AN EXPERIMENTAL ANALYSIS OF RELATIONS BETWEEN PARTS OF THE BRACHIAL SPINAL CORD OF THE EMBRYONIC CHICK[1]

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ELEVEN FIGURES

INTRODUCTION

In all vertebrate classes adequately investigated it has been demonstrated that the periphery exerts an influence on the quantitative development of various cell groups in the embryonic spinal cord and spinal ganglia (Piatt, '48, review). Furthermore, the long longitudinal fiber tracts within the cord have been shown to be without effect (Amphibia: Detwiler, '37; Zacharias, '38. Aves: Bueker, '43; Levi-Montalcini, '45; Hamburger, '46).

The present work was undertaken with a view to further investigating factors involved in determination of the basic patterns of organization in the brachial level of the spinal cord of the embryonic chick. Since the brachial spinal cord can develop normally when isolated in situ from the rest of the central nervous system (Hamburger, '46) it was reasoned that any intracentral factors involved in the differentiation of this level would be localized within the level. Such local intracentral factors which have not been analyzed heretofore are:

First, the role of the short range fibers which constitute the majority of the fibers in the embryonic avian cord (Hamburger, '46). These include the commissural fibers which

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connect one side of the cord with the contralateral side, and the association fibers which connect the cells of the alar plate with those of the basal plate on the ipsilateral side.

Second, the interaction between adjacent parts of the gray matter.

In the present investigation the role of these factors was analyzed by extirpating various regions of the neural tube in the brachial level, followed by study of the spinal cord in later stages. It has been demonstrated that short range fibers have a negligible effect, and that interaction between parts of the gray matter is not a factor in the differentiation of the brachial level of the spinal cord. In addition, the results suggest that by the end of the second day there exists in the neural epithelium of the basal plate a mosaic pattern, with each component being independent of the other, incapable of regulation or regeneration, and capable of producing only a limited number and certain types of cells.

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EXPERIMENTAL PROCEDURE

In general, the operative procedure used was that of Hamburger ('42). Eggs were incubated for approximately 44 hours; the somite number varied from 15 to 25. Operations were performed under a binocular dissecting microscope using a magnification of approximately 80 ×.

The region of the cord involved in the operation was that part adjacent to somites 17 through 21, since it has been determined by other workers (Bueker, '45; Yntema and Hammond, '45) that the first 4 or 5 somites counted at these stages disappeared later, and since our first experiments, performed

in the region adjacent to somites 13 through 17, all showed defects in the cord anterior to the brachial level.

The spinal cord was separated from the somites on the side of operation, then the cord was split in the mid-dorsal line.

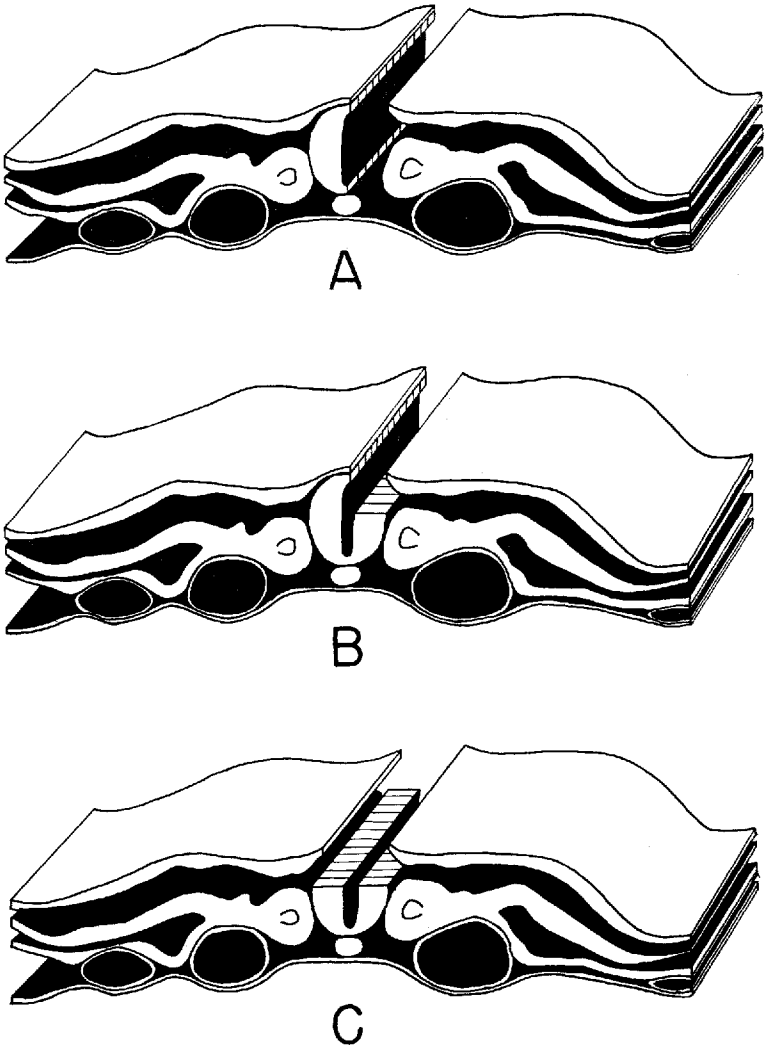


Fig. 1 Diagram illustrating operations performed. A, Removal of the right half of the cord. B, Removal of the right dorsal quarter of the cord. C, Removal of the dorsal half of the cord.

The two halves of the cord separated, so that the dorsal cut surfaces lay over the somites and the floor plate (and notochord) could be seen in the mid-ventral line at a lower level. The desired part was extirplated with a glass needle, and any small pieces adhering to the cut surface were removed with watchmaker forceps. Three types of operations were performed (fig. 1):

a. Removal of the right half of the cord.
b. Removal of the right dorsal quarter of the cord.
c. Removal of the dorsal half of the cord.

The embryos were returned to the incubator for an additional 6 to 10 days (total of 8 to 12 days). They were fixed in Bouin's fixative, run up and embedded, using a modification of the amyl acetate technique (Drury, '41), sectioned serially at 10 μ and stained with Heidenhain's iron hematoxylin.

At fixation a large number of embryos were found with spina bifida in the region of operation (see table 1). This was probably due to the fact that the dorsal parts of the cord separated readily at operation and remained relatively far apart. In later operations the occurrence of spina bifida was lessened to a considerable degree by the removal of excess saline from the surface of the embryo immediately after operation (using a Spemann pipette). This allowed the two sides of

TABLE 1

Data on operations

AGE AT FIX.	8 DAYS		9 DAYS		10 DAYS		11 DAYS		12 DAYS		TOTAL	
Type of oper.	Sp bif	Healed	Sp bif	Healed	Sp bif	Healed	Sp bif	Healed	Sp bif	Healed	Sp bif	Healed
Dorsal quarter	28	11	1	..	1	4	2	2	2	1	34	18
Right half	3	4	..	..	1	1	..	1	..	..	4	6
Dorsal half	..	1	1	..	..	3	..	2	..	..	1	6
Total	31	16	2	..	2	8	2	5	2	1	39	30

the cord to remain in a more nearly vertical position and in closer contact. No cases of spina bifida were included in the present study. Sixty-nine chicks survived beyond the 8th day. Of these, 30 showed no gross malformations; 14 of this latter group were chosen for a detailed analysis.

Graphic reconstructions of spinal cord, ganglia and plexus were made for all embryos, using every third section (Hamburger, '39). The borders between segments were determined by using the point halfway between the exit from the spinal cord of the last motor rootlets of one nerve, and the exit of the first motor rootlets of the next one. The lateral motor cells in every third section were counted with a Veeder counter, and at the same time drawn with the aid of a camera lucida. Wherever possible, the total number of cells was determined for each segment as well as for the brachial level (segments 14 to 16).

Area measurements were made in one case of lateral half removal and in one control in order to compare the relative amounts of gray and white matter. Projection drawings of every section of the segments involved were divided into right and left halves and the respective areas of gray and white matter determined on each side. To divide the cord the following procedure was necessary because of its asymmetry. The edge of the floor plate lining the neurocoel and the ventral edge of the anterior commissure were bisected and these two points connected by a line. A similar procedure was followed with respect to the roof plate and the dorsalmost edge of the roof plate fibers. The total area and the area of the gray matter were determined using a polar planimeter, and the relative values obtained for each section. The area of the white matter was determined by subtraction. All individual values were totaled for the entire segment.

EXTENT OF DEFECTS

The spinal cords of all embryos showed defects, the anterior-posterior extent of which could be correlated with the length of the piece of neural tube removed at the time of operation

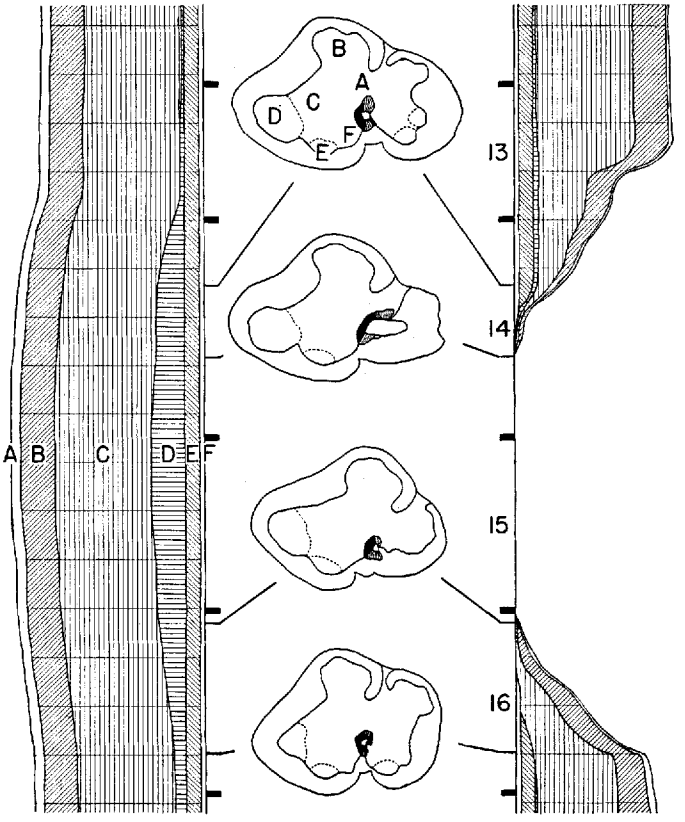


Fig. 2 Graphic representation of the spinal cord of chick 476 (lateral half removal). The left side of the drawing represents the left side of the spinal cord. The spinal cord is represented as having been split and laid out flat so that the dorsal edge of the cord is lateral in the diagram. The heavy black lines are segment borders; the numbers of the individual segments are indicated. The following regions, starting from the dorsal side, are, in order:

A Cells between the sensory horn and the roof plate fibers.
B Sensory cells.
C Cells of the intermediate region (internuncial cells).
D Lateral motor cells.
E Mesial motor cells.
F Cells between the mesial motor column and the floor plate.

These regions are represented in the same manner on the right side. The curves were constructed from values obtained by comparing the right and left sides of every third section, the right side being noted as a certain proportion of the left (considered as normal).

The outline drawings are accurate projection drawings of representative cross-sections, showing the relation between the gray matter and the ependyma (black). The roof and floor plates are lined. Guide lines connect the individual sections to their position on the graph.

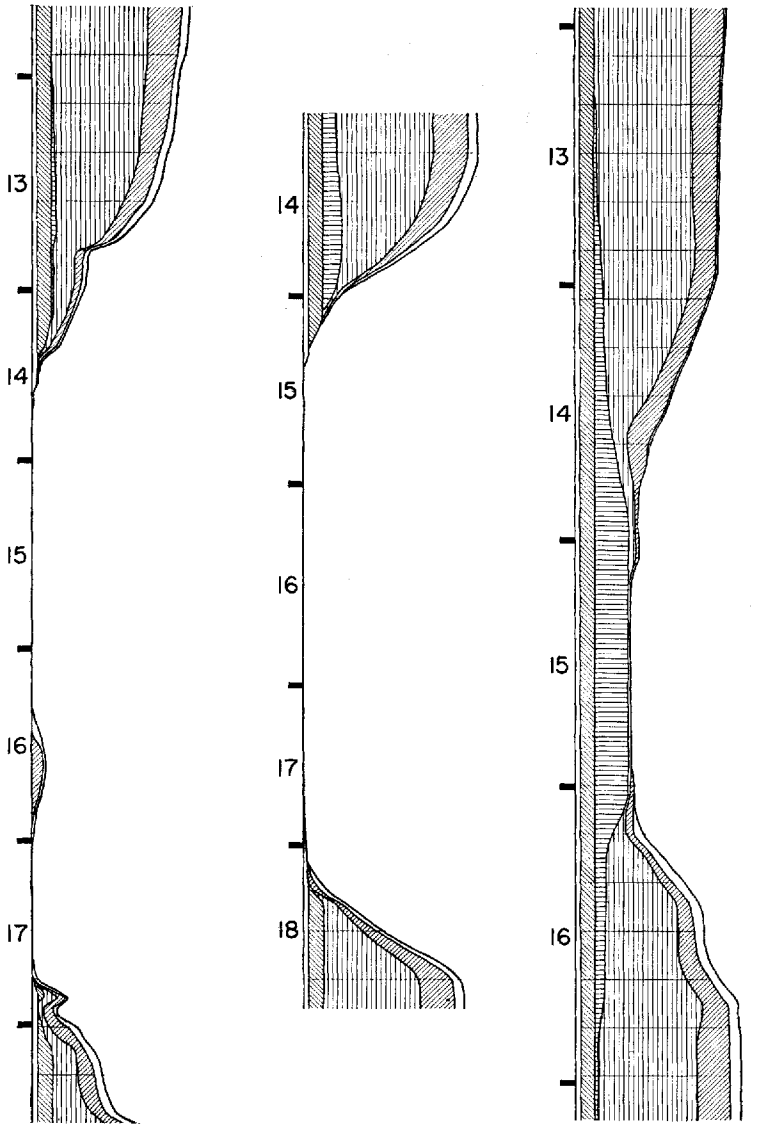


Fig. 3 Graphic representation of the operated sides of the spinal cord of chicks:
566 (lateral half removal), left
324 (lateral half removal), center
384 (dorsal quarter removal) right
Regions represented as in figure 2.

(6 to 9 days previously). (See table 2.) The dorsoventral extent of the defect also showed a satisfactory correlation with the amount of material removed at the time of operation. Normal cross sections were found in regions where there had been no injury to the spinal cord, i.e., anterior and posterior to the operation. Lateral half removal resulted in spinal cords

TABLE 2

Summary of operations

NUMBER OF CHICK	TOTAL SOMITES	LEVEL OF OPER. SOMITES	AGE AT FIX. DAYS	PLEXUS NERVES		DEFECT FOUND NERVE SEGMENTS
				Right	Left	
Removal of the right half of the neural tube						
324	21	17–21	8	13–15	13–16	14–18
342b	17	17–20	8	13–15	13–17	14–17
476	20	17–20	10	12–16 [1]	12–17	13–16
566	24	16–21	10	13–14	13–17	13–18
Removal of the right dorsal quarter of the neural tube						
233	16	16⅓ seg. pl.	8	13–17	13–17	12–16
233a	15	16 part seg. pl.	8	13–16	13–16	12–16
394	17	17–19	10	13–17	13–17	13–17
514	22	17–21	10	13–17	13–17	12–17
531	22	17–21	10	13–16	13–16	12–18
384	18	17–19½	11	13–17	13–17	12–16
Removal of the dorsal half of the neural tube						
502	16	16½ seg. pl.	8	12–17	12–17	14–18
465	21	17–21	10	13–16 [2]	12–16	12–17
479	21	17–21	11	12–16	12–16	14–17
491	22	17–21	11	12–17	12–17	12–17

[1] Supernumerary nerve and ganglion anterior to segment 12.

[2] Supernumerary nerve anterior to segment 13.

devoid of that half (fig. 4); dorsal quarter removal resulted in a cord devoid of that quarter (figs. 5 and 6); dorsal half removal resulted in a cord devoid of that half (fig. 7). It should be pointed out, however, that the nature of the operation was such that it was impossible to remove exactly the desired amount throughout the brachial level. This was noted particularly in the right dorsal quarter and dorsal half removals and at the ends of the operated regions. A more exact

idea of the nature and amount of this variation can be obtained by reference to the graphic representations of several of the cases (figs. 2 and 3).

RESULTS

A. Differentation of the lateral motor column

A quantitative study was made of the lateral motor column in 10 operated cases; of these 4 were cases of lateral half removal, 5 of dorsal quarter removal, and one of dorsal half removal. (See table 3 for results.)

Removal of the lateral half. Data for 9 segments are available. This includes the number of lateral motor cells in the brachial level (segments 14–16) of two chicks. The total counts for the brachial level, as well as counts for individual segments agree satisfactorily with control counts and with the previously collected data of Hamburger ('34, '46, unpublished). The conclusion is reached that a numerically normal lateral motor column can form in the absence of the contralateral side of the cord.

Removal of the right dorsal quarter. Data on the number of lateral motor cells in 9 segments are available, on the operated side as well as on the unoperated side. Chicks 394 and 531 lack less than the right dorsal quarter; the counts of these chicks are considered to be within the normal range. One of the clearest cases of dorsal quarter removal is segment 15 of chick 384 (fig. 3, right and fig. 6); the number of lateral motor cells on the two sides clearly checks within less than 10%. In the middle of segment 14 of chick 233 (fig. 5) the dorsal quarter of the cord is lacking; counts using only this portion of the segment check well with counts of a similar portion of the same segment on the contralateral side. In chick 514 more than the dorsal quarter of the cord is lacking; counts on the operated side are lower than those on the unoperated side. The conclusion is reached that the normal number of lateral motor cells is found, provided no more than the dorsal quarter of the cord is lacking; and further, that the absence of more

TABLE 3

Lateral motor cell counts

TYPE OF OPERATION	NO.	AGE	SEGMENT 14		DIFFERENCE % of left	SEGMENT 15		DIFFERENCE % of left	SEGMENT 16		DIFFERENCE % of left	TOTAL		DIFFERENCE % of left
			Left	Right		Left	Right		Left	Right		Left	Right	
		days												
Control	8n 11 [1]	8	1739	1746	+ 0.4	1881	1876	— 0.3	1481	1623	+ 9.7	5101	5245	+ 2.8
	10dn	10	1286			1852			954			4092		
Right half removal	476	11	1374	....		1310	....		662	....		3346	....	
	324	8	....	....		2020	....		1346	....		....	....	
	566	10	1122	....		1518	....		1324	....		3964	....	
	342b	8	....	....		....	....		1522	....		....	....	
Right quarter removal	394	10	....	....		1374	1294	— 5.8	....	....		....	....	
	233	8	1020 [2]	1010 [2]	— 0.9	....	....		....	....		....	....	
	531	10	1337	1176	— 12.8	1402	1387	— 1.0	868	943	+ 8.6	3607	3506	— 2.8
	514	10	1402	579	— 58.7	1429	1106	— 22.6	1191	394	— 66.9	4022	1943	— 48.3
	384	11	....	....		1540	1660	+ 7.8	....	....		....	....	
Dorsal half removal	491		....	....		....	....		....	....		1481	2521	

[1] 8 n 11, same chick used by Hamburger, '48.
[2] Counts made on 10 out of a total of 21 sections.

TABLE 4

Area measurements

NO.	TYPE OF OPERATION	AGE	TOTAL AREA		DIFFERENCE % of left	GRAY MATTER		DIFFERENCE % of left	WHITE MATTER		DIFFERENCE % of left
			Left	Right		Left	Right		Left	Right	
		days									
8n 11	Control (segments 15 and 16)	8	421.14	440.08	+ 4.5	246.67	260.16	+ 5.5	174.47	180.02	+ 3.2
324	Right half removal (segments 15 and 16)	8	401.04	41.58	— 89.6 — 4.9 [1]	257.61	16.72	— 93.5 + 4.4 [1]	143.49	24.86	— 82.7 — 17.8 [1]

[1] Left side of 324 compared to left side of 8n 11.

than the dorsal quarter (i.e., the absence of part of the ventral quarter) is associated with a decrease in the number of lateral motor cells.

Removal of the dorsal half. Counts are available for the brachial level as a whole (segment borders could not be determined) of chick 491. As can be seen in table 3, the total number of lateral motor cells for the three segments is less than normal. Throughout the brachial level there are regions where more than the dorsal half is missing; these regions are correlated with the greatest reduction in the number of lateral motor cells. Thus, the only conclusion derived from this case is that lateral motor cells can be produced in the absence of the dorsal half (actually more than the dorsal half) of the cord; little can be concluded regarding the number of lateral motor cells produced in the absence of only the dorsal half.

B. Intermediate and sensory regions

Area measurements were made of segments 15 and 16 of one case of lateral half removal (chick 324; fig. 3, center) to determine the relation between the total area of the gray matter and the white matter of the unoperated side and the parts remaining on the operated side. Comparable measurements were made of each side of the cord in a control chick (8n 11). (See table 4.)

The control chick showed no significant difference between the two sides. Despite some distortion in shape of the unoperated side of chick 324 (fig. 4) there was less than 5% difference in the area of the gray matter between the latter and the left side of the control chick, but 17.8% difference in the area of the white matter. It follows that a relatively small part of the total amount of the white matter consists of commissural fibers and over 80% of ipsilateral association fibers. In some portions of the operated side, small amounts of gray matter were found, associated with relatively large amounts of white matter. The increased proportion of white matter to gray matter (table 4) is probably due to the presence of commissural fibers

derived from the contralateral unoperated side. Since some of the white matter on the operated side extended for short distances as fairly large trunks in the mesenchyme, and since these were not included in the measurements, it is difficult to estimate exactly how much of the white matter is derived from the contralateral side of the cord.

These area measurements indicate that the normal amount of gray matter remained on the unoperated side in the case of lateral half removal. The mesial motor column was determined by inspection to be normal when compared with that of 8n 11. Since it has been shown (previous section) that the lateral motor cells occur in normal numbers, it follows that the internuncial and sensory cells must also have been formed in normal amounts. No further data of this type were collected. It was considered that this one case provided sufficient evidence to warrant the conclusion that the sensory and internuncial cells, as well as the motor cells, could develop normally in the absence of the contralateral side of the cord (and in the absence of 17.8% of the white matter of the ipsilateral side).

C. Spinal ganglia and sensory horns

No definite attempt was made to remove all of the neural crest at the time of operation; the complete removal of the crest without injury to the spinal cord was found to be impossible. We have found a great variation in the size of the ganglia, from the presence of a single ganglion cell to ganglia apparently normal in size. This variation probably reflects, at least in part, the variation in the amount of crest removed at operation. In all cases, removal of the lateral half of the spinal cord resulted in absence of the spinal ganglia on that side.

We have found that whenever any of the alar plate is present, a sensory horn is present. The size of the sensory horn can in some cases be correlated with the size of the spinal ganglion, but the absence of the spinal ganglion it not accompanied by a concomitant absence of the sensory horn. The observa-

tion of a correlation between the size of the spinal ganglia and the size of the sensory horn is not new; it merely confirms the observations of Shorey ('09) and others.

D. Plexus

A study of reconstructions of the operated cases revealed that, in most cases, the arrangement of the nerves to form a plexus is much the same on the operated as on the unoperated side (table 2). The fact that only small ganglia are present does not hamper the ability of the nerves to form a plexus. The individual nerves may be smaller (the decrease in diameter of the nerves being correlated with the decrease in the size of the ganglia), but these small nerves form a normal plexus pattern. The absence of the lateral half of the cord results in the absence of lateral motor cells and consequently of the motor portion of the spinal nerves. The presence of part of the spinal ganglia is sufficient to allow the formation of a nerve which is capable of joining with other brachial nerves to form a plexus. The absence of one or two of the brachial nerves does not prevent the remaining nerves from following the normal pattern for that part of the plexus. It is evident that with regard to plexus formation, the various components (sensory, motor or single brachial nerves) are independent of one another.

E. Sympathetics

Sympathetic ganglia and chains have been noted in all embryos, even in the absence of the lateral half of the cord and of the spinal ganglia on that side. This does not, however, provide any significant evidence concerning the origin of the cells of the sympathetic ganglia and will, therefore, not be discussed further.

F. Feathers

The feathers in the brachial level are, in most cases, deficient in number. Since the removal of ectoderm in the course of the operation results in defects in the feather tracts of the brachial

region, it seems reasonable to conclude either that the skin is determined as regards the production of feathers at least 5 to 6 days prior to the appearance of the definitive feather germs, or that regenerated ectoderm (the operated region of all cases considered in this study was covered with ectoderm) does not possess the capacity for producing feather germs. Saunders ('48, p. 384) notes that removal of a portion of the ectoderm of the early wing bud of the chick may result in the absence of feather tracts.

DISCUSSION

A. Absence of regeneration in the spinal cord

It has been shown that extirpation of various regions of the central nervous system of Ambystoma before the closure of the neural folds can result in a normal spinal cord or brain (Burr, '16; Detwiler, '44, '46; and Harrison, '47 for parts of the brain; Detwiler, '47 for the brachial region of the spinal cord). This reconstitution is accomplished by a higher proliferative rate of neural epithelium cells derived from the unoperated side. On the other hand, there is evidence that the dorsal giant cells of Ambystoma (Rohon-Beard cells) are determined by stage 15 or earlier (Hutchinson, '36; DuShane, '38).

Extirpation of parts of the anuran cord does not necessarily result in the reconstitution of a normal cord. Harrison ('24) demonstrated in Rana esculenta that the absence of the dorsal part of the neural tube the Rohon-Beard cells were absent while the ventral part of the cord and ventral roots were found, and further that the motor cells could be eliminated while part of the sensory cells were left intact. Hamburger ('29) reported in Bombinator pachypus and Rana fusca that extirpation of the lateral half of the newly closed medullary tube (from somites 5 or 6 through 12 or 13) resulted in the regeneration of the spinal cord in 70% of the cases by the time the animals were old larvae or had metamorphosed, but the motor region was not completely reconstituted. (See his fig. 7.)

Our results agree with those on the Anurans; in no case was any operated cord reconstituted. On the contrary, successful removal of the right lateral half of the brachial neural tube in a 15- to 25-somite embryo resulted in a cord devoid of that half (476, 14 and 15; 324, 15, 16 and 17; figs. 2, 3, and 4) and removal of the right dorsal quarter resulted in a cord devoid of that particular region (384, 15; 233, 14; figs. 5 and 6).

Our results also agree with those of other workers on the chick. Lillie ('04) found no regeneration of the neural tube after cauterization of the posterior end of 29-somite embryos. Levi-Montalcini ('45) found no regeneration of a 3- to 4-somite piece of neural tube removed in the 12- to 18-somite stage. Yntema and Hammond ('45, fig. 13) and Hammond and Yntema ('47) extirpated the cervical neural crest and part of the adjacent neural tube in 15- to 23-somite embryos and noted later that the spinal cord ended abruptly in the region of extirpation, but that the remainder of the cord appeared to be normal.

One might suggest that the main difference between reconstitution in the chick and in Ambystoma is due to the fact that, in the latter, the cord healed, i.e., the unoperated side fused with the part remaining on the operated side so that the neurocoel was closed and completely lined by neural epithelium. That this is not a crucial factor is demonstrated by the fact that despite this type of healing in the anuran cord, defects resulted. In the chick Yntema and Hammond ('45) found some cases of healed cords with many extravasated cells, but no reconstitution of the extirpated portion. Further, in some of our cases (465, 479, 502, dorsal half removal; 514, 233, dorsal quarter removal), the cut surfaces of the two sides fused together so completely that it was impossible to detect the exact line of fusion; in these cases, too, defects in the gray matter are readily discernible. One would have expected that reconstitution, if it were to occur, would have taken place by 8 to 11 days (since the period of active cell proliferation in the avian cord, by that time, has already come to and end, Hamburger, '48). It is clear, that under the particular conditions imposed

by our type of operation (age, technique, etc.) the lateral half of the spinal cord cannot be reconstituted by the remaining half; neither can the dorsal quarter be reconstituted by the remaining three-quarters, nor can the dorsal half be reconstituted by the ventral half.

B. Relative self-differentiation of the lateral motor column

Hamburger ('34, '46, and unpublished data) considers that the range in the number of lateral motor cells found in the brachial level (segments 14 to 16) normally varies from 3100 to 4800. It is considered that by 7 days the entire complement of lateral motor cells has been formed and that the number does not change appreciably in later stages (Bucker, '47; Hamburger, '48). Since our total counts on the brachial level of various operated chicks (table 3) have been within these limits, the lateral motor columns are considered to be normal. In embryos in which cell counts were not obtained for the entire brachial level, for various reasons, the counts compared favorably with those of comparable segments or parts of a segment in chicks known to have normal total counts (segments 14 to 16).

After removal of the lateral half of the spinal cord the contralateral side was found to contain the normal number of lateral motor cells. It was, therefore, concluded that the development of the lateral motor column of one side was independent of the presence of the other side. This agrees with, and in fact goes beyond, the finding of Hamburger ('46) that the number of cells found on the left side of specimens which had undergone changes in the periphery on the right side (either wing extirpation or transplantation of a supernumerary wing) compares favorably with counts of normal unoperated embryos. He considered, therefore, that it was possible to use as a control the unoperated side of such animals, since the two sides obviously reacted independently. On this basis, we have considered it justifiable to use as a control the unop-

erated side of animals in which one side of the cord had been modified. A further justification for this procedure comes from the observation that the numerical variation between the right and left sides of one embryo is lower than the variation between the same sides of different embryos of comparable incubation age.

It has been demonstrated that the long-range fiber tracts are without effect on the proliferation and differentiation of the lateral motor cells (Bueker, '43; Levi-Montalcini, '45; Hamburger, '46). Since the same investigations permit the conclusion that the white matter of 7- to 8-day chicks is composed primarily of fibers of the short range type, it becomes of interest to determine whether these fibers are a factor in the normal differentiation of the lateral motor cells.

Short range fibers are of two types: (1) commissural fibers, whose cell bodies are located on one side of the cord, while they make synaptic connections with cells on the contralateral side of the cord, and (2) association fibers, whose cell bodies and synaptic connections are both on the same side of the cord.

Both of these cell types originate early, at 3 days or earlier, and are apparently indistinguishable from one another at that time, either by place of origin, stainability or morphological characteristics (Levi-Montalcini, personal communication). Furthermore, since these cells may undergo considerable migration during development, one has to be cautious in evaluating the relative amounts removed by any one type of operation. An added difficulty is the impossibility of distinguishing between the two types of cells at the time of analysis (at least with the staining methods used).

Extirpation of the lateral half of the cord, of course, would remove all the various types of cells normally found in that particular level, including all commissural and association cells. Since the remaining side of the cord would be lacking synaptic connections with the commissural cells of the missing contralateral side, it would be legitimate to draw conclusions concerning the role of these cells in its development.

In several cases of removal of the lateral half of the cord the unoperated side was shown to contain the normal number of lateral motor cells (table 3). Thus, the absence of the commissural fibers and of their synaptic connections with the lateral motor column is shown to be without effect on the differentiation, qualitatively as well as quantitatively, of the lateral motor column (through 11 days).

Extirpation of the right dorsal quarter of the cord would probably have removed at least part of both types of cells. Since a normal lateral motor column can be formed in the complete absence of the commissural cells, and since probably not all of the commissural cells were removed by this type of operation, no further information can be gained concerning the role of these cells in development. We can, however, get some information about the role of the association cells, since in successful operations the alar plate has been removed with consequent interruption of the synaptic connections of these cells.

Since the lateral motor cell counts of chicks 233 and 384 (table 3) are normal, it can be concluded that the role of these association cells is also negligible.

Removal of the dorsal half of the cord resulted in lateral motor columns devoid of synaptic connections with association cells, and, in addition, with at least some of the commissural cells.

Whether the complete removal of both types of cells would interfere with the development of the remaining parts of the cord could be investigated by the removal of a lateral half of the cord and, in addition, removal of the dorsal quarter of the contralateral side. Several operations of this type were performed, but the chicks did not survive sufficiently long to permit analysis. However, this situation was realized in short regions of cases of dorsal half removal, e.g., chick 502, segment 15, where almost the entire left side was missing in addition to the dorsal quarter of the right side. In view of the fact that the lateral motor cells on the right side appeared to be normal, it seems reasonable to assume that the development of

the lateral motor cells is independent of synaptic connections with both the commissural and the association neurons.

C. Relative self-differentiation of non-motor regions of the mantle

Accurate quantitative data on cell counts can be obtained only where the cells are relatively large, few in number and readily distinguishable from other cell types. Such is the case with the lateral motor cells. It was decided that some idea of the normality of other regions of the cord could be obtained by comparing area measurements of operated and normal spinal cords.

As was shown above, the relative volume of the gray matter on the unoperated side of chick 324 (lateral half removal) compares favorably with that of the normal chick of comparable age (table 4). This, in itself, might have very little significance since the overall results might obscure changes in the relative numbers of motor cells and internuncial cells. However, since the counts of the lateral motor cells in these two chicks (table 3) are well within the range of variability for chicks of this age, and since (by inspection) no differences could be detected in the mesial motor columns, the conclusion appears justified, that there is no detectable quantitative difference between the gray matter in a lateral half of a complete spinal cord and a lateral half of a spinal cord in which the contralateral side had been extirpated.

However, a slight difference was noted in the amount of white matter present. Hamburger ('46) has shown that in a 7- to 8-day chick spinal cord isolated from ascending and descending fiber tracts, no decrease in the amount of white matter was detectable (by area measurements similar to ours). He concludes that the white matter must be derived from local areas of the gray matter (see his discussion for references to the literature). Since the long fiber tracts do not account for a detectable amount of white matter, the small difference in the ratios of white matter to gray matter (71% for the control,

60% for chick 324) must be due to local differences. Such local differences might be the lack of commissural fibers whose origin would have been in the extirpated right half of the cord. The major portion of the white matter (somewhere in the neighborhood of 82.2% as shown above) is derived from the gray matter on the ipsilateral side of the cord.

It can be concluded, therefore, that the role of the short range fibers, at least through 8 to 11 days of incubation is unimportant in the development of the basic patterns of organization of the chick spinal cord.

It remains to be seen whether the integrity of the individual neurons lacking synaptic connections can be maintained beyond this age. Levi-Montalcini ('49) found, after removal of the otocyst and associated ganglia, that the cells of the secondary centers of the brain were not able to complete their differentiation or maintain themselves. She suggested a quantitative correlation between the degree of effect and the number of synaptic connections which the cells established. No measurements of cell or nuclear size were made in our cases. The possibility of cellular atrophy under the conditions of the experiment, particularly in older embryos, remains to be investigated.

D. Relation between various parts of the mantle

The following observations, with representative examples, demonstrate that the presence of any one region of the mantle (fig. 2, a–f) does not depend on the concomitant presence of any other region, nor does the absence of any of these regions of the mantle depend on the concomitant absence of any other region. Chick numbers are followed by segment numbers.

1. The mantle between the mesial motor column and the floor plate (fig. 2, f) may be present in the absence of all remaining parts of the mantle of the ipsilateral side (324, 15; fig. 8).

2. The mesial motor column and the mantle between it and the floor plate (fig. 2, e and f) may be present in the absence of the remainder of the mantle of the ipsilateral side (324, 15).

3. The mesial and lateral motor columns (fig. 2, d and e) may be lacking in the presence of the remainder of the mantle (476, 14 and 16; fig. 9).

4. The lateral motor column (fig. 2, d) may be absent in the presence of the remainder of the mantle on the ipsilateral side (476, 16; fig. 10).

5. The internuncial cells in the mid-region and the lateral motor cells (fig. 2, c and d) may be lacking in the presence of the remainder of the mantle (566, 17; fig. 11).

6. Sensory cells and the cells between them and the roof plate fibers (fig. 2, a and b) may be present in the absence of the remainder of the mantle (476, 16).

7. Only some of the cells found between the sensory horn and the roof plate fibers (fig. 2, a) may be lacking (476, 13).

8. Only the cells found between the sensory horn and the roof plate fibers (fig. 2, a) may be present in the absence of the remainder of the mantle on the ipsilateral side (476, 14; 342b, 16).

9. Absence of the roof plate (502, 14).

On the basis of these data it seems reasonable to conclude that interactions between adjacent parts of the mantle are unimportant in the development of the various cell types characteristic of the embryonic chick spinal cord (at least of 8 to 11 days of incubation).

E. Local autonomy of sectors of the neural epithelium

Since it has been shown that the basic patterns of organization of the gray matter of the chick spinal cord are not dependent on the presence of long range fibers or of short range fibers, it becomes obvious that there must be some pre-organization of these patterns in the neural tube at the time of operation. Further, it has been shown that the various regions of the mantle can develop independently of other regions of the mantle. This suggests that, at the time of operation, there are already localized differences in the potentialities of adjacent sectors of the neural tube.

All of the nervous elements of the cord are derived from the original neural epithelium constituting the wall of the neural tube at the time of operation. At this time the first motor and association neuroblasts are differentiating at the lateral margins of the tube; Levi-Montalcini (personal communication) dates their first appearance at 38 hours of incubation; Tello ('23) at 46 to 48 hours of incubation. Hamburger ('48) described the ventro-dorsal differentiation of the ependyma as the mitotic activity of the neural epithelium declines, and as the various cell types differentiate in the mantle. The ependyma, then (by 8 to 11 days of incubation) is apparently a differentiated remnant of the original lining of the neurocoel.

Differences between the alar and basal plates have been noted in the past. Hamburger ('48) has shown that the mitotic patterns of the alar and basal plates differ very markedly from one another ($2\frac{1}{2}$ to $8\frac{1}{2}$ days of incubation). He has suggested the possibility that the two regions are independent in this respect. Moog's work ('43) on the localization of phosphatase in the embryonic spinal cord of the chick has indicated a difference in the content of alkaline phosphatase in these two regions from the 4th to the 8th day. Our work confirms and extends that of these two investigators, demonstrating experimentally that this relative independence of the alar and basal plates extends to small units of the neural epithelium and to earlier stages (15 to 25 somites).

Two cases (chicks 233 and 514) were noted in which the removal of the dorsal quarter of the neural tube resulted in a decreased number of lateral motor cells on the side of operation (table 3). In seeking an explanation for these results it was discovered that there was a correlation between the amount of ependyma present and the size of the lateral motor column. The lining of the neurocoel adjacent to the deficient motor column was found to be partially or completely lacking. A further study of local areas in which not exactly one-quarter or one-half of the spinal cord was absent, which condition oc-

curs in some parts of all embryos, leads to the following generalizations:

1. That whenever a certain region of the mantle is lacking, the ependyma medial to that part is likewise lacking, and further,

2. That whenever a certain region of the mantle is differentiated, the ependyma medial to that part of the mantle is present, but no other part of the ependyma need be present.

The following data substantiate these generalizations for localized areas. Chick numbers are followed by segment numbers. An example of this type of data for chick 476 is illustrated in figure 2.

Basal plate. The appearance of a small amount of the ventralmost ependyma (immediately adjacent to the floor plate) is associated with the presence of the cells (fig. 2, f) which occupy a position between the mesial motor column and the floor plate (fig. 8; 324, 15 and 17; 476, 16; 566, 14 and 17; 502, 15; 465, 15 and 16; 233a, 14). When these cells are not found, the ependyma in this region is absent (476, 14 and 15; 324, 16; 566, 14, 15 and 16; 233a, 13). On the other hand, the presence of this particular ependymal area alone (in the absence of the adjacent dorsal area) is not sufficient for the formation of mesial motor or lateral motor neurons (476, 16; 324, 16 and 17; 566, 17). It is therefore concluded that the ventralmost ependyma, immediately lateral to the floor plate, is derived from neural epithelium determined to give rise to these particular cells of the mantle (f, fig. 2), and that this part of the ependyma can supply no other cells. A slight increase in the dorsal extent of the ependyma is found to be associated with the presence of the mesial motor column (476, 16; 324, 15; 566, 17; 233a, 14). If the ependyma extends still further dorsad, the lateral motor column is found (324, 15; 566, 17; 465, 14). In one instance, the absence of this column was associated with the absence of a small, well-circumscribed area of the ependyma (figs. 2 and 10; 476, 16). The mid-region of the ependyma, namely the region located in the dorsal level of the basal plate, seems to be necessary for the formation of the

large number of internuncial cells found in the intermediate region (c, fig. 2) of the mantle. When this part of the ependyma is lacking, most of the internuncial cells are absent (324, 17; 566, 14 and 17; 514, 16; 233, 14; 384, 15; 465, 13; decreased in 394, 15). It is clear that in the basal plate, the various parts of the neural epithelium give rise to specifically different cell types, and that they do so independently of each other.

Alar plate. The absence of the dorsal quarter of the ependyma is associated with the absence of the mantle in the alar plate (fig. 5; 233, 14 and fig. 6; 384, 15). Analysis of the reactions of small sectors of the neural epithelium in this region is complicated by two sources of difficulty.

First, by 8 days the central canal is already reduced in its dorsoventral extent, and subsequent to this time the reduction continues at a more rapid rate (Hamburger, '48). This reduction involves a lengthening of the roof plate and the disappearance of progressively more of the alar plate ependyma from its position lining the neurocoel, while the floor plate and immediately adjacent regions of the mantle remain relatively unaffected. Small, darkly staining cells (probably alar plate ependymal cells) can be seen lateral to the dorsal median septum in older embryos, but it is impossible to make any meaningful correlations between them and the cells of the mantle. One finds, on the other hand, in younger embryos (6 days) in which the neurocoel has just begun to shorten, that the sensory horns are not yet clearly defined (Hamburger, '48). Our observations were made on embryos intermediate between these extremes.

Second, it has been established by limb extirpation experiments (Hamburger and others) that the quantitative development of the sensory horn is controlled by the size of the spinal ganglia. Even the internuncial cells may be affected, though only in cases of radical leg extirpation and over relatively long periods of time (Bucker, '47). Bucker found a 12% decrease in their area and an 8% decrease in their number three months post hatching, although he had noted that the two sides of the cord were approximately symmetrical in similar cases fixed at

9 to 11 days of incubation. In the case of the sheep, Barron ('45) found hypoplasia of the sensory horn and of the internuncial cells following removal of the distal forelimb segment in utero after the termination of mitotic activity in the spinal cord. In our cases the neural crest was often injured at the time of operation, producing direct effects on the quantitative development of the spinal ganglia, with presumably indirect effects on the quantitative development of the sensory horn. Since in none of the cases cited above was the general pattern (i.e., presence of particular cell groups) of the cord affected, it is reasonable to assume that changes in this pattern observed in our experiments must be due to other factors.

Our cases of reduced or lacking spinal ganglia (p. 63) confirm the above-mentioned observations that the size of the sensory horn is influenced by the size of the spinal ganglia. Under certain circumstances, however, the size of the sensory horn can vary independently of the ganglia. The sensory horn can be lacking in the presence of a spinal ganglion (233, 14; 465, 14 and 15; 502, 15 and 16) in cases where the alar plate ependyma is lacking. The sensory horn can be present in the absence of a spinal ganglion (479, 15, the sensory horn on the left side is smaller than the one on the right side, although the ganglia are absent on both sides). The size of the sensory horn can vary within one segment (476, disappearance of horn in 14, reappearance in 16, ganglion 14 normal, ganglion 16 very much reduced; 233, disappearance of sensory horn in 14 and reappearance in 15, ganglia present in both segments though somewhat reduced). It thus becomes evident that some factor, other than the presence, absence or size of the spinal ganglia, must play a role in the development of the sensory horn. Such a factor is probably the amount of neural epithelium which remained in the alar plate after the operation.

The question then arises as to whether the formation of the sensory horn is localized in a dorsal portion of the alar plate neural epithelium, or whether the entire neural epithelium of the alar plate is capable of producing sensory cells. There is some indication that the second alternative is the correct one.

In one case of an embryo with a large neurocoel (chick 233, 14) a small sensory horn is present in the absence of the dorsal portion of the alar plate ependyma. Furthermore, the sensory horn is found in all sections in which part of the alar plate ependyma is found. When this alar plate ependyma disappears completely, the sensory horn also disappears. The decrease in the size of the latter is always accompanied by a decrease in the number of internuncial cells (324, 14; 233, 13 and 14; 233a, 13; 502, 14; 384, 14 and 16; 476, 13). We did not find a single instance in which either the sensory horn or the dorsal internuncial cells were present in the absence of the other. This would suggest that there are no separate regions in the neural epithelium responsible either for sensory horn formation alone, or for internuncial cell formation alone. Rather it would seem that any part of the neural epithelium of the alar plate can produce both components. Our material does not give conclusive evidence for this assumption either because of healing of the cut surfaces of the cord or of decrease in the extent of the neurocoel which does not permit an exact identification of the missing part of the ependyma. While one can state that only a small portion of the alar plate neural epithelium is sufficient for the formation of a sensory horn, one cannot, on the basis of the evidence at hand, critically evaluate either the specific capacities of any particular sector of the alar plate neural epithelium or the quantitative relation between the neural epithelium and the size of the horn.

As indicated above, correlations have been found between defects in the mantle and defects in the ependyma in the basal plate. As can be seen from the data, the evidence strongly suggests that the basal plate of the chick spinal cord (at the time of operation) constitutes a fairly rigid mosaic, with each part giving rise to a certain type or types of cells and presumably in a certain amount.

There is evidence for the existence of the following independent neural epithelial units:

1. Ventralmost basal plate which gives rise to the cells between the mesial motor column and the floor plate.

2. Ventro-lateral basal plate which gives rise to the mesial motor column.

3. Lateral basal plate which gives rise to the lateral motor column.

4. Dorsal basal plate which gives rise to internuncial cells. (Regions 2 and 3 probably also produce internuncial cells found medial to the motor columns.)

5. Alar plate which gives rise to the sensory horn and internuncial cells of the alar plate.

6. The roof (and probably also the floor) plate.

As stated previously, the evidence for the determination of small parts of the neural epithelium is derived primarily from the anterior and posterior ends of the operated regions where the level of the cut obviously varied from what had been planned. Further operations are in progress, which, it is hoped, will lead to a more clear-cut demonstration of the autonomy of local regions of the basal plate, and a more direct correlation of these regions with specifically delimited regions of the ependyma. It is also hoped to clarify the reactions of the alar plate and to extend these observations to other regions of the cord.

SUMMARY AND CONCLUSIONS

In order to study the local intracentral factors involved in the development of the normal patterns of organization in the spinal cord of the embryonic chick, lateral halves, dorsal quarters, and dorsal halves of the neural tube were removed in the brachial level of 15- to 25-somite embryos. A detailed study was made of 14 operated cases ranging in age from 8 to 11 days of incubation.

It is concluded that:

1. Short range fibers play a negligible role in the development of the normal pattern of the mantle.

The normal number of lateral motor cells can develop in the absence of the lateral half of the cord, the dorsal quarter of the cord, and probably also in the absence of the dorsal half of the cord.

The lateral motor column can develop normally in the absence of synaptic connections with commissural cells and in the absence of at least some of the association cells.

The entire mantle (as determined by area measurements) can develop normally in the absence of the contralateral side of the cord. The entire mantle can develop, therefore, in the absence of synaptic connections with commissural cells.

2. Interactions between adjacent parts of the mantle are of negligible importance in the development of their normal patterns.

3. It is suggested that the neural epithelium, at the time of operation, constitutes a mosaic with at least the following independent neural epithelial units:

(a) Ventralmost basal plate which gives rise to the cells between the mesial motor column and the floor plate.

(b) Ventro-lateral basal plate which gives rise to the mesial motor column.

(c) Lateral basal plate which gives rise to the lateral motor column.

(d) Dorsal basal plate which gives rise to internuncial cells. (Regions b and c probably also produce internuncial cells found medial to the respective motor columns.)

(e) Alar plate which gives rise to the sensory horn and internuncial cells.

(f) The roof (and probably also the floor) plate.

LITERATURE CITED

BARRON, D. H. 1945 The role of the sensory fibers in the differentiation of the spinal cord in sheep. J. Exp. Zool., *100:* 431–443.

BUEKER, E. D. 1943 Intracentral and peripheral factors in the differentiation of motor neurons in transplanted lumbo-sacral spinal cords of chick embryos. J. Exp. Zool., *93:* 99–129.

———— 1945 The influence of a growing limb on the differentiation of somatic motor neurons in transplanted avian spinal cord segments. J. Comp. Neur., *82:* 335–361.

———— 1947 Limb ablation experiments on the embryonic chick and its effect as observed on the mature nervous system. Anat. Rec., *97:* 157–174.

BURR, H. S. 1916 The effects of the removal of the nasal pits in Amblystoma embryos. J. Exp. Zool., *20:* 27–57.

DETWILER, S. R. 1937 Does the developing medulla influence cellular proliferation within the spinal cord? J. Exp. Zool., *77:* 109–122.

———— 1944 Restitution of the medulla following unilateral excision in the embryo. J. Exp. Zool., *96:* 129–142.

———— 1946 Midbrain regeneration in Amblystoma. Anat. Rec., *94:* 229–237.

———— 1947 Restitution of the brachial region of the cord following unilateral excision in the embryo. J. Exp. Zool., *104:* 53–68.

DRURY, H. F. 1941 Amyl acetate as a clearing agent for embryonic material. Stain Tech., *16:* 21–22.

DUSHANE, G. P. 1938 Neural fold derivatives in the Amphibia: Pigment cells, spinal ganglia and Rohon-Beard cells. J. Exp. Zool., *78:* 458–503.

HAMBURGER, V. 1929 Die Entwicklung experimentell erzeugter nervenloser und schwach innervierter Extremitäten von Anuren. Arch. f. Entw.-mech., *114:* 272–363.

———— 1934 The effects of wing bud extirpation on the development of the central nervous system in chick embryos. J. Exp. Zool., *68:* 449–494.

———— 1939 The development and innervation of transplantated limb primordia of chick embryos. J. Exp. Zool., *80:* 347–389.

———— 1942 A Manual of Experimental Embryology. University of Chicago Press.

———— 1946 Isolation of the brachial segments of the spinal cord of the chick embryo by means of tantalum foil blocks. J. Exp. Zool., *103:* 113–142.

———— 1948 The mitotic patterns in the spinal cord of the chick embryo and their relation to histogenetic processes. J. Comp. Neur., *88:* 221–284.

HAMMOND, W. S., AND C. L. YNTEMA 1947 Depletions in the thoraco-lumbar sympathetic system following removal of neural crest in the chick. J. Comp. Neur., *86:* 237–266.

HARRISON, R. G. 1924 Neuroblast versus sheath cell in the development of peripheral nerves. J. Comp. Neur., *37:* 123–205.

———— 1947 Wound healing and reconstitution of the central nervous system of the amphibian embryo after removal of parts of the neural plate. J. Exp. Zool., *106:* 27–83.

HUTCHINSON, C. 1936 Reconstitution in the nervous system following unilateral reversal of the dorsoventral axis in part of the spinal cord of Amblystoma punctatum. J. Comp. Neur., *63:* 465–487.

LEVI-MONTALCINI, R. 1945 Corrélations dans le développement des différentes parties du système nerveux. II. Corrélations entre le développement de l'encephale et celui de la moelle épinière dans l'embryon de poulet. Arch. Biol., *56:* 71–83.

———— 1949 The development of the acoustico-vestibular centers in the chick embryo in the absence of the afferent root fibers and of descending fiber tracts. J. Comp. Neur., *91:* 209–242.

LILLIE, F. R. 1904 Experimental studies on the development of organs in the embryo of the fowl (Gallus domesticus). II. The development of defective embryos, and the power of regeneration. Biol. Bull., *7:* 33–54.

MOOG, F. 1943 The distribution of phosphatase in the spinal cord of chick embryos of one to eight days incubation. Proc. Nat. Acad. Sci., *29:* 176–183.

PIATT, J. 1948 Form and causality in neurogenesis. Biol. Rev., *23:* 1–45.

SAUNDERS, J. W., JR. 1948 The proximo-distal sequence of origin of the parts of the chick wing and the role of the ectoderm. J. Exp. Zool., *108:* 363–403.

SHOREY, M. L. 1909 The effect of the destruction of peripheral areas on the differentiation of the neuroblasts. J. Exp. Zool., *7:* 25–64.

TELLO, J. F. 1923 Les différenciations neuronales dans l'embryon du poulet, pendant les premiers jours de l'incubation. Trav. Lab. Recherches Biol. Univ. Madrid, *21:* 1–93.

YNTEMA, C. L., AND W. S. HAMMOND 1945 Depletions and abnormalities in the cervical sympathetic system of the chick following extirpation of neural crest. J. Exp. Zool., *100:* 237–263.

ZACHARIAS, L. R. 1938 An analysis of cellular proliferation in grafted segments of embryonic spinal cord. J. Exp. Zool., *78:* 135–157.

PLATE 1

EXPLANATION OF FIGURES

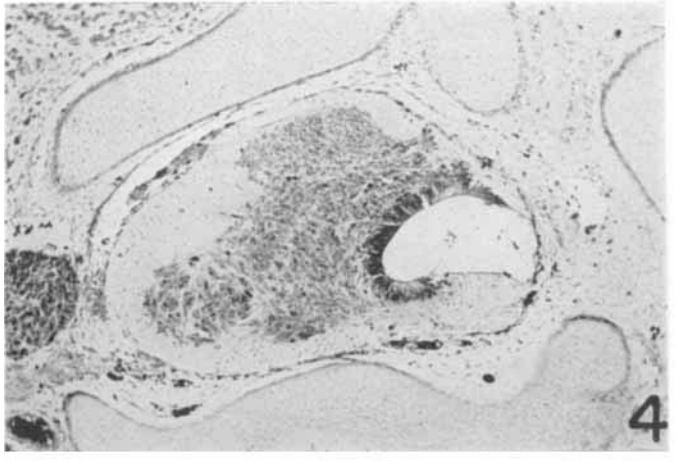
All sections magnified 80 ×. Right side of spinal cord appears on right.

4 Chick 324, segment 15. Absence of mantle on operated side. Absence of ependyma.

5 Chick 233, segment 14. Absence of sensory horn in presence of ganglion. Absence of alar plate ependyma on operated side.

6 Chick 384, segment 15. Absence of dorsal quarter of mantle. Absence of alar plate ependyma on operated side.

7 Chick 465, segment 13. Presence of ventral half of cord. Presence of basal plate ependyma.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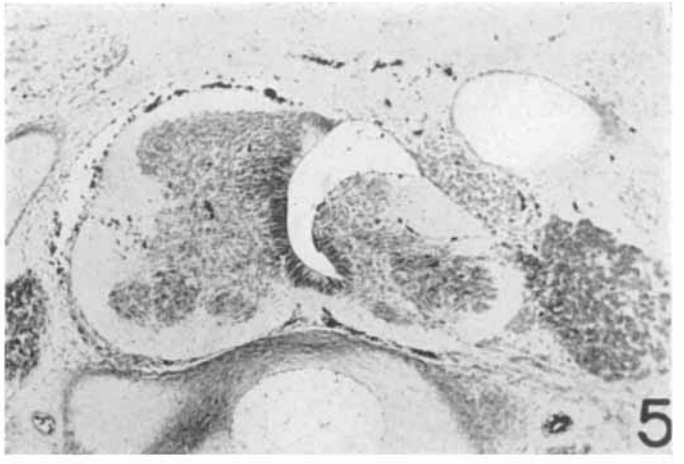
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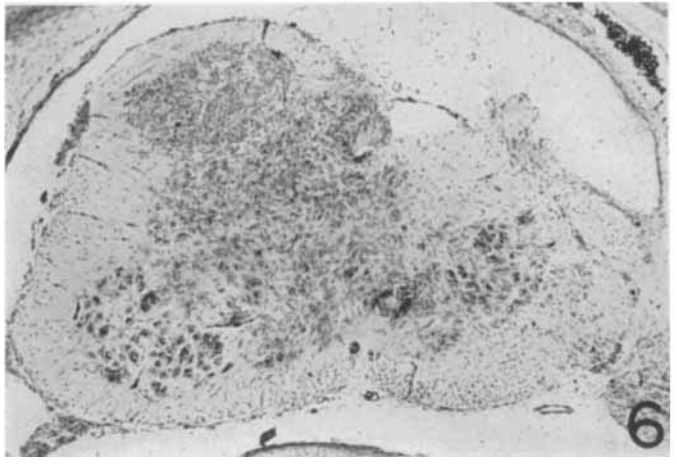
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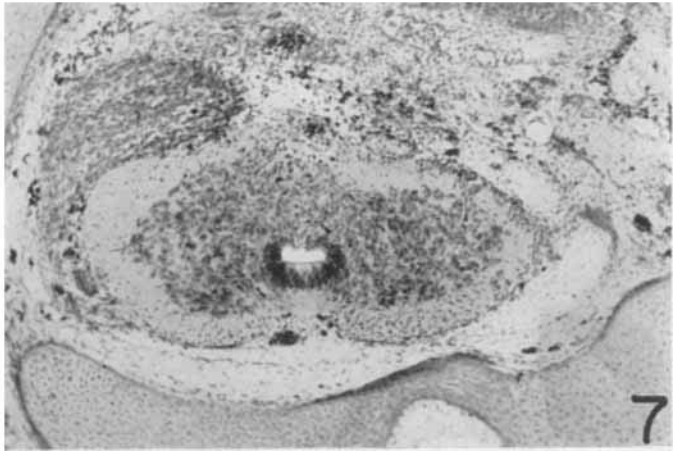
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PLATE 2

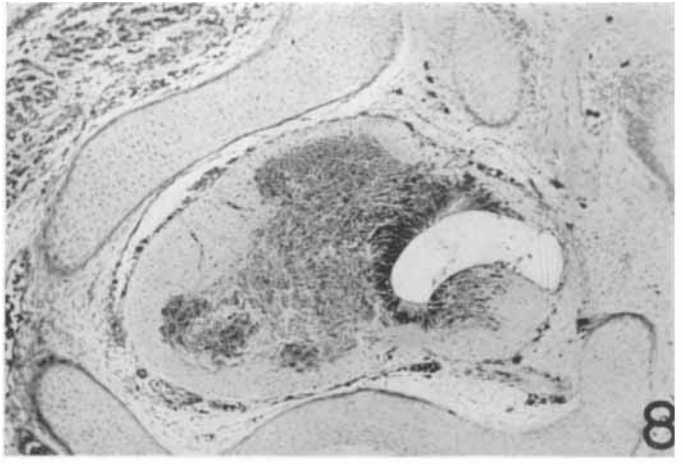
EXPLANATION OF FIGURES

8 Chick 324, segment 15. Presence of cells found between mesial motor cells and the floor plate. Presence of ventralmost ependyma on the operated side.

9 Chick 476, segment 16. Absence of mesial motor and lateral motor cells. Defect in ventro-lateral and lateral ependyma on the operated side.

10 Chick 476, segment 16. Absence of lateral motor cells. Defect in lateral ependyma on the operated side.

11 Chick 566, segment 17. Absence of lateral motor cells and internuncial cells. Defect in mid-region and lateral region of ependyma on the operated side.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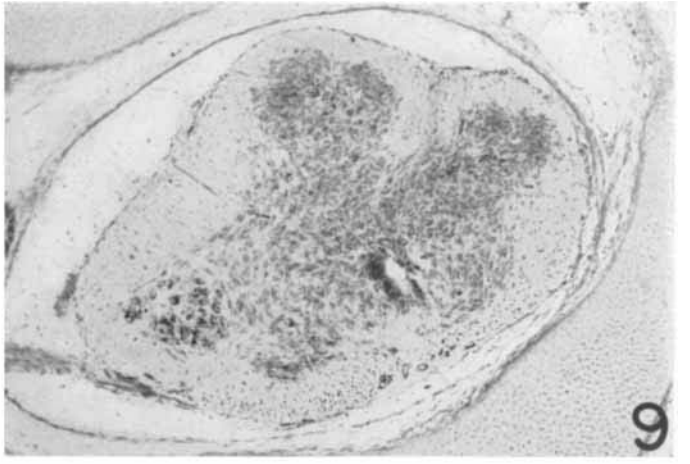
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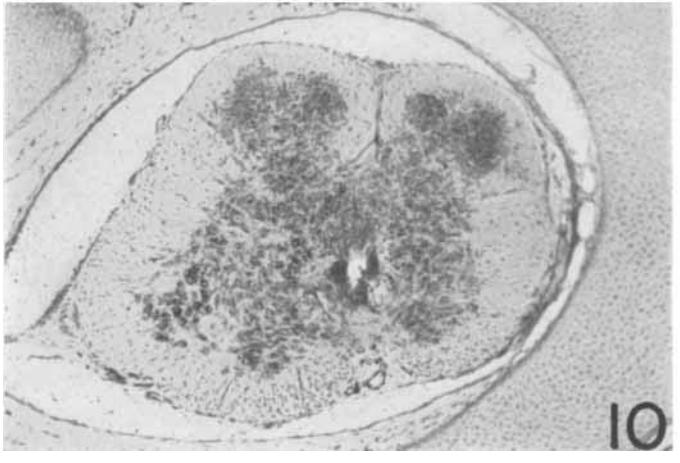
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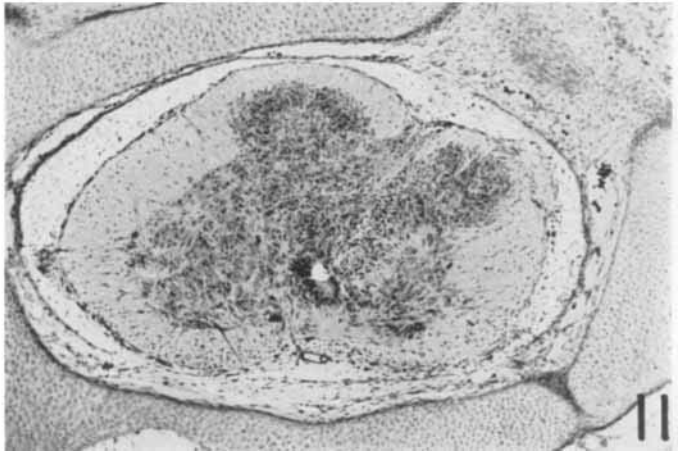
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