

DETERMINATION OF STRUCTURAL PATTERNS IN
THE SPINAL CORD OF THE CHICK EMBRYO
STUDIED BY TRANSPLANTATIONS
BETWEEN BRACHIAL AND
ADJACENT LEVELS¹

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THIRTEEN FIGURES

INTRODUCTION

One of the most challenging problems of experimental embryology concerns the differentiation of complex patterns from previously homogeneous cells or groups of cells. By the use of suitable operative procedures it can frequently be demonstrated that differences leading to widely divergent final patterns may become established before any visible changes have taken place in the cells concerned, i.e., the cells are said to have become determined.

While final analysis of the nature of determination must depend upon refined biochemical and, perhaps, biophysical investigations, these must necessarily be preceded by elucidation of biological relationships responsible for the observed phenomena. This requires not only demonstration of causal agents or relationships involved, but also observations as to the time at which any given change takes place.

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The central nervous system of vertebrates has proven to be one of the most fruitful fields for this type of investigation. The most striking result of primary organizer action is, of course, the brain and spinal cord and probably more is known about factors influencing their subsequent development than is the case for any other system.

Shortly after discovery of the organizer it was demonstrated (Spemann, '31; Holtfreter, '33; Hall, '33) that different levels of the chordamesoderm induced preferentially either brain or spinal cord depending upon whether they represented anterior or posterior parts. These observations suggest that at least the major divisions of the amphibian central nervous system, brain and spinal cord, may be determined at the time of primary induction of the medullary plate, although the possibility is not excluded that such differences may arise later as the result of prolonged contact with the underlying mesoderm.

More recently Roach ('45) in repeating and extending some earlier experiments of Spemann ('12) has shown that in *Ambystoma* the anterior-posterior arrangement of brain parts is already determined in pre-neurulae (stage 13) though the medio-lateral organization is still labile.

In somewhat later stages, Detwiler ('43) has shown that the medulla develops according to origin when transplanted in place of part of the spinal cord.

An extension of the analysis to the different levels of the larval salamander spinal cord is rendered very difficult by the fact that (with usual techniques) there are no qualitative differences serving to distinguish different levels in this form. Quantitative differences do, however, exist and Detwiler and his associates have contributed a great deal of work which seems to indicate the very early existence of differences between brachial and other levels of the salamander spinal cord. Data of this type are, however, very difficult to evaluate and the picture is still not entirely clear.

It is well known, on the other hand, that in the spinal cord of higher vertebrates can be recognized a number of regions each

characterized by particular cell types and patterns of arrangement. This is most strikingly evident in the brachial and lumbosacral enlargements which are characterized mainly by the presence of very large motor cells arranged into the pronounced lateral motor columns.

In the chick where clear-cut differences in pattern serve to distinguish the various levels of the spinal cord, much less work of this nature has been done than in Amphibia. Rudnick ('44) aptly summarized the situation by the statement, "In the case of intramedullary or neural crest pattern, the field for speculation is entirely unencumbered by experimental facts."

With respect to early determination little has been added since that time though a few pertinent observations have been made. Levi-Montalcini ('45), Yntema and Hammond ('45), Hammond and Yntema ('47) have noted a marked failure of the central nervous system of the chick to reconstitute parts removed during early somite stages. E. Wenger ('50) has further demonstrated that the neural epithelium of the brachial region represents a relatively rigid mosaic by the time of closure of the neural folds. This observation indicates that the cellular pattern for this level is already determined but does not give any information as to differences between it and other levels.

In addition to early determination or any later influence of the immediate environment, a given part of the central nervous system is subject to influences mediated through its nervous connections with the periphery.

That the periphery does exert a marked influence on the development of the central nervous system was shown first by Braus ('06) on the anuran cord and by Shorey ('09) who extended the observations to include urodeles and the chick. These observations have since been confirmed many times, extended to other parts of the nervous system and analyzed further (see Piatt, '48).

The question eventually arises as to what extent the strikingly different patterns characteristic of various spinal cord levels in the late embryo (or adult) are determined by the

time of formation of the neural tube and prior to the outgrowth of any nerves and to what extent the entire spinal cord at this stage represents a uniform tube of equal potentialities. In the latter case development of the final pattern characteristic of each level would depend upon development or suppression (including degeneration) of particular units under the influences of peripheral structures at that level and mediated by nervous connections with these structures.

The present study represents an attempt at identifying which of these factors is primarily responsible for establishing the different patterns. It consists of transplanting brachial spinal cord, prior to any visible differentiation, to the adjacent levels (cervical and thoracic) and the reciprocal operations.

The results obtained indicate that as early as they can be identified with reasonable accuracy (13 to 24 somites), the levels of the spinal cord involved (cervical, brachial, thoracic) are determined with respect to recognizable cell types and patterns of cellular arrangement. Peripheral structures can only influence the quantitative development of existing cell types or patterns.

In addition, some information is contributed concerning factors involved in plexus formation and neural segmentation.

The author wishes to express his gratitude to Dr. Viktor Hamburger for suggesting this problem and for advice and encouragement during the course of the work.

MATERIAL AND METHODS

Operative procedure. The operative procedure was essentially that described by Hamburger ('42) with a few modifications which will be mentioned. All operations were performed on New Hampshire Red eggs obtained from a commercial hatchery, and incubated for about two days.

An elliptical hole approximately 18 mm in greater diameter is cut in the shell using a dental drill with a carborundum disc. By making the shape of the ellipse correspond to the shape of the egg, a hole is obtained whose entire circumference lies ap-

proximately in the same plane, thus facilitating sealing with a flat cover glass. It was found necessary to turn the egg over and make the cut on the lower side while yolk and embryo float upward. This prevents injury which frequently results from cutting directly over the embryo. A small cut is made through the shell at the large end of the egg and the membrane over the underlying air space punctured. When this is done the embryo occupies a lower position in the egg, sinking away from the overlying shell. This permits removal of the shell and underlying membranes in one piece without damage to the embryo.

The general plan of operation is shown in figure 1; slight variations in individual cases are indicated in table 2.

Cutting instrument. To cut out the long, narrow piece of neural tube without disturbing it unduly poses a considerable problem. The most satisfactory instrument has proven to be a slight modification of the vibrating needle described by Drury ('41a). It consists of a steel blade made to vibrate in the field of an electromagnet activated by ordinary alternating current.

The only significant modification of Drury's instrument consists in bending the blade about 45° out of line with the shaft in a plane perpendicular to the plane of vibration. This angle facilitates greatly the task of making horizontal and vertical cuts and is particularly important in work on chick embryos where the operation has to be performed through a relatively small opening in the shell. The bend is made after removing the temper of the needle with heat; the blade is retempered prior to final sharpening.

Spinal cord levels. The levels considered (cervical, brachial, thoracic) are based upon differences in characteristics of the spinal cord and do not correspond to the usual regions (cervical, thoracic) determined on the basis of skeletal structures. The normal brachial plexus is formed by spinal nerves 13 to 16 with usually a small contribution from 17 and occasionally one from 12. Thus, the brachial region is considered to include the 5 nerve segments, 13 through 17. Similarly for purposes of the present discussion the cervical region is considered to in-

clude segments 1 to 12 and the thoracic region includes segments 18 to 22. An additional segment was usually included on each end of the transplant to shield the pertinent segments against traumatic effects of the operation.

At the stage of development of the embryos used (13 to 24 somites), wing buds have not yet appeared, and it is necessary to identify levels in terms of adjacent somites. The figures in row 2 of table 2 give the levels of operation as determined in this manner. These do not correspond to the nerve segment numbers of older embryos since the anterior somites disappear during development. Bueker ('45) found that three or 4 anterior somites disappear while Yntema and Hammond ('45) give the number as 5. Results of the present investigation agree with those of the former author. If the operation was performed on embryos whose somite number was less than 20, the difference between somite and nerve segment numbers was more frequently 4; if performed on embryos with a somite number of more than 20, the difference was usually three.

Since the attempt was made to perform the operation at the earliest possible stage, many of the transplants involving brachial and thoracic cord were from (or into) levels in which segmentation of the segmental plate had not yet taken place. The level of the anterior cut was usually reasonably certain and (unless otherwise stated in table 2) the posterior cut was made to include an estimated number of 7 segments.

As a rule a given number of brachial somites corresponds in length to an equal number in the thoracic level. Since cervical somites are smaller at the time of operation, 9 of them were found in general to be equivalent to 7 brachial somites. However, considerable size variation was found between individuals of the same incubation and somite age. The number of somites involved in each case is given in table 2 along with other operation data.

Operation. Host somites were left intact except in a few cases where small parts adjacent to the neural tube were accidentally removed or damaged. Host notochords were usually

not disturbed though in several cases they were inadvertently removed. Also a donor from a previous experiment in which the notochord had been removed with the graft was occasionally used as a host. Grafts were made with or without

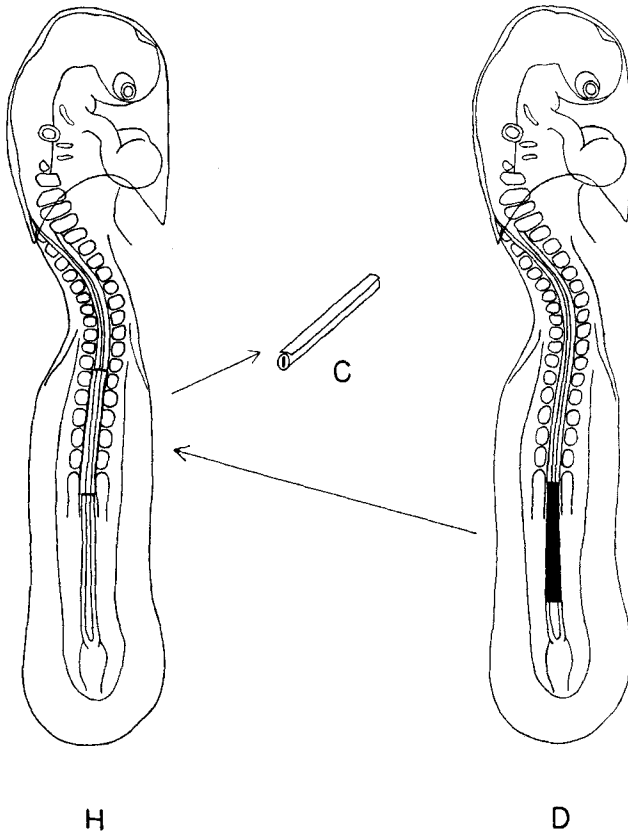


Fig. 1 Diagram of operation showing thoracic spinal cord implanted in the brachial region. D = donor with graft removed; H = host with graft in position; C = host spinal cord removed from implantation site.

notochords or parts of somites. The amount of each involved in individual cases is given in the table. The diagram (fig. 1) shows the transplantation of thoracic spinal cord into the brachial level. It represents a case in which only the neural

tube, and overlying ectoderm were grafted, without notochord or somites.

Hosts and donors were of approximately the same age. However, when a graft was made to a more posterior position (e.g., brachial to thoracic), the donor was usually selected to be a few somites younger³ than the host. Conversely, when the graft was made to a more anterior level, the donor was a few somites older.

Contrary to the experiences of some other workers, hemorrhage was not considered a serious problem. Some cases in which hemorrhage at operation was so extensive as to cause loss of all visible blood and slowing of heart beat almost to stopping, survived and are included in the analysis. The only observed defect resulting from extensive hemorrhage was reduction or absence of the left eye noted at the times of fixation. Cairns ('41) obtained somewhat similar results by interfering with the peripheral circulation and discusses the matter further.

The excised cord was transferred from donor to host with a Spemann micropipette (Hamburger, '42), and maneuvered into place. Excess saline was removed with the pipette to prevent the graft from floating away. Care had to be exercised in removing fluid since excessive drying of the surface tends to interfere with normal amnion formation.

After the desired period of incubation, the egg was carefully opened into a dish of warm saline. Observations were made on spontaneous movements of the limbs as well as reaction of the limbs to mechanical stimulation.

Most of the embryos were fixed in Bouin's fluid; a few were fixed and stained with silver according to the method of De Castro (see Levi-Montalcini, '49). The Bouin-fixed embryos were stained *in toto* with iron hematoxylin.

³ Eggs were put into the incubator at intervals during the day so as to have embryos of approximately the same age throughout a day's operating. This provided a graded series of embryos from which to select. It would be unwise, as Bueker ('45) has pointed out, to use embryos of the same incubation age that were noticeably retarded.

Staining procedure. The hematoxylin stain used was developed by the author to eliminate the time-consuming mordanting and destaining of sections necessary with the Heidenhain method usually employed for similar material. It will be described since it differs somewhat from any standard technique.

After fixation the embryos were washed in repeated changes of 70% alcohol (Li_2CO_3 may be used) until no more color comes out, then placed in a solution of 1 volume normal HCl to 19 volumes 70% alcohol for an hour or so, then into the following solution:

70% alcohol	14 parts
N HCl	1 part
4% FeCl_3 (aq)	1 part
2% hematoxylin in abs. alc.	4 parts

These ingredients are mixed in the order given since the last two will precipitate if mixed in concentrated or neutral solution.

Eight- to 10-day embryos are stained for two days in enough of the above solution to cover. Smaller embryos will stain in less time; longer staining will do no harm. The solution can be used several times.

After staining, the embryos are washed in repeated changes of 70% alcohol. The first two or three changes are made at 15 minute intervals (critical — longer time intervals will result in excessive destaining), the remaining changes are made as often as the liquid becomes noticeably colored. This is repeated until no more stain comes out on several hours' standing.

The resulting stain is comparable with respect to selectivity and contrast to well differentiated Heidenhain's iron hematoxylin. Considerable variation in the technique is possible without seriously affecting the results. The precise limits of variation as well as the applicability of the technique to other tissues are under investigation and will be reported at a later date.

Sectioning. When all excess stain had been removed, the embryos were embedded according to the amyl acetate technique of Drury ('41b) and sectioned serially at 10 μ ,⁴ through the graft region. In addition the host region corresponding to the donor level of the graft was similarly sectioned and used as a control.

Graphic reconstructions. Graphic reconstructions of the spinal cord, ganglia, and proximal nerves were made by pro-

TABLE 1
Summary of operations

TYPE OF OPERATION	NO. OPERATED	SURVIVAL		SEC-TIONED	GRAFT ABSENT OR DIS-ORGANIZED	USABLE CASES	
		No.	%			No.	% (of survivors)
Cervical to brachial	68	7	10.3	6	3	3	42.9
Thoracic to brachial	67	15	22.4	8	2	6	40.0
Brachial to cervical	84	4	4.8	3	1	2	50.0
Brachial to thoracic	30	8	26.7	7	3	4	50.0
Total	249	35	14.0	24	9	15	42.9

jecting every 5th section on graph paper and marking the edges of each structure on the line representing the particular section. Magnification of the projection was so adjusted that lateral dimensions were on the same scale as longitudinal dimensions, giving a reconstruction of accurate proportions.

Since it was desirable to reveal any distortions in axial structures, the latter could not be used for orientation of the sections as is usually done. Orientation was achieved by sec-

⁴ Chick 55 was sectioned at 8 μ .

tioning, with the embryo, longitudinal markers which, when aligned with appropriate lines on the paper would orient each section in the proper position with respect to the whole embryo. These markers were made by embedding longitudinal strips of stained fibrin clot with the embryo.

A summary of the operations performed is given in table 1. All embryos which lived until the time of fixation (7 to 10 days' total incubation) are included as survivors. In general, only those cases which showed no gross malformations of body or limbs were sectioned for study. Three of the chicks included as usable cases showed malformations which involved parts of the host other than the regions bearing the graft.

RESULTS

Fifteen of the 24 chicks sectioned were found to have grafts in sufficiently good condition to permit identification of cellular patterns. Protocol data for these animals are summarized in table 2. Where additional information or clarification is necessary, it is given in the discussion.

Healing of grafts

The degree of healing between graft and host varies from complete union to a condition in which there are considerable gaps between graft and host cord which may or may not be bridged by fiber bundles. However, even in the best cases, damage to the ependyma and a certain amount of disorganization in the adjacent gray matter make it possible to identify the transplant accurately. The injured parts of the graft or host cord are frequently characterized by extravasation of cells from the gray matter or by bundles of fibers which ramify for short distances into the surrounding tissue. As pointed out by Hamburger ('46) it is probable that these fibers end blindly without making functional connections.

In cases showing the best healing of the graft, vertebrae in the operated region appear to be entirely normal with complete neural arches and typical separation into segments. They

139	23 + (e7) to 16-22 (7)	23	22	Yes	No	0	0	D	D	8	+	+	0	0
255	21 + (e7) to 15-21 (7)	24	20	No	No	0	0	0	D	10	0	+	+	+
72	15 + (e7) to 8-14 (7)	15	16	Yes	No	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	9	+	+	0	0
243	16 + (e6) to 5-12 (8)	18	18	Yes	Yes	0	0	D	D	9	0	0	0	0
39	15 + (e7) to 22 + (e7)	16	21	Yes	No severed post.	$\frac{1}{2}$	$\frac{1}{2}$	D	D	8				
55	15 + (e7) to 22 + (e7)	19	24	No	No poorly visible	$\frac{1}{4}$	$\frac{1}{4}$	0	0	9	+	+		
123	16 + (e7) to 22 + (e7)	13	17	No	No	0	0	0	0	9				
254	15-21 (7) to 21 + (e7)	20	24	No	No	0	0	0	0	8	+	+	0	0

* First line, somite level of donor at which graft was taken; second line, level of host at which graft was implanted, followed in each case by the number (in parentheses) of segments involved; e indicates that the number of segments had to be estimated.

also show the usual relationship to ribs and to roots of spinal nerves. In less favorable cases, neural arches and the centra may show incomplete separation or complete lack of division into individual vertebrae. Even in cases where somite material was included with the graft, vertebrae appear to be typical of the host region. Ribs are usually found to be normal both in form and in relation to vertebrae and sternum. Abnormalities such as fusion or displacement are found, as well as cases in which ribs failed to connect with the sternum. In no case were supernumerary ribs found following inclusion of thoracic somite material in the graft. The vertebrae and ribs, however, were not investigated *per se* but only as landmarks to help identify levels of the body.

Visceral ectopia among operated animals was common, but it is assumed that this has no effect upon development of the spinal cord.

In general, only those embryos were studied whose body form and limbs were normal. In case 243, however, the head and neck which contained the graft were normal while the remainder of the body was badly stunted. This chick was used because of the shortage of cases of this operation (brachial to cervical). An adjacent hydrocoel had caused distortion of the graft; nevertheless, the latter showed excellent histological detail and typical cell pattern. Similarly, chick 254 was used although the right wing was missing. This is considered justifiable in view of the fact that the operation (brachial spinal cord transplanted to the thoracic region) did not involve the brachial region of the host.

Identification of segments in grafts

While location and recognition of the grafted cord as a whole proved to be an easy matter, identification of a given level of the original donor cord in the final graft presents much greater difficulties.

The complications caused by disappearance of the posterior head segments have been mentioned above. As was indicated,

subtraction of either 3 or 4 from the number of the somite level at operation gives the corresponding level in the adult (or late embryo). This system gives good agreement in the host where it can be checked by other topographic features such as nerve plexuses, ribs, etc. It also checks satisfactorily in the case of brachial cord grafts where the presence of lateral motor columns can be used to identify the level.

Identification of posterior levels in many grafts was further complicated by the fact that several grafts were taken, at least in part, from neural tubes adjacent to the segmental plate of the donor where the somite level could only be estimated at the time of operation. In all cases, the first segment of the graft was identified at the time of analysis according to the system outlined above and the remainder arbitrarily numbered in series.

Segment boundaries of the spinal cord were determined on the basis of motor rootlets as described by Hamburger ('48). The midpoint between the last motor rootlet of one nerve and the first rootlet of the next nerve posteriorly is located for each side. There is no evidence, however, that the limits of a segment found in the graft necessarily correspond to those of a donor segment as determined by boundaries of adjacent somites. The identification of segments in the graft in terms of donor segments represented must, therefore, be considered as only approximate.

Those cases in which segments could be identified with reasonable accuracy are presented in table 3.

Since segment identification in the graft is not always exact, no conclusions are based on this identification which would be substantially altered if the level were considered to differ by one segment in either direction. However, the identifications were made only after careful examination of the material and it is believed that the only cases where identification may be in error by this amount are those where the number of segments found differs from the number included in the transplant. This is known to have occurred in chick 164 and may have occurred in some of the cases where somite level had to be estimated.

Factors involved in segmentation of the graft

In the process of identifying segment levels in the operated chicks, some observations were made which tend to bear upon the question of factors involved in segmentation of the chick spinal cord.

In the case of chick 164 (cervical spinal cord grafted to the brachial level) the operation was estimated to involve extirpation of 9 host segments, and 9 donor segments were known to have been included in the graft. Analysis after 8 days' total incubation shows, however, that only 8 host segments are missing and the graft is uniformly divided into 8 segments with a corresponding number of ganglia all harmoniously spaced with respect to the host segmentation (fig. 2). It is probable that the estimate of the number of host segments removed was in error, and that actually only 8 segments were removed. Since it is known, however, that 9 donor segments were included in the graft, the latter appears to have segmented according to the host.

In all other cases where the number of donor segments transplanted is definitely known, either the number involved in the donor and host is equal or segmentation of the graft is too irregular to permit drawing any conclusions. No attempt to analyze the factors responsible for irregularities in segmentation would appear to be justified, in view of the limited material.

Plexus formation

Ventral rami of all spinal nerves were included in the reconstructions. They were plotted for a sufficient distance laterally to determine whether they made major plexus-like anastomoses.

Distribution within the wings was not included in the reconstructions. It was investigated superficially by tracing through the sections and found to be essentially normal in all cases where a reasonably normal plexus was formed.

Non-brachial cord in the brachial region. In all cases where non-brachial cord grafted to the brachial level showed any de-

gree of normal organization, it innervated the host wing and those nerves which entered the wing, though smaller than normal brachial nerves, made definite plexus-like anastomoses. All cases in which the host and graft segments involved could be determined with reasonable accuracy are presented in table 3 to show the location of the plexus and normality of its pattern.

While it is apparent that there is a great deal of variation in the degree to which a graft plexus approaches the normal, little can be said with respect to the factors responsible. Variations in number of nerves in the plexus can usually be correlated with the number of nerves emerging from the graft at the limb level. Distortion of patterns of anastomoses can be correlated with irregularities in formation of vertebrae, in separation of spinal ganglia, and in outgrowth of ventral roots. The point to be made is that either thoracic or cervical cord *can* give rise to nerves which form a brachial plexus having the normal pattern. In those cases where this is demonstrated (cases 164, 139; figs. 2 and 4) vertebrae are regular and separate, ganglia are separate and well spaced, and nerve roots emerge at regular intervals. Slight variations such as abnormal spacing anteriorly and asymmetrical segmentation (chick 139) and absence of a ganglion on the right (chick 164) are correlated with slight irregularities in the plexus patterns in these cases.

Brachial cord in the thoracic region. Brachial cord transplanted to other regions varies in different cases with respect to plexus formation. The three cases analyzed are summarized in table 3. In two cases (55 and 123) brachial cord grafts transplanted to the thoracic level gave rise to nerves which run laterally in a manner characteristic of normal thoracic nerves, without plexus formation. In chick 123 (fig. 5 and table 3) the graft extends posteriorly into the lumbar region of the host. Nerves 18 and 19 (donor thoracic nerves) of this graft form a partial lumbar plexus but the entire brachial region gives rise only to typical thoracic nerves. Chick 55 differs somewhat in that graft nerves 16 and 17 contribute to the

TABLE 3
Cell columns and nerve plexuses formed by spinal cord grafts

TYPE OF OPERATION	CASE NO.	SOMITE LEVELS INVOLVED	NERVE SEGMENTS IDENTIFIED IN GRAFT AT THE LEVEL OF HOST SEGMENTS																										
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Normal																													
Cervical	82	L 8-14 (7) to 15-21 (7)																											
to																													
brachial	164	L 6-14 (9) to 16+ (e9) R																											
Thoracic	47	L 22+ (e7) to 15+ (e7) R																											
to																													
brachial	139	L 23+ (e7) to 16-22 (7) R																											
Brachial	72	L 15+ (e7) to 8-14 (7)																											
to																													
cervical	243	L 16+ (e6) to 5-12 (8) R																											
	39	L 15+ (e7) to 22+ (e7) R																											
Brachial	55	L 15+ (e7) to 22+ (e7) R																											
to																													
thoracic	123	L 16+ (e7) to 22+ (e7) R																											
	254	L 15-21 (7) to 21+ (e7) R																											

Somite levels involved in the operation are indicated as in table 2; the columns numbered from 1 to 27 represent host segment levels. Numbers inserted in these columns indicate graft segments located at that level. Plus (+) signs indicate host segments adjacent to the operated area. A space between a + and a graft number signifies a gap between grafts and host spinal cord (usually bridged by fiber bundles). The remaining space represents normal host cord. A straight line between numbers indicates that nerves from these segments make plexus-like anastomoses, but do not form a typical pattern. Nerves of segments connected by an arc join to form a plexus characteristic of that host level. A dotted line (or arc) indicates that the nerves converge similarly but do not unite. The letters L and R represent the left and right sides of the embryo respectively.

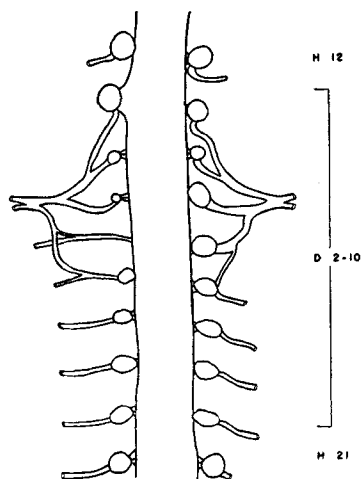


FIG. 2

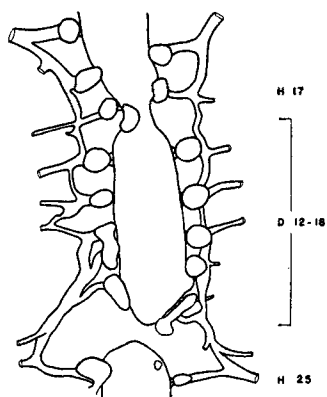


FIG 3

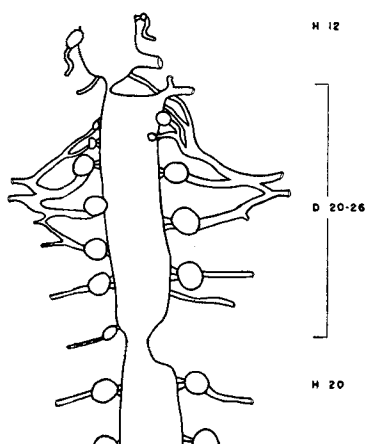


FIG. 4

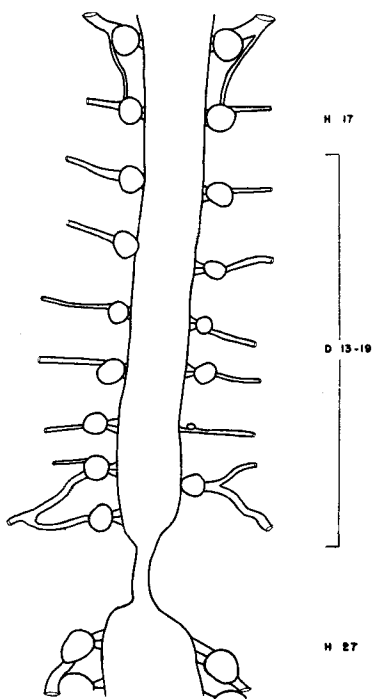


FIG. 5

Figs. 2-5 Graphic reconstructions of the operated regions, showing spinal cord, ganglia and proximal distribution of spinal nerves. $\times 21$. The brackets indicate the extent of the graft, numbers in the brackets indicate donor nerve segments represented by the graft. Numbers preceded by the letter H indicate host segment levels. Fig. 2, chick 164, cervical spinal cord transplanted to the brachial level. Fig. 3, chick 39, brachial spinal cord transplanted to the thoracic level. Fig. 4, chick 139, thoracic spinal cord grafted to the brachial level. Fig. 5, chick 123, brachial spinal cord grafted to the thoracic level.

lumbar plexus of the host in a manner characteristic of normal nerves 23 and 24 (lumbar). The first three brachial segments, however, give rise only to nerves with a typically thoracic pattern.

The other case of brachial cord in the thoracic level, chick 39, presents a strikingly different picture. It can be seen from table 3 and figure 3 that not only do nerves from the graft contribute to both the brachial and lumbosacral plexuses of the host, but also that all nerves of the graft are connected near the base by plexus-like anastomoses.

Brachial cord in the cervical region. In both successful cases of brachial cord grafted into the cervical region a tendency to plexus-formation can be observed (see table 3). The anterior part of the graft of chick 72 was discarded by mistake, but nerves from donor segments 13 to 16 are found to form irregular anastomoses. Most of the nerves from this plexus either make connection with surrounding organs or end in neuroma-like masses suggesting those found by Hamburger ('34) following wing extirpation. A small branch contributes to the brachial plexus of the host. The graft of chick 243 was placed farther anteriorly in order to isolate it more completely from the host brachial region. The first two nerves from the graft pass laterad and craniad into the anterior neck and posterior head region in a manner similar to that of the first two cervical nerves of the normal chick. The distribution was not followed, so it cannot be stated whether they make connections normal for these nerves. Graft nerves 15 to 18, on the other hand, turn sharply caudad to converge at a point opposite the posterior end of the graft. On the left side, nerves 15, 16 and 17 unite to form a plexus; 18 converges with them but makes only a minor connection. The nerves on the right converge in a similar manner but form no major anastomoses. Distal to this plexus, the nerves break up quickly into small branches distributed to the surrounding region. The host tissues in the region are richly supplied with small nerve twigs, but no nerves could be traced for any great distance. It is certain that no nerve of appreciable size made contact with the host brachial plexus.

Function of limbs

Prior to fixation all chicks were observed to determine whether the limbs showed evidence either of spontaneous movements or response to mechanical stimulation (table 2). In all cases where wings were innervated by nerves from foreign cord, either spontaneous movement or reaction to stimulation or both were observed. Too much significance should not be attached to a failure to respond at this stage since normal embryos of the same age frequently fail to show any response to similar stimulation.

Spinal cord patterns

Any analysis of determination in the different levels of the spinal cord requires, necessarily, a knowledge of those cell types and patterns characteristic of each level and sufficiently distinctive to be recognized in heterotopic grafts.

There exists in the literature no satisfactory comparison of the levels of the spinal cord of chicks at this stage (8 to 9 days). Direct comparison is therefore made either with the corresponding region of the host or of another embryo of the same age.

While a thorough study and comparison of cell types in the different levels might have revealed additional bases for comparison, it is outside the scope of the present work. Lateral motor columns and columns of Terni contain the only cell types, detectable by the techniques used, that are suitable for characterizing the levels under consideration. Fortunately, differences with respect to size and arrangement of various elements are also present and can be used for this purpose. A brief description will be given of those features in each of the levels which have been used to characterize that level. Obviously, no attempt is being made to give a complete description of any level.

Sections of the brachial cord of the chick up to 8½ days of incubation have been described by Hamburger ('48) and this work will be used as a basis for comparison. The 8-day chicks

considered here compare closely to his 8n 11 while the 9-day embryos differ from 8 dn mainly in a slightly greater reduction of the neurocoel. The characterizations apply equally well to either the 8- or 9-day chicks, although differences between levels are considerably more marked in the latter. Regions of transition from one level to another tend to show an intermediate picture. Transitional levels are usually avoided in making comparisons not only for this reason but also because in the graft they are more likely to have been injured by the operation.

The ependyma with adjacent floor and roof plates and the entire region of white matter, while showing quantitative regional differences, provide no satisfactory criteria for identification of levels.

The three cell columns which are clearly identifiable, namely sensory, lateral motor, and mesial motor, will be considered and the entire remaining mantle regarded as a 4th unit. In addition the preganglionic columns of Terni ('24) will be discussed in one case.

Normal cervical patterns (fig. 6). Cross sections through the cervical spinal cord have a small, rounded appearance. The mantle is narrower than at either of the other levels and the sensory horns are relatively small and inconspicuous. Mesial motor columns are small and occupy a position near the ventral commissure. In occasional sections one sees large cells having the appearance of motor cells protruding laterally from the mantle. These are considered to be the columns of von Lenhossék though they cannot be identified absolutely in hematoxylin preparations. While their appearance in occasional sections suggests that of lateral motor cells, they do not form continuous columns as do the latter.

Neither lateral motor columns nor columns of Terni are found in the cervical region.

Normal brachial patterns (figs. 8 and 9). In the brachial region the spinal cord becomes much larger as compared with the cervical cord and its overall shape is that of a trapezoid. The mantle is wider and the sensory horns larger and more con-

spicuous. Little difference is seen in either the position or size of mesial motor cells while the most striking change over the cervical region is in the presence of the large lateral motor columns. In silver impregnated specimens, preganglionic columns of Terni are found in the posterior segments (16 and 17).

Normal thoracic patterns (fig. 7). The picture of thoracic sections differs considerably at the two ends; intermediate levels present an intermediate condition. Anteriorly the overall shape is rectangular with the mantle somewhat narrower and the sensory columns smaller than in the brachial level. Mesial motor columns are about the same size as those of the brachial region and continuous with them. As one proceeds posteriorly, the mesial motor columns become larger, occupy a more lateral position and appear to be continuous with the lateral motor columns of the lumbosacral enlargement (see Bueker, '45). No lateral motor columns are found in the thoracic level but it is characterized by the presence of most of the preganglionic columns of Terni.

Patterns of grafts. In all cases of the present investigation where the graft was in sufficiently good condition to permit identification, patterns formed in grafted spinal cords were qualitatively characteristic of the donor level from which the graft was taken. All cell types recognized by the techniques employed and characteristic of the donor level of origin are found in each graft and always in the normal position relative to the rest of the cord. Quantitative differences between grafts and controls may well exist and undoubtedly do, but, as will be pointed out later, the material at hand is not suitable for a satisfactory quantitative analysis.

Since identification of patterns involves a certain amount of subjective evaluation, outline projection drawings of representative sections are included with controls for position and origin (figs. 6 to 9).

Wherever possible the origin control was taken from that segment of the host which corresponds to the particular donor segment of the graft. Position controls were taken from an-

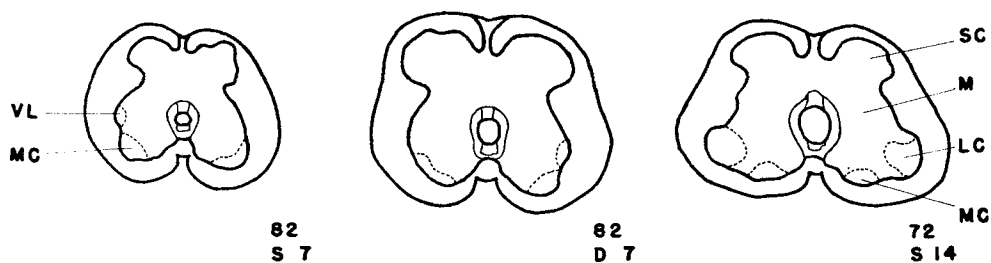


FIG. 6

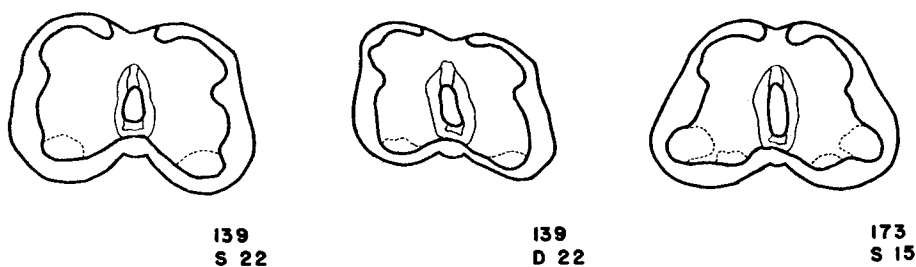


FIG. 7

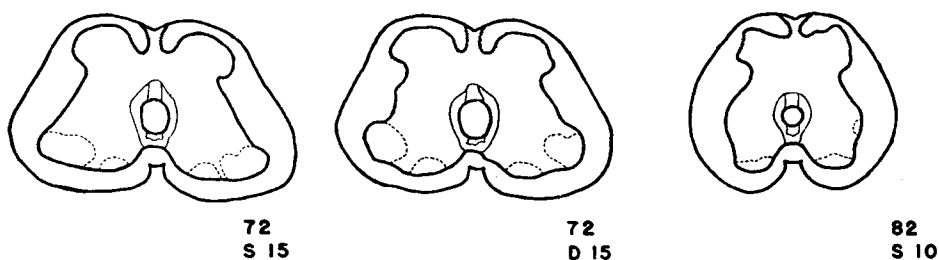


FIG. 8

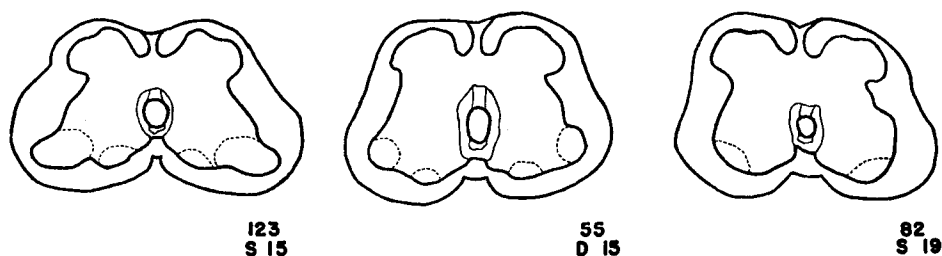


FIG. 9

Figs. 6-9 Cross sections of graft spinal cords (center) compared with origin control (left) and position control (right), $\times 58$. Symbols at the lower right of each drawing indicate, on the first line, the protocol number of the chick from which that section was taken and on the second line, the segment number of the animal (S) or donor segment number (D) of the graft. Cell groups recognized for the purpose of identifying levels are: sensory columns = SC; mantle = M; lateral motor columns = LC; mesial motor columns = MC; cells tentatively identified as columns of von Lenhossék = VL. Fig. 6, chick 82, cervical to brachial. Fig. 7, chick 139, thoracic to brachial. Fig. 8, chick 72, brachial to cervical. Fig. 9, chick 55, brachial to thoracic.

other animal of the same age (within the experimental group) and from a level corresponding to the position of the graft segment in the host. The segment from which the section was taken is indicated in each case.

Use of other operated animals for controls is justified by Hamburger's ('46) demonstration that adjacent levels of the cord have no effect, quantitatively or qualitatively upon development of the brachial cord. Furthermore, this procedure tends to minimize differences that might arise between experimental animals and unoperated controls as a result of the effect of the operation on the animal in general.

Photomicrographs of representative graft sections are presented in figures 10 to 13 to demonstrate the excellent histological differentiation found in the grafted spinal cords.

Lateral motor columns are seen to be present on only the right side of chick 123. In removing this graft from the donor embryo, the ventrolateral edge of the tube was sliced off on the left. This injury can be seen in figure 13 as a gap in the ependyma and is associated with lack of the lateral motor column and most of the mesial motor column of that side. A similar correlation was observed by E. Wenger ('50) following removal of parts of the brachial spinal cord.

In the cases illustrated it will be seen that all cell groups characteristic of a given region are found in the grafts from that region and always in the normal position. This situation prevails equally well in the cases not illustrated. In cases 242, 254 and 255, the alar plate is damaged. However, all qualitative criteria used for identifying levels are found in the basal plate, so the statement holds in these cases as well. All show basal plate patterns characteristic of the level of origin.

The longitudinal extent of lateral motor columns in all cases involving transplanted brachial spinal cord is shown in table 3. In addition, the preganglionic columns of Terni are shown for the one case (chick 254) which was impregnated with silver.

It can be seen that in all cases, lateral motor columns are found in and are limited to those graft segments taken from the region of the donor in which they are normally found. This

statement holds equally well for the preganglionic columns of Terni in the one case where they are demonstrated.

DISCUSSION

Transplantation of a given region of spinal cord to another level raises a number of questions in addition to the one of pattern determination toward which the work was primarily directed. These include the problems of nervous segmentation in the chick, peripheral distribution of nerves in an abnormal environment, function of limbs with an abnormal nerve supply, and the effects of peripheral structures upon the development of the central nervous system. Since operations and analyses of material were designed primarily to provide information on pattern determination, little of a conclusive nature can be deduced regarding the other points. However, each will be discussed in its relationship to the present problem and to the extent that information is contributed by the present work.

Pattern determination

The question was presented in the introduction as to whether the strikingly different patterns characteristic of the various spinal cord levels in the late embryo (or adult) are determined by the time of formation of the neural tube and prior to the outgrowth of any nerves, or whether the spinal cord represents a uniform tube of equal potentialities capable of forming any of the different patterns under the influence of suitable peripheral connections. Such an equipotential tube would, presumably, contain over its entire length the neural epithelial units described by E. Wenger ('50) plus possible additional units. Development of the final pattern would depend upon development or suppression (including degeneration) of particular units under the influence of peripheral structures at that level and mediated by nervous connections with these structures.

Some work bearing on this problem has been done. The considerable work of Detwiler and his students on *Ambystoma* was reviewed above. Although limited by the nature of the

material to a quantitative study, they have shown that the final size relationships in the salamander spinal cord depend on a combination of intrinsic and other factors. Detwiler ('43) has also shown that the medulla of *Ambystoma* retains its normal morphological character when transplanted to the spinal cord region. Transplantation of the embryonic chick medulla to the chorio-allantoic membrane by Reddick ('45) showed that cell groups differentiate normally in the isolated medulla but that normal separation of the latter into nuclei and formation of nerves depends upon the presence of normal mesodermal structures.

Bueker ('45) transplanted different levels of the chick spinal cord between the hind limb and lumbosacral cord of the host in order to test the effect of the limb periphery on foreign cord. Although he obtained motor and sensory hyperplasia in segments from non-limb levels, the general pattern was apparently not markedly altered.

It can be seen that previous work on the chick either has subjected the experimental part to highly abnormal situations or, in the case of limb extirpation and transplantation, has not completely removed it from all possible effects of the normal location. The present operation was conceived to avoid the possible effects of such factors. The neural canal of another level should provide the optimal heterotopic situation for development of a spinal cord graft, yet it is a situation in which cord normally found in that level develops a very different pattern. While this operation can give no information as to determination of patterns common to all levels of the spinal cord, development according to origin of all grafts studied indicates that at the time of operation (13-24 somites) each level differed from the other level by some intrinsic factor which caused it to develop a different pattern when allowed to develop under essentially identical circumstances. This constitutes our concept of determination and is consistent with the modern view that determination of a structure can only be considered relative to another structure and in terms of their different behavior when cultured under the same conditions.

The conclusion holds for the presence or absence of lateral motor columns and the general pattern of the cord as it appears in cross section. A similar conclusion with respect to the preganglionic columns of Terni must be considered tentative since their failure to develop in spinal cord from other than the thoracic level has been demonstrated in only one case and their development in thoracic cord transplanted to a non-thoracic level has not been demonstrated.

In this sense it can be stated on the basis of the present work that the demonstrated qualitative differences in cell types and patterns, existing between brachial and adjacent levels of the chick spinal cord are determined by the time the particular levels can be identified (i.e., 13-24 somites).

These findings are in accord with the views of Levi-Montalcini ('50) who, while demonstrating the presence of morphologically uniform motor columns throughout the length of the chick spinal cord at 4 days' incubation, points out that functionally they are quite different in different levels.

The exact time at which determination takes place still remains an open and very important question. In the absence of experimental data, the best that can be done is to examine work on the *Amphibia* for suggestions as to what may occur in the chick.

While work cited in the introduction suggests that major regions of the central nervous system of *Amphibia* may be determined at the time of primary induction, it has been shown (Lehmann, '35) that formation of the characteristic spinal cord patterns depends upon continued contact with the chordamesoderm and lateral (somite) mesoderm. Kitchin ('49) has shown that in the axolotl this effect is completed by the time a definite medullary plate is formed (stage 15) since subsequent development follows the normal pattern in the absence of the notochord and apparently independently of the somites.

At the present time it can only be stated that determination of the spinal cord patterns under consideration in the chick takes place some time between primary induction of the medullary plate and closure of the neural folds. Results of the am-

phibian experiments would certainly open the possibility that determination of regional patterns occurred subsequent to primary neural induction though it is not known at what time in the laying down of the embryonic chick axis this event takes place or whether it takes place at a sharply defined time as in the Amphibia.

Answers to these problems must await not only a more precise mapping of presumptive organ forming areas in the early chick embryo but also more refined operative techniques.

Effects of periphery

Wings and associated structures provide a much more extensive field for nerves growing out from the brachial level than is present at either the cervical or thoracic levels. Furthermore, it was pointed out above that grafted spinal cords innervate adjacent regions of the host in a qualitatively normal manner. The question arises as to what effects this increased (or decreased) periphery has upon development of the grafted spinal cords.

Such effects are of particular interest in view of the work that has been done with regard to the effect of peripheral structures upon development of the central nervous system (see Piatt, '48). Hypoplasia has been observed to follow extirpation experiments (Shorey, '09; Hamburger, '34; Hamburger and Keefe, '44; Levi-Montalcini and Levi, '42; Bueker, '43, '47) would lead one to expect hypoplasia of a graft transplanted to a region with a smaller periphery. Conversely, the hyperplasia that follows grafting of supernumerary limbs (Hamburger, '39b) would lead one to expect hyperplasia of spinal cord grafted into a region having a more extensive periphery. Bueker ('45) has demonstrated motor and sensory hyperplasia in spinal cord grafts from other levels under the influence of the leg periphery.

In most of the work just cited, the data were obtained by making unilateral operations such that the contralateral side could serve as a control. In the present work, on the other

hand, the operation involved the entire cord of a given level and comparison can only be made to the corresponding level of the host or another animal. This in itself does not provide as satisfactory a control. Furthermore, it was found to be virtually impossible to identify with precision comparable units of the graft and control cords.

Uncertainties were pointed out earlier as to the exact donor segment levels represented by a given segment found in the graft or the number of donor segments represented by a graft segment. It would, therefore, be of little value to attempt a direct quantitative comparison (cell counts, volume measurements, etc.) between graft and control spinal cords.

Segmentation of the chick spinal cord

Although marked dependence of neural segmentation upon that of the mesoderm has been demonstrated for urodele amphibians, it has only been assumed (e.g., Streeter, '33) that a similar situation holds in the chick.

Lehmann ('27) found that removal of somites in *Pleurodeles* prevented ganglion formation, and he concluded that normal location of the sensory and motor roots was dependent upon a normal arrangement of the mesoderm. Detwiler ('27, '36) confirmed the above observation though he also showed that in *Ambystoma* irregular ganglionic masses as well as nerve roots can be formed in the complete absence of segmental mesoderm. He further demonstrated conclusively the mesodermal influence by transplanting supernumerary somites or the equivalent segmental plate next to the spinal cord. In this way he was able to obtain as many as two additional segments, uniformly spaced and with corresponding ganglia, from the brachial cord which normally contains only three segments.

While the present operation was not planned to provide critical evidence with regard to nervous segmentation in the chick, some very suggestive observations were made in the course of analyzing the material. As has been indicated above, in case 164 (fig. 2), a graft known to represent 9 donor seg-

ments occupies an 8 segment gap in the host spinal cord. This graft was found to give rise to 8 uniformly spaced nerves with corresponding discrete ganglia (1 missing). These nerves are harmoniously spaced with respect to those of the host.

Before accepting this case as evidence that host segmentation has been imposed upon the graft, the alternative of segmentation according to donor should be examined. If one assumes the latter interpretation, the difference in segment number (8 instead of 9) can be accounted for only by assuming either that a segment from one end of the graft was lost, followed by union of the remainder with the host cord, or that part of a segment was lost from each end, followed by similar fusion. In the first place, it seems rather unlikely that an entire segment should disintegrate and disappear and the remainder of the graft shift so as to affect contact with the host cord in such a way that the segmentation would correspond to that of the host. The possibility that half a segment should be lost from each end would result in a graft whose segmentation, while being similar in number, would be one half segment out of phase with that of the host.

Since the former of these possibilities seems very unlikely while the latter would lead to a condition contrary to what is found, it is concluded that the segmentation of the graft (both spinal cord and ganglia) is according to that of the host and imposed by it.

Plexus formation

Outgrowth of graft nerves into a completely abnormal environment raises first, the question of whether nerves from non-brachial spinal cord can form typical patterns in the brachial plexus and wing, and second, whether brachial cord can form peripheral patterns characteristic of a foreign region or can only form peripheral nerve patterns characteristic of the plexus and wing. Stated differently, the problem is one of the extent to which complex patterns found in peripheral nerves are imposed on them by the peripheral structures or are inherent in the fibers themselves.

Early transplantations in anurans by Braus ('06) and Harrison ('07) showed that the former was the case with respect to nerve patterns within the limb. Normal hind limb nerve patterns were formed in posterior limbs by heterotopic spinal nerves and even by cranial nerves. These results have been amply confirmed by other workers in other Amphibia (see Detwiler, '36) and by Hamburger ('39a) in the chick. See also Piatt ('42, '48).

Less attention seems to have been paid to patterns formed by the same nerves prior to entering the limb. Plexuses formed by nerves from foreign cord entering grafted limbs normally show considerable variation in number of contributing spinal nerves as well as distortion of patterns (Detwiler, '36; Hamburger, '39a). This variation is probably due to irregularities in spatial relationships existing between the grafted limb and the spinal cord segments innervating it though the possibility of a more or less characteristic influence of intervening somite material should also be considered.

With regard to the question of somite influence, Detwiler ('36) has demonstrated normal innervation and function of the forelimb of *Ambystoma* after removal of the brachial somites. The plexus patterns were, however, somewhat abnormal.

Moyer ('43) transplanted brachial cord to the trunk region of *Ambystoma* and later grafted a limb lateral to it. This author makes no statement as to the degree of normality of the plexus supplying the grafted limb, but from her reconstructions it appears that while approaching normality very closely, the nerves, which traverse posterior trunk somite material, fail to reproduce completely the normal plexus pattern.

On the other hand, trunk spinal cord transplanted to the brachial region gave rise to nerves which formed a normal plexus pattern before entering the limb (Detwiler, '23).

In the most favorable cases of the present study, plexuses formed by nerves from either cervical (chick 164) or thoracic (chick 139) spinal cord are found to be almost perfect replicas of the normal brachial plexus pattern and could not be distinguished from it were it not for the considerably smaller diameters of the nerves.

It may be noted that in each of the two best cases just cited no donor somite material was included with the graft (table 2). In all other cases, however, irregularities can be associated either with an abnormal number of nerves opposite the wing level or with irregularities in emergence of nerves from the graft cord. It would, therefore, be without justification to conclude that the presence of part of the donor somite material either did or did not interfere with plexus formation. The available evidence tends to suggest the possibility of a specific effect of brachial somites upon plexus formation. The possible role of patterns formed by general tissue movements peculiar to a given region must, however, be kept in mind. Critical experimental evidence is needed before any conclusions can be drawn.

Brachial cord in non-limb levels. In most of the limb extirpation experiments, particularly those on chicks (Hamburger, '34), a considerable tendency is noted for limb nerves to form plexus-like anastomoses in the remaining tissues. In his extremely radical leg extirpations, on the other hand, Bueker ('43) reports cases in which no tendency toward plexus formation is observed, with the fibers ending blindly in the region of the vertebrae. In the first cases above, as Bueker ('43, '47) has pointed out, it is difficult to eliminate the possibility of some elements of the limb or girdle remaining, and, in fact, they are frequently observed (Hamburger, '34). In Bueker's experiments it might be contended that patterns inherent in the nerves might fail to be expressed because lack of suitable periphery prevented the nerves from growing out at all.

Chicks 55 and 123 of the present investigation demonstrate conclusively that nerves from brachial spinal cord can grow out in a typically thoracic pattern without plexus formation. In these cases (fig. 5, 123; table 3) the normal brachial segments (estimated 13 to 17) send out lateral nerves in a manner characteristic of the normal thoracic nerves, showing no apparent tendency to form interconnections. (Nerves 16 and 17 of chick 55 contribute to the lumbosacral plexus in a manner typical for normal lumbosacral segments 23 and 24.)

Thus it is seen that whatever capacity may be inherent in brachial nerves to form plexuses is labile, and can readily be over-ridden by the pattern-directing influence of the thoracic periphery.

It is apparent that plexus patterns just as patterns of more distal peripheral distribution are imposed upon outgrowing nerves by factors present in the mesoderm. The greater frequency of abnormalities in plexus patterns as compared to peripheral nerve distribution must be considered merely as a greater susceptibility of the latter to disturbances resulting from the usual operative procedures. The remaining cases (chicks 72, 243, brachial to cervical; 39, brachial to thoracic), on the other hand, present a strikingly different situation. In all three (table 3) nerves from the grafted brachial cords unite in plexus-like anastomoses. This suggests that although brachial nerves *may* follow patterns typical of non-limb levels, they still show an inherent tendency toward plexus formation. While the possibility must be borne in mind that the patterns found may be merely the product of uncontrolled disturbances in the mesenchyme occurring at the time of operation, it still seems necessary to postulate a greater capacity of nerves from brachial cord to follow such patterns. The question appears to be an important one, and its solution should provide valuable information bearing upon the problems of plexus formation and nerve outgrowth in general. The answer must, however, await more complete experimental data.

Symmetry. When the influence of peripheral structures on nerve patterns is recognized it becomes apparent that bilateral plexus formation can be no more symmetrical than these structures. Symmetry of the plexus in normal development is, then, to be considered as an expression of the symmetry of the periphery.

It has been shown, however, that the number of nerves entering a limb can be made to differ on the two sides of the animal (e.g., Detwiler's production of supernumerary spinal segments). In these cases, asymmetry of the plexuses reflects a difference in the two sides of the cord itself.

In the present work where the periphery was presumably symmetrical, minor asymmetries in the two plexuses can be associated with differences in number of nerves contributing to the plexus (chick 47, table 3), bilateral differences in segmentation (chick 139, fig. 3), and deficient contribution of one nerve (chick 164, fig. 2).

Thus, it is seen that normal, symmetrical plexus formation depends not only upon symmetry of the periphery but also upon symmetrical nerve contributions from the two sides of the spinal cord.

In this connection the results of Allen ('48) obtained with chorio-allantoic grafts of the brachial region of early chicks are of interest. She found that not only was the plexus formed from heterotopic cord when the wing became displaced, but that it could be formed by nerves from opposite ends of a relatively long piece of grafted cord when the latter became bent back upon itself.

Function of limbs

In all cases (see table 2) of wings innervated by nerves from foreign spinal cord, either spontaneous movement or reaction to mechanical stimulation or both were observed.

In a few cases (82, 139) small contributions to the plexus made by adjacent brachial segments of the host cord could possibly account for the observed reaction. In the other cases, however, either no contribution to the plexus by the host cord could be demonstrated, or all host segments which normally contribute to the plexus (12 to 17) were removed so that any contribution from the host would necessarily involve cervical or thoracic levels. It seems evident, therefore, that innervation from either cervical or thoracic spinal cord transplanted to the wing level is capable of producing wing movements typical for chicks of the age studied (8 to 9 days).

This is in agreement with the result of Detwiler ('23) who found that trunk spinal cord grafted to the brachial region of *Ambystoma*, is able to produce normal, coordinated movements in the host forelimbs. On the other hand, heterotopically

grafted forelimbs perform normal, coordinated movements only if they have received some nerve contribution from the brachial region of the host (see Detwiler, '36). Moyer ('43) found that heterotopically grafted brachial spinal cord was not able to produce normal movements in similarly grafted limbs of *Ambystoma*.

Conclusive data on grafted chick limbs are not available though in all cases studied, Hamburger ('39a) found that those grafted limbs which showed motility all received a contribution from the host plexus.

It should be pointed out, however, that limb movements in chicks of the age studied consist of simple, relatively non-specific twitches. The observations can, therefore, give no evidence for the production of complex, coordinated movement of wings by nerves from foreign cord. This question can only be answered by raising the operated animals to considerably later stages of development, preferably past hatching.

SUMMARY AND CONCLUSIONS

Transplantation of neural tube between brachial and adjacent levels has been performed in chick embryos of two days' incubation (13 to 24 somites). These grafts undergo excellent histological and morphological differentiation and innervate adjacent regions of the host.

The host levels recognized are based upon cellular patterns in the spinal cord rather than skeletal structures. These levels, in terms of the spinal nerve segments which they include are: cervical, 1-12; brachial, 13-17; thoracic, 18-22.

Spinal cord patterns characteristic of each level were determined on the basis of the presence, shape, and relative position of mesial motor columns, lateral motor columns, and the remainder of the mantle. Preganglionic columns of Terni were also studied in one case. In all cases investigated, cell groups characteristic of a given level are found in grafts from that level and always in the same relative positions, no matter to which host level these grafts were transplanted.

Nerves arising from cervical or thoracic spinal cord, while decidedly more slender than brachial nerves, can form a completely normal brachial plexus pattern under the influence of the normal brachial periphery. Variations are considered to be due to disturbances in either graft or periphery resulting from the operation.

Nerves from brachial spinal cord grafted to another level may produce nerve patterns typical for that level (thoracic) without forming a plexus. They do, however, demonstrate a particularly high capacity for growth and frequently make abnormal plexus-like anastomoses.

It is concluded that:

1. As early as they can be identified (by adjacent somites), the cervical, brachial and thoracic levels of the chick spinal cord are determined with respect to recognizable cell types and patterns of cellular arrangement.
2. Plexus patterns as well as patterns of more distal peripheral distribution are imposed upon nerves by surrounding tissues.
3. Wing movements typical of normal chicks of the same age (8 to 9 days' incubation) can be produced by nerves from cervical or thoracic spinal cord.
4. Segmentation of the spinal cord in the chick appears to be dependent upon mesodermal segmentation.

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PLATE I

EXPLANATION OF FIGURES

Photomicrographs of cross sections through grafted spinal cords. Iron hematoxylin. All figures $\times 87$.

- 10 Chick 82, cervical spinal cord in the brachial region, donor segment 7.
- 11 Chick 47, thoracic spinal cord in the brachial region, donor segment 21.
- 12 Chick 72, brachial spinal cord in the cervical region, donor segment 15.
- 13 Chick 123, brachial spinal cord in the thoracic region, donor segment 15.

