barrier may be overcome on the anterior side of the transplant, at least, in the course of thirty days is shown by F4 (fig. 18), but whether or not similar connections would have been made later on in all of the F series is of little moment to the point under discussion. The primary reason for lack of successful reunions in the F series would seem to be the more posterior site of the operation as compared with the other series.

General conclusions may be summarized as follows:

I. When a small section of the neural tube of *Amblystoma*, at the stage of the closed neural folds, is removed at the level of the second to fourth somites, together with portions of adjacent somites, and reimplanted at right angles to its normal position:

1. The transplanted tube continues its development and retains its original polarity.

2. The growing brain, pushing the anterior stump back against the anterior side of the transplanted tube, may be a factor in forming a union at the point of contact.

3. Nerve fibers grow back from the anterior stump into the transplant near its original anterior end.

4. The posterior stump becomes club-shaped, but shows no initial tendency to send fibers forward into the transplant.

5. A connection between the transplant and the posterior stump is brought about by the continued growth backward of fibers from the anterior stump through the transplant.

6. Ascending fibers then grow forward from the posterior stump.

7. The transplant eventually becomes absorbed in the reconstructed neural tube, but the appearance of sections shows that the cells of the transplant do not lose their original polarity as late as thirty to forty days after operation.

II. When the operation is performed at a stage just before the larva becomes sensitive, the process is the same, but the union is formed in a shorter time after operation.

III. When the operation is performed at the earlier stages, but in the region of the fifth and sixth somites, or farther back:

1. A longer time is required for the formation of nervous connections.

2. In series of twelve operations no case of complete connections between the stumps occurred in thirty days.

3. In one case at least the transplant was disintegrated at the end of thirty days.

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