

THE EFFECTS OF WING BUD EXTIRPATION ON THE DEVELOPMENT OF THE CENTRAL NERVOUS SYSTEM IN CHICK EMBRYOS

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NINE FIGURES

INTRODUCTION

The problem of embryonic correlation between the nervous system and the peripheral fields has attracted much attention during the last years. One of the earliest contributions in this field was made by Miss Shorey, who, 25 years ago, following a suggestion of Dr. F. R. Lillie, approached the problem by studying in chick embryos the effect on the growing nervous system of unilateral limb bud extirpations. Using electrocautery, she succeeded in getting a small number of wingless embryos, which were studied 1 to 6 days after operation. The operation resulted in remarkable hypoplasia in most parts of the nervous system. The spinal ganglia as well as the anterior and posterior horn of the spinal cord and the spinal nerves were smaller on the operated side. She concluded from these data that the peripheral fields play an important role in the development of the nervous system.

Since that time much work has been done on this problem. We owe the most extensive and thorough experimental analysis of the problem to Detwiler (summary, '33) who performed his investigations on the urodele *Amblystoma*. His experiments consisted of changing the size of the peripheral field by extirpating or adding limb buds and somites. In all these experiments the spinal ganglia reacted perceptibly to such

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peripheral changes by hypoplasia after extirpation and by hyperplasia after peripheral overloading. The spinal cord, however, failed to indicate the least response to the peripheral changes. This remarkable discrepancy in the results obtained in two different forms demands further investigation. Therefore, Dr. F. R. Lillie suggested that I undertake a reinvestigation of the problem.

The results obtained by Miss Shorey were fully confirmed. In chick embryos the spinal cord as well as the spinal ganglia react strikingly to a decrease in the peripheral field. A detailed discussion will be devoted to the question of how the obvious difference in the behavior of the two forms can be explained.

After positive evidence had been obtained for the fact that the growing limb bud controls the quantitative development of the central nervous system, a second problem arises at once: How is the embryonic correlation between these two systems established? This problem is highly complex and far from being solved. We approach it by comparing the quantitative variations of the hypoplastic effects in a number of operated animals.

I am deeply indebted to Dr. F. R. Lillie for his suggestion of the problem and for his incessant, stimulating interest. Furthermore, I wish to express my thanks to Dr. B. H. Willier for the kind hospitality with which he received me in his laboratory, for introducing me to the technique of operating on chick embryos and for valuable help and advice.

MATERIAL, OPERATION

This paper deals exclusively with wing bud extirpations in chick embryos. The same experiments have been performed on hind limb buds but these data will be reported later.

Most of the operations were done on embryos 68 to 72 hours old, although a few embryos were younger. The somites were not counted, but the size of the wing bud was recorded. Three different sizes were distinguished: small, medium and

large buds (fig. 1). Of these, small and medium ones were most frequently used. However, this variation in age and size of the buds did not affect the results, so that it will not be taken into further consideration. At the time of the extirpation the limb bud consists of a compact mass of mesoderm cells, which have not yet undergone visible differentiation.

The histological condition of the spinal cord of a 72-hour chick embryo has been studied carefully by Ramón y Cajal (1890, '06, '08), Tello ('22), Held ('09) and others. Large numbers of mitoses are found around the central canal. Neuroblasts in various stages of differentiation may be found in the wall. Small groups of motor neuroblasts are already

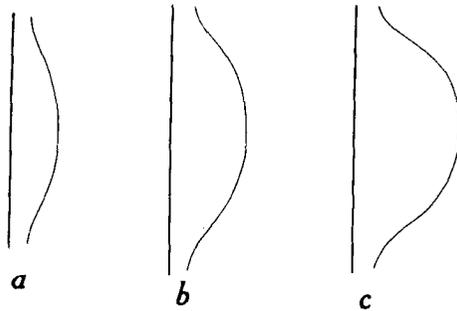


Fig. 1 Wing buds of chick embryos in the stage of operation (about 72 hours of incubation). a, small; b, medium; c, large bud.

assembled in the anterior horn region. Some of them have sent out their axones, which form a tiny anterior root. The spinal ganglia are already formed; they have established their first connections with the spinal cord by forming a dorsal root, and the first sensory fibers have joined the motor fibers. According to Tello ('22) small nerves have reached the base of the wing bud but have not yet entered it.

It must be kept in mind that the differentiation of the nervous system had begun when the experiments were performed. Removal of the wing rudiment in earlier stages in order to anticipate this process was considered but this would have involved the extirpation of certain somites, and such an operation might have resulted in direct injury to the

ganglia or even to the spinal cord itself—a danger which had to be avoided carefully. Moreover in order to check Miss Shorey's results, it was advisable to repeat her experiment as accurately as possible.

The general technique of incubating follows the usual procedure. Thermocauterization is an unsatisfactory method for extirpation experiments. The glass needle introduced by Spemann as an instrument for operating on amphibian embryos proved to be applicable as well in 3-day chick embryos. The wing bud can be easily cut off by a needle of the same shape and size as the original instrument used in operations on amphibians. A hair loop proved to be of help as an additional instrument.

The operation is performed as follows: Before the operation the embryo is exposed by sawing a rectangular window about 1 sq.cm. in size in the shell above the embryo. The shell membrane is removed by means of fine watchmaker forceps. With the glass needle, a small hole is torn in the amnion above the right wing bud. The glass needle is pushed underneath the right wing bud, between it and the somatopleure and the bud is cut through at its base by lifting the needle and rubbing it against the hair loop which softly presses the bud. Sometimes the hair loop is used only to keep the whole embryo in position. Then the bud is lifted out of the shell with the tip of the needle or with the hair loop. We endeavored to cut it out in one piece. The window-piece is then sealed in by means of fluid paraffin, the egg is gently turned on its longitudinal axis so that the embryo may be situated underneath an uninjured area of the shell, and immediately put back into the incubator. Hemorrhage was carefully avoided; embryos which suffered a loss of blood were discarded. The instruments were carefully sterilized; the glass needle and hair loop were kept in alcohol for a few hours before the operation and they were washed in sterilized warm water immediately before being used.

Most of the embryos were allowed to develop 4 to 6 days after operation. Five specimens were preserved 2 to 3 days after operation and two were raised 9 to 10 days.

The results of the experiments are given in table 1. In five cases a wing nearly normal in shape, but smaller in size had developed. It is most unlikely that regeneration had taken place. Probably small strips of tissue had been left at the base of the bud. The obvious fact of the harmonic regulation occurring in these cases deserves further attention.

TABLE 1
General results of the operation

| | WING COMPLETELY ABSENT | SMALL CONE | LIMB-SHAPED OUTGROWTH | DIED WITHOUT FURTHER DEVELOPMENT | TOTAL |
|-----------|------------------------------|------------|--------------------------|--|-------|
| Surviving | 18 | 3 | 2 | — | 23 |
| Dead | 12 | 1 | 3 | 23 | 39 |
| Totals | 30 | 4 | 5 | 23 | 62 |



Fig. 2 Chick embryo, 6 days after operation (9 days old). Right wing bud extirpated. $\times 1$.

In the cases which survived, the embryos had undergone normal undisturbed development. The wound and the amnion had apparently healed immediately after operation. In some specimens, a little knob marked the place of the lacking wing (fig. 2). In a few cases the regions of the somatopleure adjacent laterally to the wing bud had been injured during the operation and had not regenerated. As a result, the belly wall failed to close in the ventral midline of the body. Such

an opening allowed the heart and sometimes parts of the ventral intestines to protrude. This ectopia viscerum, however, did not inhibit normal development of the spinal cord and such cases were not discarded.

The embryos were fixed in Bouin's fluid and stained with Heidenhain's iron hematoxylin. The sections were cut 8 μ .

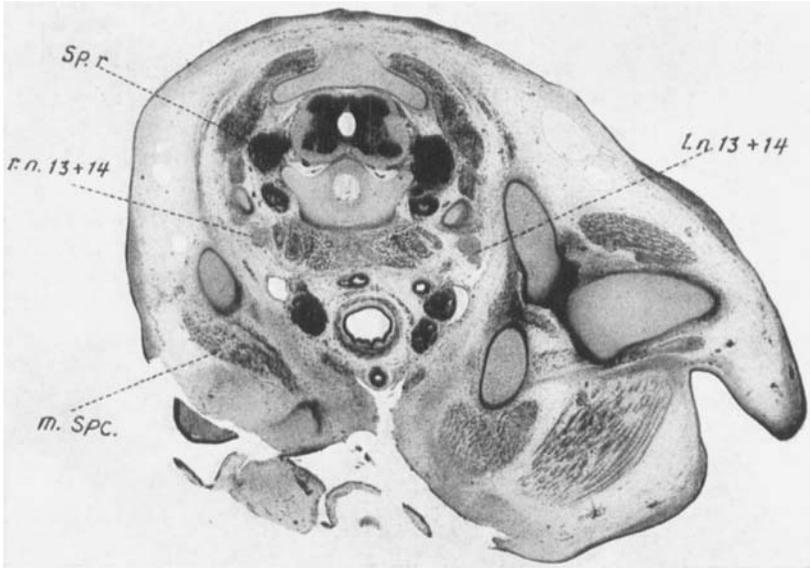


Fig. 3 S.118. Cross section through the whole animal in the level of the fifteenth spinal ganglia. sp.r., right ganglion no. 15; l.n. 13-14, left spinal nerves 13 and 14; r.n. 13-14, right spinal nerves 13 and 14 (fused); m.spc, musculus supracoracoideus.

DESCRIPTION OF A TYPICAL CASE

S. 118; 1933. The embryo was preserved 5 days after operation, that is 8 days after the beginning of incubation. It was normally developed except that the belly wall was not closed in the ventral midline; a narrow aperture was found in the wing level which increased in size posteriorly to form a wide opening through which the heart and liver protruded. The development was not disturbed.

The sections (compare fig. 3) show that not only the right wing but also a large part of the right shoulder muscles had been removed. The pectoral girdle is well intact. The

clavicula is normal and in process of ossification. The scapula and coracoid are united to form a single cartilage, as is typical for this stage. However, there is no trace of an articular cavity, and the posterior end of the coracoid does not join the sternum but ends freely in the mesenchyme. The ribs are normally formed. Of the sternum, which is known to be formed by the fusion in the ventral midline of the two lateral primordia, the left half is developed normally, whereas the right anlage is represented by two irregular cartilages, one at its anterior end and the other at its posterior end.

Most of the shoulder muscles² are absent. Only small portions of three of them are left; and they can be identified by their position which is symmetrical to the corresponding muscles of the left side. They are, 1) a small part of the *m. supra-scapularis* (fig. 3, *m.spc*); 2) a very small rudiment, probably of the *m. coracobrachialis internus*; 3) a small remnant of the *m. pectoralis major*. The latter begins and ends freely in the mesoderm. It can be shown, by comparison of the weights of limb and shoulder muscles of both sides, that only 8 per cent of the normal mass is left on the right side. The somites had not been injured in the operation; consequently the axial trunk muscles do not show the least defect.

The study of the reaction of the nervous system has been confined to the wing level of the spinal cord and the nerves of the brachial plexus. The sympathetic system and the higher levels of the central nervous system have not yet been examined to determine whether or not they are affected.

A cross section through the wing level of a normal 8-day chick embryo allows one to distinguish clearly four different areas (fig. 4). First of all, very definite anterior and posterior horns are formed. The anterior horn—lateral motor group (*l.m.*) consists of a group of large motor neurones not intermingled with other cells. They can easily be distinguished from the neighboring cells by their large size, their large nuclei, and by the dark staining of their plasma. They are small in number: thirty to eighty per section, and are found only in the brachial and lumbar swelling. The posterior horn (*p.h.*) consists of a large number of small cells which lie close together. Most of these cells probably belong to

² The names of muscles and nerves are taken from Fürbringer (1888).

the sensory system; they may also contain correlation neurones. In most of the sections, the posterior horn is clearly separated from the median part by a small cell-free line, running in direction x-y. A third group of cells is the mesial motor column (m.m.). It is situated medially to the anterior horn, between it and the ventral midline and clearly separated from the motor lateral group. The nuclei of these cells are smaller than those of the motor horn; their plasma is stained darker than that of the surrounding cells, although not as dark as that of the lateral motor cells. They are fewer in number, twenty to thirty in each section. This mesial

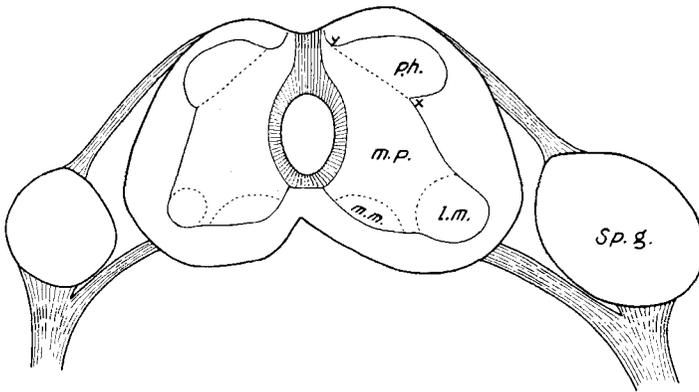


Fig. 4 Diagram of the central nervous system of an operated chick embryo (wing level). sp.g., spinal ganglion; L.m., lateral motor group (anterior horn); m.m., mesial motor group; p.h., posterior horn; m.p., median part.

group innervates the axial trunk muscles. The remaining portion of the cord (m.p.) contains cells of different size and arrangement and cannot be further divided. This part of the spinal cord, for convenience, may be called the median part. It contains mostly correlation neurones. Along the limb level of the spinal cord are found the spinal ganglia 13 to 16, whose fibers take part in the formation of the brachial plexus (fig. 6).

A cross section through the spinal cord of our experimental case (fig. 5) shows a striking difference between the two halves. The right half as a whole is remarkably smaller. An

anterior horn is present on the operated side, formed by typical large motor neurones, but their number is considerably reduced as compared with the unoperated side. In order to get an accurate picture of the hypoplasia in the motor horn, the lateral neurones were counted on both sides in every third section throughout the brachial swelling. This was done by drawing the nuclei by means of a camera lucida and at the same time counting them with a pole counter. There was

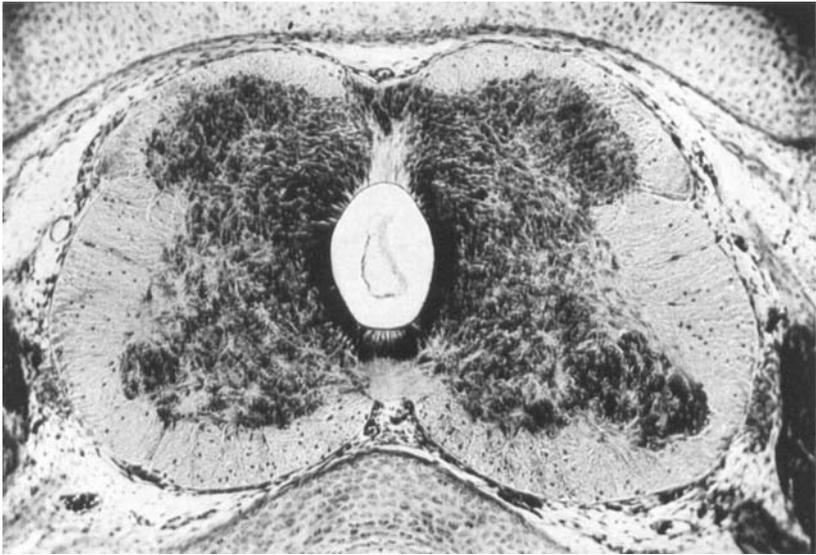


Fig. 5 S. 118. Spinal cord. Cross section in the level of the fifteenth ganglion.

found a total of 5231 cells on the left side and 2044 cells (= 39 per cent) on the operated side. Therefore, the extirpation of the wing with inclusion of the shoulder muscles has resulted in a hypoplasia in the motor horn of 61 per cent. The mesial motor group is found to be unaffected as shown by cell counts, 2240 cells were found on the left side and 2170 cells on the right side. The posterior horn is obviously smaller on the operated side. Its cells were also counted but in not more than one-eighth of all the sections. We found 7320 cells on the normal side and 5770 cells (= 78.8 per cent)

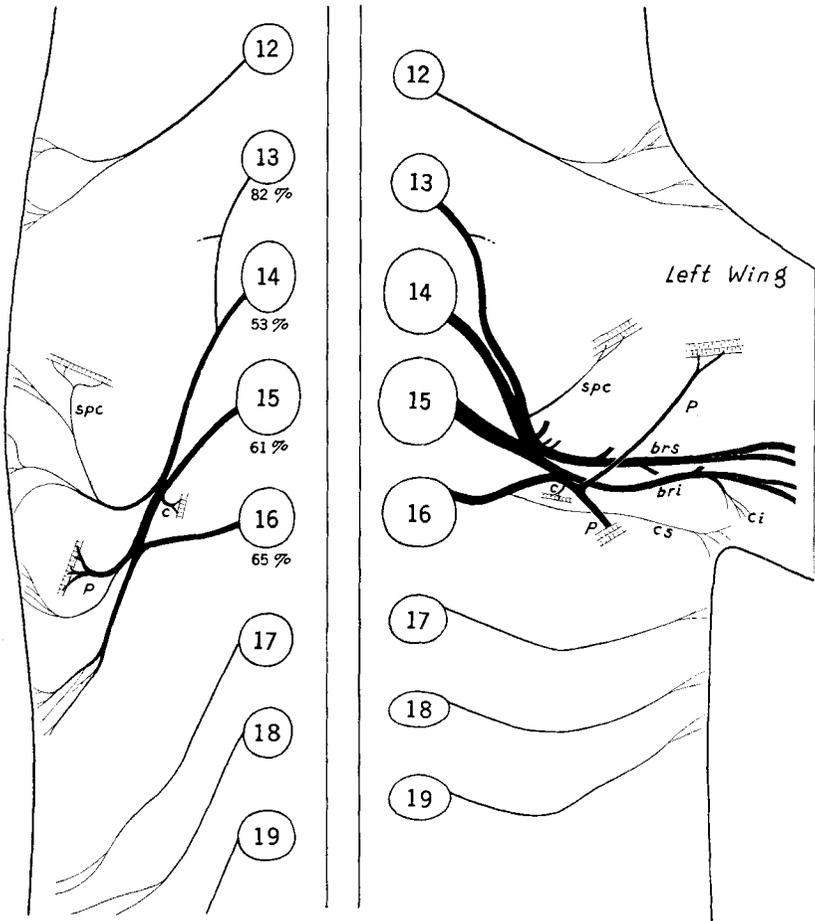


Fig. 6 Schematic reconstruction of the brachial plexus and the distribution of the peripheral nerves of case S.118. 12-19, spinal ganglia. The numbers underneath the ganglia give the volumes in per cent of the right ganglia as compared with the left (normal) ganglia. spe, n. supracoracoideus; c, n. coracobrachialis posterior; p, n. pectoralis; brs and bri, nn. brachialis superior and inferior; es and ci, nn. cutaneus brachii superior and inferior. The diagram is complete for the right side of the animal (left in figure). On the left side (right in figure) only the nerves and muscles are figured which correspond to the remnants of the operated side.

on the operated side. Therefore, the hypoplasia found in the posterior horn is evident but smaller than in the anterior horn. The median part of the spinal cord was counted³ in the same sections as in the posterior horn, that is, every eighth section: 14,490 cells were found on the left side and 14,675 on the operated side. The slight surplus of 2 per cent on the operated side has no statistical significance. Therefore, it is evident that this part of the spinal cord is not affected at all. Summarizing we state that the spinal cord is definitely affected by the loss of its peripheral field. However, this hypoplasia is strictly confined to two centers, the anterior and the posterior horn. The median part, including the mesial motor group, has not reacted at all.

The spinal ganglia of the operated side have also undergone a remarkable hypoplasia (figs. 3 and 6). For the purpose of this study it was not necessary to compare the exact number of ganglionic cells in all eight cases. It seemed to be sufficiently accurate to compare the volumes of the respective ganglia. The data were obtained by drawing with the camera lucida the outline of the ganglion in each section and measuring the areas by means of a planimeter. The total area of a given ganglion was compared with that of the other side. In our case, the losses in volume were:

| |
|----------------------------|
| 18 per cent in ganglion 13 |
| 47 per cent in ganglion 14 |
| 39 per cent in ganglion 15 |
| 35 per cent in ganglion 16 |

It is of some interest to determine whether these figures give an approximately true picture of the cellular hypoplasia or if the loss of volume is different. Therefore, in addition, the cell loss was calculated for ganglia 14 and 15 by counting the nuclei in each third section on both sides. The cellular hypoplasia amounts to not more than 28 per cent in both ganglia. This means that in the same ganglion the decrease in volume is higher than the decrease in cell number. A possible explanation of this 'shrinkage' is that also the amount of inter-

³As it is not possible to differentiate between neurones and neuroglia all cells were counted with the exception of the ependymal layer surrounding the central canal.

cellular neuropil might be decreased considerably, so that the cells lie closer together. A reduction in size of the single neurones would have the same effect.

In the spinal ganglia of the 8-day chick embryo, it is easy to distinguish cells of two different sizes. The two types are not intermingled. The small cells forming a compact group are situated in the dorso-medial corner of the ganglion near the point where the posterior root leaves; they are surrounded by the larger cells which form a cup around them. Separate counting of small and large cells shows that both types are decreased in number. In ganglion 14 the ratio of small to large cells is 1:1.2 for the left side (unoperated) and 1:1.35 for the right side; the same figures were obtained for ganglion 15.

Peripheral nervous system. The anterior and posterior roots are smaller on the operated side. However, it is difficult to get exact quantitative data for this decrease in size.

Figure 6 gives a diagrammatic drawing of the brachial plexus and of the peripheral distribution of the nerves of both sides (ventral view). For the operated side (left side of fig. 6), the complete pattern is given in detail; at the left side (right side of figure), only the points of branching are indicated. The thickness of all nerves and of the spinal ganglia is given in approximately precise proportions. The roots and the dorsal branches are omitted. (For further details concerning the construction of such diagrams see Hamburger, '28, p. 288.)

The paths taken by the nerves 13 to 16 and the configuration of the plexus are normal on the operated side. Apparently the proximal pattern including the plexus itself was already laid down at the time of the operation. However, the distribution of the nerves distal to the plexus is completely abnormal. The nerves 13 to 16 are much thinner on the operated than on the normal side (compare also fig. 3; r.n. 13 and 14 and l.n. 13 and 14). The three rudiments of the shoulder muscles, mentioned above, are innervated by three nerves. These nerves cannot be homologized with the nerves

supplying the corresponding muscles of the other side for the following reasons: The nn. supracoracoideus (spc.) and pectoralis (p.) are mixed nerves on the right side, carrying masses of sensory fibers, whereas the true spc. and p. are pure motor nerves. Moreover, the nerve spc. takes a completely abnormal course. N. coracobrachialis posterior (c.) branches off from the plexus not at its usual place at the fusion of nerves 15 and 16 but more proximally at the point where nerves 14 and 15 cross the rudiment of m. coracobrachialis posterior. The three small muscles are abundantly supplied with nerves, and one receives the definite impression that they are overloaded with nerve fibers far beyond their normal requirement. However, it is very difficult to prove this observation by quantitative data.

The sensory pattern differs even more from that of the normal side. There are several strong sensory nerves running to the skin, lateral and ventral to the operated region. They have no homology among normal nerves. Whereas on the normal side only the small n. cutaneous brachii inferior (c.i.) innervates the ventral base of the wing, on the right side several thick nerves end in the same ventrolateral region. Therefore, the skin is certainly overloaded with sensory fibers. The apparent shifting backward of the caudal nerves of the operated side is probably due to a bending of the embryo.

This information concerning the quantity and distribution of the peripheral nerves running to the region of operation will be of some importance in the later discussion of the quantitative variation of hypoplasia.

BRIEF SURVEY OF THE RESULTS OBTAINED IN EIGHT OTHER CASES

Eight more cases have been studied in the same way and since they corroborate without exception the results just reported for one typical case, it would be of no use to present them in detail. The following brief report will include the general results and some individual variations which are of interest for the later discussion.

All the embryos were preserved 5 to 6 days after operation, that is, 8 to 9 days after the beginning of incubation. Thus the quantitative data are comparable.

In most of these specimens the extirpation proved to be much less radical with regard to the shoulder muscles and the pectoral girdle than was the case in S. 118. Only one case

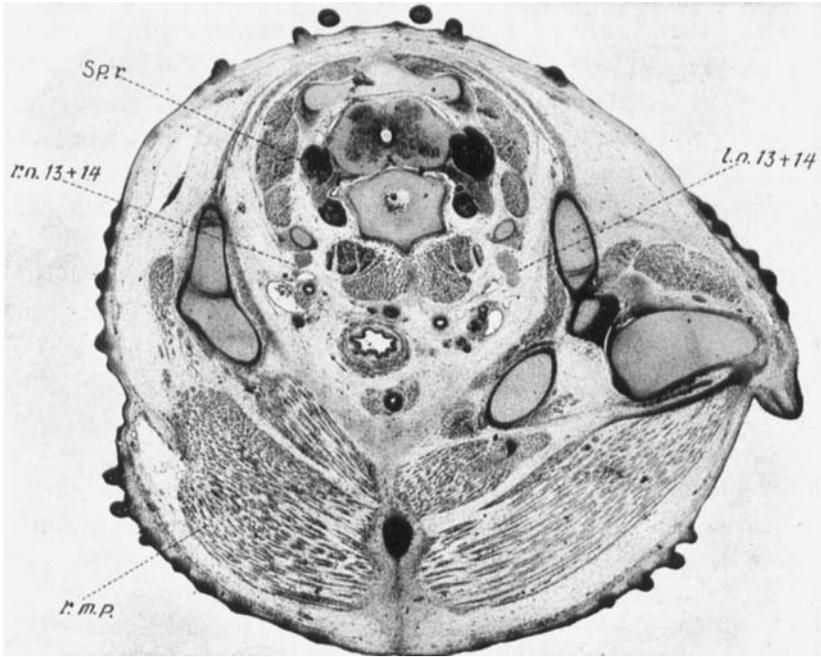


Fig. 7 S. 151. Cross section through the whole animal in the level of the fifteenth spinal ganglia. sp.r, right spinal ganglion; l.n 13-14 and r.n 13-14, left and right nn. 13 and 14; r.m.p., right musculus pectoralis.

was found in which the loss of shoulder muscles was even more extensive (S. 200); in this case there was not more than a small fraction of the m. pectoralis left; the right half of the sternum was completely absent; and the ventral belly skin was damaged on both sides so that a large hole was formed.

In all the other cases, skeleton and muscles were more complete than in case S. 118 (fig. 7). Clavicula, coraco-

scapula and sternum were always present and the coracoid was articulated in the sternum with a joint. In several cases, even a small proximal part of the humerus had developed. It was either fused abnormally with the coraco-scapula in the region where the joint was to be expected or it was articulated in a typical shoulder joint. The shoulder muscles presented a wide variation in number and size. The cases described above (118 and 200) rank as extremes of heavy loss. They are in contrast to others in which the complete set of brachial musculature is developed in full size (compare fig. 7, r.m.p.), and only the distal wing muscles are absent. Other cases display an intermediate deficiency. (In table 2, the cases are arranged according to the muscle loss, the cases of heaviest loss are found at the top.)

In the frequent cases where the brachial muscles are deprived of their normal points of insertion at the humerus, they are found either terminating freely in the mesenchyme or attached to the skin or fused with their inner ends in a tendinous ligament.

The reactions of the nervous system to the extirpation differ only in quantity from what was reported for case S. 118. In every case, the decrease in size of the right half of the spinal cord is obvious (fig. 8), though not always as marked as in figure 5. The hypoplasia again is confined to the anterior and the posterior horn. The mesial motor group and the median part of the cord are not affected at all, as was shown by counting the nuclei in three more cases (compare table 2). The hypoplasia of the lateral motor group was studied in every one of the eight cases, by counting the cells of this group in each third section. Practically every one of these sections displays an asymmetry. Among approximately 720 sections counted there were not more than a dozen in which the number of motor cells of the operated side equaled or surpassed that of the unoperated side. These facts definitely disprove an objection raised to the illustrations in the paper of M. Shorey. Her photographs are certainly not taken from sections selected to show a variation of the cell number

in favor of the non-operated side; on the contrary, they illustrate the typical situation to be found throughout the whole wing level of the cord.

In this connection it was of some interest to find whether or not this hypoplasia is regularly distributed all over the brachial swelling. For this purpose graphical curves were constructed which give the number of motor cells found in each section (fig. 9, for case S. 118). They were constructed

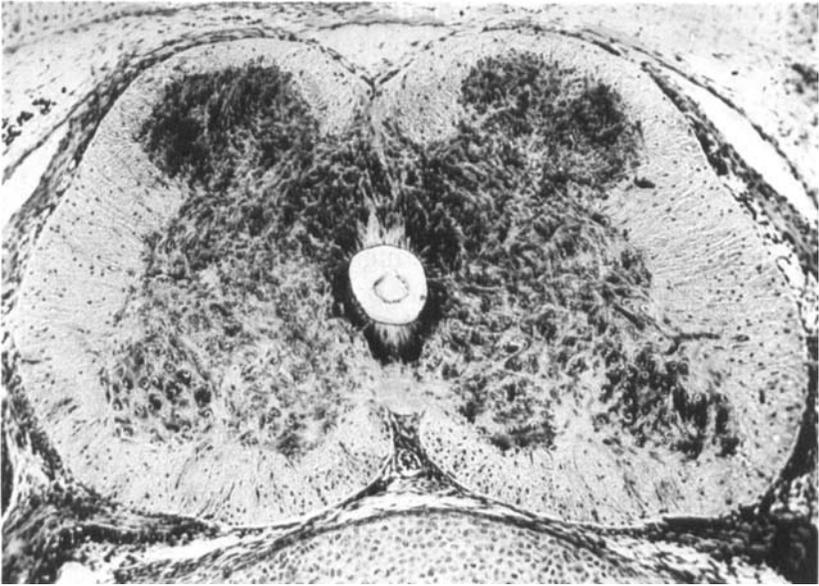


Fig. 8 S. 151. Spinal cord. Cross section in the level of the fifteenth ganglia.

in the following way: The average was calculated for three neighboring sections, thereafter one section was omitted and a new one added and so forth. Curve A gives the amount of motor neurones of the normal (left) side, curve B gives the same for the operated (right) side, curve C gives the proportion of left:right side in per cent. Evidently, the curve for the right side (curve B) follows that for the normal side (curve A) rather accurately, thus indicating that the percentage of hypoplasia (curve C) remains approximately the

TABLE 2
Effects of extirpation of right wing bud (chick)

| NO. | MUSCLES | | | LATERAL MOTOR GROUP | | | POSTERIOR HORN | | | MESIAL MOTOR GROUP | | | MEDIAN PART | | | SPINAL GANGLIA | | | | |
|--------|---------------------------|-------|---------------------------------|---------------------|-------|-------------------------|-------------------|-------|-------------------------|--------------------|-------|-------------------------|-------------------|-------|-------------------------|---------------------------------|---------|---------|---------|---------------------------------|
| | Weight of models in grams | | Reduction of volume in per cent | Number of cells | | Hypo-plasia in per cent | Number of cells | | Hypo-plasia in per cent | Number of cells | | Hypo-plasia in per cent | Number of cells | | Hypo-plasia in per cent | Reduction of volume in per cent | | | | |
| | Left | Right | | Left | Right | | Left | Right | | Left | Right | | Left | Right | | Ggl. 13 | Ggl. 14 | Ggl. 15 | Ggl. 16 | Average hypo-plasia in per cent |
| S. 200 | 17.1 | 0.7 | 96 | 3991 | 1686 | 58 | 7320 | 5770 | 21.2 | 2240 | 2170 | 3 | 14490 | 14675 | + 2 ³ | + 20 | 36 | 63 | 53 | 43 |
| S. 118 | 17.6 | 1.4 | 92 | 5231 | 2044 | 61 | 6310 | 5500 | 13 | 1417 | 1409 | 0 | 12640 | 12710 | + 0.5 | 18 | 47 | 39 | 35 | 38 |
| S. 113 | 16.3 | 3.8 | 76.7 | 4767 | 2339 | 51.2 | 5085 | 2675 | 47.4 | 2476 | 2479 | 0 | 2494 ² | 2532 | + 2 | 8 | 50 | 59 | 48 | 45 |
| S. 96 | 30.4 | 8.7 | 71.4 | 5085 | 2675 | 47.4 | 2102 ¹ | 1730 | 18 | 1941 | 1921 | 1 | 11580 | 11600 | 0 | 32 | 55 | 47 | 42 | 46 |
| S. 132 | 20.3 | 6.8 | 66.5 | 4842 | 2714 | 43.5 | 6600 | 5670 | 14 | 3829 | 2629 | 33 | 4006 | 2797 | 30.2 | 33 | 44 | 54 | 51 | 48 |
| S. 150 | 40.4 | 22.8 | 43.5 | 3929 | 2629 | 33 | 3869 | 3027 | 22 | 4006 | 2797 | 30.2 | 3869 | 3027 | 22 | 53 | 63 | 55 | 50 | 54 |
| S. 151 | 71.6 | 40.6 | 43.3 | 4006 | 2797 | 30.2 | 4012 | 3137 | 22 | 4012 | 3137 | 22 | 4012 | 3137 | 22 | 22 | 44 | 37 | 36 | 37 |
| S. 129 | 22 | 14.1 | 36.5 | 3869 | 3027 | 22 | | | | | | | | | | | | | | |
| S. 148 | 60.8 | 41.9 | 31 | 4012 | 3137 | 22 | | | | | | | | | | | | | | |

¹ Only eight sections counted.

² Only six sections counted.

³ + means hyperplasia.

same throughout the swelling. In cases where the pectoral muscles were present, the hypoplasia was higher in the posterior region of the wing level. This coincides with our conception that the centers for the proximal parts of the limb are located anteriorly to those for the distal parts. However, only four cases were studied in this way and these data will have to be corroborated by more material.

If one compares the average hypoplasia of the lateral motor cells found in the nine different cases, a striking variation is

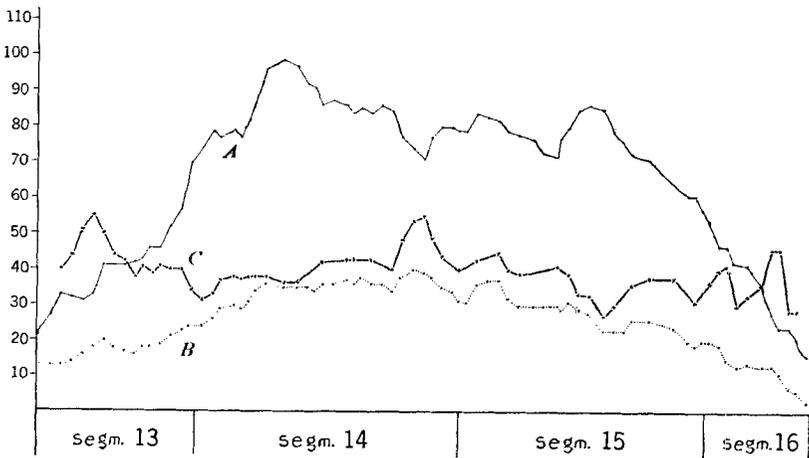


Fig. 9 Distribution of the motor neurones of the anterior horn in the limb region (segments 13 to 16) of case S.118. A, number of motor neurones found on the left side (normal); B, number of motor neurones found on the operated side; C, ratio B:A in per cent. The ordinate gives the number of cells for the curves A and B and the percentage for the curve C.

revealed (compare figs. 5 and 8 and table 2). The case described above shows the most radical degree of hypoplasia (61 per cent); the lowest percentage is 22 per cent. It was soon discovered that this variation runs parallel with that of the loss of muscles (compare figs. 3 and 7). This observation seemed of importance for the analysis of the correlative factors which are at work here and will therefore be fully discussed later (p. 469). At this moment we confine ourselves to the statement that the motor horn undergoes a hypoplasia ranging from 22 to 61 per cent.

The cell numbers in the other three areas of the cord were studied in detail in three more cases. The mesial motor cells were counted in each third section and the posterior horn and median part in each tenth section. The posterior horn is decreased in size about 13 to 21 per cent. The mesial motor group and the median part are found to be unaffected (table 2). No degenerated neurones were found in the areas affected. Therefore we are justified in considering the effect as a real hypoplasia, that is a primary blocking of the growth of the nerve centers. No measurements of the sizes of the single neurones have been made, but it looks as if there were no difference in diameter between the nuclei of the right and left side.

The spinal ganglia 13 to 16 are hypoplastic in every case (fig. 7, sp.r.), except in ganglion 13 in case S. 200 which shows a slight hyperplasia. This is probably explained by the fact that this case has suffered a heavy bilateral destruction of the ventral belly skin. As a result, also the ganglia of the left side have undergone a hypoplasia and, therefore, cannot be used as a control for the right ganglia. In all the other cases, the loss of skin in the brachial region is confined to the operated side. The average hypoplasia for all four ganglia is rather uniform in the nine specimens, ranging from 37 to 54 per cent. We can, therefore, state that loss of the skin covering of the wing produces an average hypoplasia of about 45 per cent in the ganglia supplying this region. The individual hypoplasia of the different ganglia involved, however, is very variable, as may be seen from table 2. For case S. 118, for instance, the percentage of hypoplasia is 18 per cent for ganglion 13, 47 per cent for ganglion 14, 39 per cent for ganglion 15 and 35 per cent for ganglion 16. The same figures for case S. 150 are: 53 per cent, 63 per cent, 55 per cent, 50 per cent. This variation apparently does not follow any rule. The irregularity may be partly caused by the very irregular and abnormal distribution of sensory nerves in the operated region, as will be described now. In general, the ganglia 13 are less affected than the other ones.

The spinal nerves. The plexus and peripheral nerves of all the eight cases were reconstructed in the same manner as figure 6. The proximal parts of the nerves 13 to 16 and the formation of the plexus are normal in all cases. Only in one case, nerve 16 does not take part in the plexus but runs directly to the skin. However, the number and the paths of the nerves emerging from the plexus are completely irregular and cannot be compared with the situation found in a normal pectoral region. Only a few general data may be given. The details are interesting for the problem of determination of nerve paths which, however, cannot be discussed here. We are concerned with their distribution only in so far as it may help us to explain the quantitative variations found in the central organ. We find that whenever a rudiment of a muscle is present, it is innervated. In some cases of high muscle loss, one gains the impression that the muscle tissue is overloaded with fibers (see p. 461 for case S. 118). However, this is not true when the muscle has its approximately normal size. The path of a motor nerve is more or less normal if the muscle is situated in its normal location, and abnormal if the muscle is dislocated or if only part of it is present. Sensory fibers are found in all cases to furnish an unusually high supply to the skin; however, they are never as abundant as they were described for case S. 118 (fig. 6). The paths taken by these sensory nerves are always abnormal and the factors conditioning the quantitative development as well as the distribution of such abnormal sensory fibers are obscure.

Summary. If a wing bud of a 72-hour chick embryo is extirpated, the spinal cord and the spinal ganglia react to this decrease of peripheral fields by a marked hypoplasia.

The spinal ganglia are reduced in volume to 40 to 50 per cent. Within the spinal cord the hypoplasia is most pronounced in the lateral motor group (anterior horn), amounting to 22 to 60 per cent. The posterior horn is affected to an amount of 13 to 21 per cent. The mesial motor group and the median part of the cord are not affected.

The brachial plexus is formed normally by the nerves 13 to 16. If muscles are present in the brachial region, they are always innervated. The skin of the brachial region is well innervated by abnormal sensory nerves of varying size and distribution. Apparently, muscles are overloaded if they are represented by small rudiments. These statements are corroborated by the detailed study of nine cases.

In judging these results, one should always remember that they are obtained on chick embryos of 8 to 9 days, when the development of the nervous system is in full progress and by no means at its end. Therefore, all the quantitative data are of only relative value. It will be of interest to study similar cases raised to the end of development.

QUANTITATIVE VARIATIONS OF THE REACTION AND THE PROBLEM OF THE MECHANISM OF THE CORRELATION

The existence of a reaction of the nervous system to changes in the peripheral fields is proved for the chick embryo. The next problem to be approached is this: What kind of mechanism determinates this embryonic correlation? The problem is a complicated one; it comprises the following three questions:

1. In what way does the wing bud, as the acting system, exert its influence?
2. In what way does the nervous tissue, as the reacting system, answer the stimulus?
3. How is the stimulus transferred?

The first question concerns the role of the wing bud. How does it stimulate the central nervous system? Three possible explanations offer themselves.

1. The growing bud as a whole, in its capacity as a center of high physiological activity, stimulates the development of those parts of the spinal cord which are situated in its immediate neighborhood.

2. The wing stimulates the central nervous system by way of a 'reflex arc.' Only its sensory fields are the acting parts. They send nervous excitations centripetally to the spinal

ganglia and via the posterior roots into the spinal cord and thus affect the growth of the nervous tissue.

3. The action of the wing is not restricted to its sensory field, but every structure within the growing limb, muscles as well as sensory organs, send stimuli to the central nervous system. Each part of the peripheral field controls directly its own nervous center, i.e., the limb muscles affect the lateral motor centers, the sensory fields control the ganglia, etc.

Instead of discussing each one of these three assumptions, we will give positive evidence for the third one. The same material, with other experimental data available from the literature, will then enable us to rule out the other explanations. As was mentioned above, the hypoplasia in the different parts of the spinal cord was studied by cell counting. When the nine cases were compared, a high variation was found in the hypoplasia of the lateral motor group. The percentage of hypoplasia ranges from 61 per cent to 22 per cent. It was soon discovered that this variation is in quantitative relation to the degree of destruction of the muscles in the operated region. A loss of limb muscles with an additional loss of shoulder muscles corresponds to a high percentage of hypoplasia in the anterior horn, whereas in cases where the shoulder muscles are left intact, the anterior horn has a considerable size. In order to obtain exact data for this relation, the loss of the muscles in the different cases was measured quantitatively in the following way: the outlines of all limb and shoulder muscles of every section were drawn on cardboard by means of a camera lucida and the drawings were cut out and weighed. This was done for the right and left side of each individual and the weights of both sides were compared. Table 2 gives the weights in grams and the per cent of the loss in mass. If this percentage is compared with the percentage of hypoplasia in the anterior horn, a striking relation is to be found. The increase in the mass of muscles is closely proportional to the increase in number of lateral motor cells, one being almost a linear function of the other. For instance, case S. 96 shows a loss of muscles twice as high

as is shown in case S. 129; accordingly, the per cent of hypoplasia of motor cells in S. 96 amounts to double that in case S. 129; compare also case S. 118 with S. 150. These facts suggest very strongly the interpretation that the development of the motor horn is in quantitative dependence on its own peripheral field, the muscles. In one point, however, the relationship is not consistent. An almost radical destruction of muscles (in cases S. 200 and S. 118) does not result in the complete lack of an anterior horn; on the contrary, not less than 40 per cent of the motor cells are present under this condition. This amount of 40 per cent, apparently is developed independently of the peripheral fields and has to be regarded as an elementary constituent; only the growth of the horn beyond this primary size is under the influence of the limb muscles and follows the quantitative relation stated above (see discussion, p. 476).

The variation in the hypoplasia of the spinal ganglia is rather fluctuating in the single ganglia of the same specimen (compare table 2), and no general rule can be given. The sensory innervation of the operated region is abnormal and different in each case, and the ganglia 13 to 16 participate in this innervation in an irregular manner. However, if one calculates the average amount of hypoplasia for all ganglia of a given specimen and if one compares the nine cases, one will find a low variation ranging from 37 to 54 per cent (table 2). This variation does not show the least relation to that of the motor cells and has nothing to do with the size of the motor field. Incidentally, a high loss in the volume of the spinal ganglia is found in the case of least motor hypoplasia (S. 129), and a slight reduction in the ganglia is linked with the highest motor hypoplasia (S. 118). The situation finds its simple explanation in the assumption that the spinal ganglia are under the direct control of their own peripheral fields, mainly the sensory organs of the skin. In our experiments, the loss of skin is considerable, but almost the same in size in the different specimens. It involves the skin covering the wing and is independent of the presence or absence of

pectoral muscles. Therefore, we have to expect a considerable but not highly variable hypoplasia of the ganglia. The material is not favorable for proving whether this relationship is also of a quantitative nature. Experiments are planned in which measurable variations in the loss of skin area would be produced. Then, also, the question of proprioceptive fibers will have to be considered.

The mesial motor group gives a further illustration of the principle which we want to establish: that each peripheral area controls its own center. The mesial motor cells are the center of the axial trunk muscles: Since the primordia of these muscles are situated in the somites, they were not injured in the operation, thus they were fully developed in our cases. Therefore, we would expect their motor centers to be unaffected and that is exactly what appears in the cell countings.

The variation in the hypoplasia of the posterior horn is low, ranging between 13 and 21 per cent, and does not display any relation to that of the motor fields. It is not unlikely that the spinal ganglia have some influence on these centers, but the cases are too few to allow us to establish such a correlation.

With these data at hand it is not difficult to rule out the other two possible explanations stated above. A supposed mass action of the wing bud as a whole, affecting the neighboring level of the central nervous system would be expected to result in a general non-specific stimulation of the whole system. Yet the different centers within the nervous system react in a very specific way, each one typically different and independent of the others. The only way out of this difficulty would be to ascribe to each center its own intrinsic, characteristic manner of reaction. Such an assumption would be acceptable were it not in contradiction to our experience, that these specific reactions of the single centers (lat. motor group, ganglia, etc.) have something to do with quantitative changes in their own appropriate peripheral fields. Thus we are entangled in insoluble difficulties, whereas our first assumption gives a simple and intelligible explanation of the situation.

Another strong experimental evidence against a mass action is given in Detwiler's experiments in which a limb anlage was shifted several segments caudally ('23). In such cases not all the spinal ganglia of the trunk region in the neighborhood of the transplant were increased in size, but only those showed hyperplasia which were actually connected with the limb. The same is true in limb transplantations in chicks (Hamburger, '33, results unpublished). In one case studied in detail, an additional limb was transplanted in the neighborhood of the sixteenth to twentieth segment. Only the nineteenth and twentieth ganglia supplied the transplant with nerves, and only these two ganglia showed hyperplasia.

Arguments of the same kind invalidate the conception of a 'reflex arc.' If we presume that the stimulus enters the spinal cord only by the way of the spinal ganglia and the dorsal roots, it is difficult to explain in an intelligible way, why the motor groups vary quite independently of the spinal ganglia and evidently in dependence on the motor area. Experimental evidence against this view is given by Miss Shorey; she described two cases of somite extirpation in which the spinal ganglia had been removed incidentally ('09, p. 46). Although no sensory fibers entered the spinal cord, the motor column had reacted to the peripheral changes in the usual way. Furthermore, students of normal neurogenesis agree, that the peripheral outgrowths of the spinal ganglia cells are developed later than the first motor fibers; therefore, a reflex arc is not yet established at the time when the anterior horn is being formed.

Summarizing, we state that our present experimental evidence supports strongly the following conception of the mechanism of correlation. The different peripheral structures while growing, are in some direct connection with their appropriate centers in the central nervous system. Thus, they are enabled not only to control the growth of their own centers in general but even to regulate this growth in quantitative adaptation to their own progressing increase in size. This conception necessarily leads one to the interesting con-

clusion that, from the viewpoint of physiology of development, the central nervous system is, in some respects, not an integrated unit with its parts in close developmental correlation. On the contrary, at least the five parts marked in figure 4 are independent of one another in their variation (with the possible exception of ganglia and posterior horn) and separately linked to extracentral structures.

We have to discuss the other two problems stated above. It would be interesting to know in what manner the nerve cells respond to the stimuli arriving from the peripheral fields. The cells situated within the spinal cord have the following development: They undergo mitoses in the epithelium around the central canal, then start differentiation and while differentiating migrate to their final position. Any one of these three steps: proliferation, differentiation, migration, may be the point at which the extrinsic stimuli are interfering. We hope that a study of what happens in the nervous system during the first days after wing bud extirpation will throw some light on this question. The situation is somewhat different in spinal ganglia, where proliferation and differentiation probably occur at the same place.

Our experiments do not permit any conclusion concerning the nature of the stimuli involved in this correlation. Therefore, we will not discuss this problem but confine ourselves to raising the question of how the stimuli might be transmitted from the peripheral structures to the central nervous system? Miss Shorey ('09) advanced the hypothesis that the products of the metabolism of the muscles filter into the lymph surrounding the spinal cord and act as a stimulus. This suggestion makes it difficult to understand why certain parts of the spinal cord react and others do not. One would have to imply, either that several qualitatively different substances are released or that the different nerve centers answer specifically differently to the same unspecific stimulus. Even if we admit one of the possibilities, we have not yet explained why the centers react in quantitative relation to the growth of their fields proper. It is much easier to understand the

whole situation if one assumes a direct substantial bridge between each field and its own center; and the most suggestive assumption is that the nerve fibers themselves serve as mediators between the two links of the correlation. This gives a simple explanation for the strictly specific paths which the stimuli follow: from limb muscles to lateral motor cells, from sensory fields to spinal ganglia, and so forth. Such a notion does not necessarily imply the idea that the nerves are fulfilling this task by transmitting common nervous excitations. Such an assumption is not even very likely. For it has repeatedly been pointed out that functional excitations do not play any role in the early development of the nervous system (compare Coghill, '29, p. 83). However, the problem is not settled.

The foregoing discussion of the mechanism of correlation leads us to a hypothetical and provisional picture of the process which we may be allowed to outline in a few words.

Our starting point is the fact that at the time of the operation a number of neurones are already in the process of differentiation, and that their fibers, motor as well as sensory, have arrived at the base of the wing bud. We must charge the end organs of these first pathfinders with the double task of locating the peripheral field, and, in some way, 'reporting' back centripetally to the central organ the approximate size of the field to be innervated. The fibers would communicate the result of their exploration to their own cell bodies which thus would become the first relay station for the stimulus to be transmitted. Under the influence of the stimuli these nerve cells, which are not yet fully differentiated, when they have sent out their axones, would undergo a morphological or physiological change. One possibility of understanding this would be: Under normal conditions, that is, if plenty of muscle fibers have to be supplied, the exploring fibers ramify rapidly and abundantly. Consequently, their cell bodies proper will undergo further differentiation, whereas in the case of poorly developed peripheral fields some fibers may even undergo partial resorption and their respective cell

bodies be blocked in their further differentiation. By such a kind of mechanism, or by transmission of true nervous excitations or of substances, stimuli must be transferred to the growing nerve centers. These centers on their part would thus be put into a state of corresponding physiological activity and that condition would enable them to induce presumptive neighboring neuroblasts to join their group. (As pointed out, it remains to be settled, whether this latter effect is produced by inducing proliferation, differentiation or migration in the neighboring cells.) More and more cells would be added to each center as long as it receives stimulation from the periphery, and its growth would be blocked automatically at the moment when the peripheral stimuli stop.

THE SYNERGETIC PRINCIPLE IN THE DEVELOPMENT OF THE
SPINAL CORD

As has been pointed out, an almost complete destruction of the musculature of the limb and the pectoral region does not result in the complete absence of an anterior horn; in such cases (table 2, S. 200 and S. 118) a remarkably large number of lateral motor neurones, amounting to 42 per cent respectively 39 per cent of the normal number is found to form a typical horn, located in its normal position. M. Shorey ('09) has found exactly the same percentage of motor cells present under the same circumstances.

Some of the fibers sent out by these neurones have entered the few vestiges of muscles left in the operated region. But although these muscle remnants seem to be overloaded with nerves, it is not likely that they harbor 40 per cent of the fibers which under normal conditions supply a whole limb. It may be, that each fiber has formed less branches than it does normally; or that single fibers which cannot be made visible by the haematoxylin stain are spread over the operated region. Whatever solution this question may find, it is certain that peripheral factors cannot be held responsible for the formation of this large amount of 'useless' neurones. How shall we explain their presence?

A number of motor neurones were already differentiated when the operation was performed, and one might assume that still heavier defects would be obtained by operating in earlier stages. M. Shorey has done this experiment ('09, p. 45). The somites of the future wing region were extirpated in embryos incubated for 45 to 60 hours, and thus the development of somites and wing inhibited. The results were the same as in later operations: "even when the primordium of all the musculature of a single somite is destroyed some motor cells and motor fibers will develop within the medullary tube of this segment" ('09, p. 48). The same applies to the spinal ganglia, in which about 50 per cent of the cells are developed, even if the whole skin covering of the wing is absent.

It is not difficult to find the factors which in all probability are cooperating with those studied in our experiments. First, Coghill (summary, '29), studying the growth of the nervous system in *Amblystoma*, discovered in the spinal cord a definite pattern of localized centers of proliferation and differentiation which apparently is determined in the earliest stages of development of the medullary tube and which therefore has to be regarded as the fundament upon which all the later processes of growth are based. Second, Detwiler (summary, '33) has demonstrated, by exchanging segments of the embryonic spinal cord of different levels in *Amblystoma*, that the development of one part of the spinal cord is definitely influenced by neighboring parts. He thinks that it is likely that this correlation between neighboring segments is produced by intracentral ascending and descending fibers. That such intracentral correlations are at work also in the chick embryo has been proved by Williams ('31). He isolated the lumbar region of the spinal cord in a 56-hour chick embryo by extirpating a piece of cord anterior to this level and cutting out the tail bud. The ingrowth of fibers from anterior regions was prevented by putting small pieces of shell into the gap. A few animals were raised beyond hatching. Quantitative data are given for only one case: here the total number of cells within the gray matter was reduced by 41.4 per cent.

These data offer the simplest way to explain the presence of the large number of motor neurones (and of large ganglia) in spite of heavy peripheral loss: we have to ascribe an important determining role to both of these groups of factors which are at work in the central nervous system from the beginning of its development. They have to be made responsible for the pattern of distribution of the motor, sensory and correlation neurones, and they regulate the proliferation and differentiation to a large extent. The peripheral fields are nothing but an additional factor taking part in the control of the quantitative development of their own centers. The influence of these external factors is restricted in still another respect: they only take part in the growth regulation of the spinal ganglia and of the horns of the spinal cord, leaving the whole central mass under the exclusive control of the intracentral factors.

Thus our experimental results fit well into the general picture of the physiology of development of the central nervous system which is based on earlier studies. Its development follows the synergetic principle (Spemann, '31). Its final structure is the result of the cooperation of several factors each of which has its definite task in the determination and the control of the growth of the system. Our material is favorable in so far as it will allow us to determine the quantitative share of each of these cooperating factors.

DISCUSSION OF THE RESULTS OBTAINED IN OTHER EXPERIMENTS

Birds. As mentioned in the introduction, the original motive for carrying out these experiments was to verify the results obtained by M. Shorey in 1909. This became necessary because Detwiler, in his experimental work on the development of the nervous system of the urodele *Amblystoma*, obtained different results. It was desirable to find out whether there are really fundamental differences in the two groups with respect to the factors determining the nervous system and, if so, how the discrepancies could be explained.

The experiments described above repeat those done by Shorey as accurately as possible. The stage in which the operation was done, the area destroyed, and the age of the embryo when preserved were the same. A minor difference was that, instead of electrocautery, a mechanical method of extirpation was used which proved to be much more satisfactory. Miss Shorey's results are corroborated in every detail by a larger amount of material than she had presented. (She had described not more than three cases.) Our results are in agreement in the following points:

In both sets of experiments the extension of the muscle loss was fluctuating. In two of her cases considerable parts of the shoulder musculature had been left, in the third case "only the merest remnants are present" (p. 36). This latter case corresponds to the cases S. 200 and S. 118 of our experiment.

She found in all three specimens the right side of the spinal cord reduced in size. "The most evident difference is in the ventral horn, this being decidedly smaller throughout the operated region" (p. 34). By counting the anterior horn cells and measuring their size, she also found that this decrease is due to reduction in cell number and not in size of the single cell. Numerical data are given for two cases. The hypoplasia in the motor horn amounts to 50 per cent and to 58 per cent, respectively. The former figure refers to a case in which some shoulder muscles are present, the latter, to the one mentioned above, in which only the merest remnants are left. In comparing this latter case with my two cases S. 200 and S. 118 in which approximately the same amount of muscle fibers is present, we find the most striking coincidence: They display exactly the same percentage of hypoplasia, 58 per cent and 61 per cent, respectively. The spinal ganglia of her cases also showed a remarkable hypoplasia. Unfortunately, quantitative data are given in diameter and therefore not comparable to our figures. However, one point is evident. The hypoplasia is about the same in both cases, although the muscle loss is different. Thus two of our principal results are in full agreement: 1) that the size of of the motor region is varying

in relation to the degree of muscle loss, whereas the hypoplasia of the spinal ganglia remains constant; 2) that even in the case of practically complete loss of the peripheral motor field, nevertheless 40 per cent of the motor neurones develop.

In agreement with our findings, she also observed in her cases that "the loss in the posterior horn is less but still evident" (p. 35) and that the median part is not affected. Cell counts, however, were not made to confirm this statement. No attention was paid to the mesial motor group; but figures 15 and 16 of her paper show definitely that it was not at all reduced. Miss Shorey found no degenerating neurones and regards the effect as a typical hypoplasia; the same conclusion was reached in our studies.

As in the cases described in this paper, the brachial plexus on the operated side was normally formed and each muscle or part of a muscle present in the area of the shoulder girdle was innervated. Miss Shorey also had the impression that if only small portions of a muscle are left, they are overloaded by motor fibers, whereas muscles of normal size are supplied with the normal amount of fibers. "The smaller the muscle the greater is the proportional size of the nerve" (p. 50). This interesting point deserves further attention. Aberrant sensory fibers running to the skin of the operated region are not mentioned.

To summarize: The observations made by M. Shorey were confirmed in every detail and there is not the least deviation in the experimental results. Some of the conclusions which she derives from these results have already been discussed.

Mammals and man. Occasionally nature performs a similar experiment in mammals and man and there have been described a large number of cases with congenital deficiency of one or several limbs. A few students of such malformations were interested in the structure of the nervous systems. Their observations give us a welcome indication as to whether or not we can generalize from our results.

Messner ('09 and '12) presents a careful compilation and brief reports of the cases scattered in the literature up to that

time and I refer to his list of references. Among the twenty-odd cases mentioned by him, there are fourteen in which the histological details of the spinal cord were studied carefully enough to give a contribution to our problem. (Troisier, Pick, Edinger, Variot, v. Kahlden, Tschernitschoff (two cases), Souques et Marinesco, Klippel et Bouchet, Flatau, Perrero, Elders, v. Leonova—v. Lange, Salmons.) He adds five further cases of abrachia in goats and pigs studied by himself. Since that time, four more cases have been described: the studies of a case of bilateral abrachia in man by Curtis-Helmholz ('11) and of a unilateral abrachia in sheep by Krediet-Schultze ('26) and the papers of Strauss ('28) and Lukjanow ('29). Altogether twenty-three specimens have been described, including both human and mammalian malformations (calf, goat, sheep, pig), both complete and partial congenital lack of 1 to 4 limbs, both newly born and adult cases.

Fortunately, the authors are in agreement concerning the major points in which we are interested. They found, 1) that in cases of unilateral abrachia, one side of the swelling of the spinal cord (lumbar respectively brachial) was reduced in size, and that the whole cord was smaller in cases of bilateral deficiency. The motor horn was far more affected than any other part of the gray matter. In several cases the cells have been counted, and from the data given by Curtis-Helmholz, Messner, Krediet-Schultze, it is possible to calculate a hypoplasia of 77 per cent, 38 per cent, 60 per cent, respectively.

2) In ten cases the different cell groups of the motor region were studied separately (in mammals and man at least four groups can be distinguished, two lateral and two mesial ones) and in each single case the lateral groups were found to be affected and the mesial ones unaffected.

Cells of the mesial groups agree throughout in location, number and size with those of the controls. The lateral groups on the contrary do not at all correspond. The antero-lateral and postero-lateral groups of cells are enormously decreased in number, in striking contrast with the abundant number of cells in the antero-lateral and postero-lateral groups in analogous segments of the control cord (Curtis-Helmholz, '11, p. 332).

3) The posterior horn in every case was also found to be reduced in size but the hypoplasia was smaller than in the anterior horn.

4) The spinal ganglia were studied by only two authors. They found them to be hypoplastic.

5) In fifteen cases special attention was paid to the histological state of the neurones. This examination showed in twelve cases no trace of a degeneration or decrease in size of the neurones and only three authors (Edinger, v. Kahlden, Leonova) reported that they found degenerated and atrophic cells. However, the majority, and among them the most careful recent studies, are in agreement that the defect in the cord is entirely due to decrease in number of cells, which means that the effect observed is a primary hypoplasia.

It is obvious that all these data are in complete agreement with what we have reported for the experimental cases in chick embryos. We are inclined to extend to mammals and man our statement, that the peripheral fields control the development of the central nervous system. If we hesitate to take these teratological cases as a definite proof, we do so more for formal reasons, for conclusions concerning embryonic correlations which are based on malformations are always open to this objection: If two organs or systems are found to be deficient in a late stage of development, such cases allow a threefold interpretation. Either one of the two may have suffered a primary defect and may have influenced the other one, or both may have been influenced independently by a third factor. In our case we are justified in excluding one of the three possibilities; that the limb defect may be due to a primary defect in the nervous system, for we have experimental evidence that the limb is able to develop and differentiate even in complete absence of any nerve supply (for frog: Hamburger, '28; for chick: Hamburger, unpublished). On the other hand, many details are in favor of the view that the hypoplasia in the spinal cord is the direct result of a primary deficiency in the limb; for instance, the very specific, circumscribed losses in definite areas of the cord, which indi-

cate the same kind of correlation between special peripheral areas and their own centers that were described in our experiments—whereas a direct damage of the cord produced by a third factor would be expected to result in a more general, non-specific, perhaps not even unilateral, destruction.

To summarize: It cannot be proved beyond doubt, but is most likely, that in mammals and man the loss of a limb in embryonic stages results also in hypoplastic development of the spinal cord corresponding in all details to what we have found in the chick experiments.

Amphibia. In anurans, two authors, Dürken and May, have obtained a positive reaction of the spinal cord following changes in the peripheral fields. Both authors obtained reactions of the spinal ganglia also, which, however, will not be discussed here.

Dürken ('11) performed extirpations of one anterior and of one posterior limb bud, respectively, in larvae of the frog *Rana fusca*. The spinal cord was found to be decidedly smaller in the limb region of the operated side, and it is explicitly stated that the asymmetry is caused chiefly by the underdevelopment of the motor horn ('11, p. 240 and fig. 22, pl. 11). The posterior horn was also found to be hypoplastic, though in a lower degree. The formation of the plexus was normal on the operated side, but all the roots and the peripheral nerves were remarkably decreased in size (fig. 21, pl. 11). Thus far, the results are in agreement with those obtained in the chick, and different from the situation in *Amblystoma*. Dürken, furthermore, claims to have observed that the single neurones were smaller in size. However, no quantitative data are given.

May ('33) produced both peripheral defect and overloading by extirpating or transplanting hind limb primordia in the anuran *Discoglossus pictus*. In the first series of experiments, one hind limb anlage was excised in the early tail bud stage. The spinal cord was found to be asymmetrical in the lumbosacral region, and again, the motor horn was found to be most affected. Quantitative data based on cell counting of the

ventral half are given for three cases. They show an amount of hypoplasia of 20 per cent, 10.7 per cent and 8.6 per cent, respectively. These data, however, are of little absolute value, as only ten sections were counted in a limited level of the lumbo-sacral region. Still they show that the decrease in number is very low. This fact is doubly surprising in view of the amazing statement made by the author, that, with the exception of the poorly developed eighth nerves, the whole lumbo-sacral plexus is lacking. If this means that no nerves at all are formed, it would be of interest to know what has happened to the sensory and motor fibers of the neurones which, as the author shows, are present in so large a number. In another series of experiments peripheral overloading was produced by implanting an additional hind limb anlage near the normal hind limb region. Again the results are positive. Hyperplasia was found in the corresponding part of the spinal cord, and a distinct increase of motor neurones was established by counting cells.

In contrast to these results is the observation reported by Weiss ('31). He studied an adult frog which possessed two fully grown additional fore limbs on one side. To judge from the very complete formation of these limbs they must have originated in very early stages of development. All three limbs of the one side were innervated by the one normal brachial plexus. Despite this overloading, the roots and proximal parts of the nerves were not visibly increased in size, and the spinal cord was apparently symmetrical. However, no cell counts were made. The compensation for the added limbs was not obtained by increase of the number of cells but by increased ramification of the peripheral axones, as is shown by counting. Braus ('06), also, did not find a remarkable decrease in size in the peripheral nerves following unilateral extirpation of a fore limb bud in *Bombinator*. The situation needs further investigation.

Urodeles. Divergent in some important points from these data are the results obtained by Detwiler in his extensive studies on the development of the nervous system of the

urodele *Amblystoma*. The experiments have been reviewed by Detwiler ('26 a, '33) and by Mangold ('28) and need no detailed description. In the first series of experiments ('20 a and b, '23) the right fore-limb rudiment of an early tail bud stage was shifted one to five segments caudally and thereafter made nervous connections partly with original limb nerves, partly with spinal nerves belonging to the region of implantation. Both together formed a plexus and entered the transplanted limb, whereas the anterior limb nerves were deprived of their peripheral field. Thus the effect on the developing nervous system of the extirpation as well as the addition of a limb could be studied in the same animal. In the affected region cell counts were made in the two halves of the spinal cord and in the spinal ganglia of both sides; furthermore, the weights of paper models of the spinal ganglia, the gray matter and the dorsal and ventral roots were compared.

Concerning the spinal ganglia, Detwiler's results are in full agreement with those obtained in chick embryos. The brachial ganglia deprived of a limb undergo a hypoplasia of about 50 per cent, and those of the trunk region react to overloading with an additional limb with a hyperplasia of about 60 per cent. Detwiler had the same experience that we have when comparing the cellular hypoplasia and the loss of volume (weight) for the same pair of ganglia. He also found the percentage loss of volume to exceed that of cells ('20 a, '24 b). As pointed out, this may be due either to decrease of the size of the cells or to an extensive loss of the neuropil situated between the cells.

The spinal cord, on the other hand, did not display the least reaction in its rate of proliferation, either to excision or addition of a limb (numerical data given in '20 a, '23, '24 a and b, and '29). In *Amblystoma* the motor cells are not arranged in a ventral horn and not distinctly different in size from other cells; therefore, they cannot be isolated from other cell groups and cell counts were made of all cells of the spinal cord, or of its ventral section. These results were corroborated by comparison of the weight of paper models of

the same region. He checked the results in still another way: the ratios of cell numbers were calculated for segment III: VII, segment IV: VIII and segment V: IX of the spinal cord; segments III to V forming the brachial region, segments VII to IX being indifferent trunk segments. If this is done for two animals, one with both limbs intact, one with the right limb extirpated, the ratios are found to be practically identical ('23, table 7). It may be added that even after bilateral extirpation of the anterior limb blastema the typical number of cells was found in the brachial region of the cord. A third series of experiments approaching the same problem consisted in the complete extirpation of three somites in the trunk region of one side ('29). Again not the least hypoplasia was found in the level of the spinal cord normally innervating the operated region.

However, the neurones of the spinal cord are not completely unaffected by such radical changes in their peripheral fields. Both the size of the motor nuclei and the size of the motor (anterior) roots are reduced after a decrease in the peripheral field. The former point was established in the following way (Detwiler and Lewis, '25): The areas of the median plane of a large number of ventral neurones, most of them presumably motor neurones were measured by means of a polar planimeter. This was done in a case of unilateral and in a case of bilateral extirpation of a limb rudiment. The data obtained for different levels were compared. The figures revealed a reduction in size of the motor neurones amounting to 8 per cent in the case of unilateral extirpation and to 24 per cent in the case of bilateral extirpation. The weights of paper models of the motor roots differed from the normal by 24 per cent after unilateral excision and by 32 per cent after bilateral excision of the limbs ('24 b, '25).

The clear result of these experiments is that in *Amblystoma* the rate of proliferation within the spinal cord is not controlled by peripheral fields. Only the size of the neurones

and the numbers of fibers is to some extent affected by peripheral changes.⁴

It is evident that whereas the reaction of spinal ganglia to changes in the peripheral field is found to be identical in chick and *Amblystoma* embryos, the situation is very different concerning the spinal cord. How can this discrepancy be explained?

In our discussion we will refrain from taking into account the following possible explanation: in Detwiler's papers dealing with limb bud extirpation and transplantation, no data are given concerning the fate of those limb nerves which enter the limbless region; nor did he state whether or not part of the shoulder muscles had been developed. If this were the case, a number of motor axones would have made connections with these muscles, and the normal development of the spinal cord could at least in part be attributed to this fact. However, this explanation cannot hold for the experiments of myotomectomy. Here the operated region is described as devoid of any muscles with the exception of a few ventral muscles. Also the lack of hyperplasia of the cord following peripheral overloading does not agree with this interpretation. Therefore, we hope that we are justified in not considering seriously this explanation.

Another way of interpretation, though as unlikely as the one just mentioned, is this: the total number of cells within the spinal cord is certainly unchanged, but nevertheless, the number of motor neurones might be decreased and, as a compensation, the number of other neurones be increased, for motor neurones in *Amblystoma* cannot be definitely distinguished from other neurones. However, the equal number of

⁴ M. Shorey ('09, p. 57) reported three cases of unilateral extirpation of hind leg buds in *Amblystoma tigrinum*. They were studied 10, 14 and 33 days after operation; a slight hypoplasia was found in the ventral motor cells of the operated side. The difference in results may be due to the difference in species, the difference in the age at operation or the age when preserved or to the fact that she had extirpated hind limbs, whereas Detwiler's results were obtained on fore limbs. As the material is very small and no quantitative data were given, these results will not be taken into account.

large ventral cells in normal and operated animals invalidates such an interpretation.

Therefore, it is more likely that the discrepancy found in *Amblystoma* and chicken concerning the reaction of the spinal cord is not due to such masking effects but is of fundamental character.

For the convenience of the discussion, we will consider only the motor horn. Two different hypothetical explanations will be offered. The one is based on the outstanding morphological difference in the spinal cord of the two forms: In the chick, the motor neurones innervating the limbs are concentrated in anterolateral columns projecting from the oval outline of the gray matter: the motor (anterior) horn. In *Amblystoma* no such horns exist. The motor neurones, though also found ventrally and ventrolaterally, are mixed with other cells and do not differ markedly from other neurones either in size or in staining. According to Ramón y Cajal ('29, p. 39), the anterior horn is formed in chick embryos on the fourth day. The presence of such a horn during the early stages of development of the spinal cord in chick embryos might be likely to create a special situation in the system which is lacking in *Amblystoma*.

As has been pointed out, the experimental results suggest the assumption that the anterior horn is a system which under the influence of external stimuli is able to increase its own size by inducing proliferation or differentiation in neighboring neuroblasts or simply by attracting them toward the horn. The lateral motor group, then, would represent a center of high physiological activity, and, in this quality, would be an indispensable link in the whole mechanism of correlation. It may be that for the production of such an inductive effect, it is necessary that the neurones be aggregated in compact masses such as represented in horns, whereas neurones which are scattered over a large area and mixed with other cells would be unable to do so. Consequently, in *Amblystoma* with the horns lacking, the whole mechanism would be incomplete and no similar effect could be expected.

A second attempt to explain the situation is based on the following consideration: In *Amblystoma*, the motor neurones are located within the central part of the spinal cord and not separated from the correlation apparatus. Furthermore, Coghill ('13, '26, '29) has discovered that, in *Amblystoma*, the motor neurones in the early stages of their development actually take part in the function of integration. Each single cell is a motor cell and a coordination cell at the same time:

. . . . nerve cells are arranged in a longitudinal series in the spinal cord in such a way that they conduct excitations from the head tailward and that from these nerve cells side branches go to the muscle segments to excite them to contraction. . . . Having attached themselves to the muscle segment, . . . they, by other branches grow on beneath and beyond the muscle segment and invade the territory of the limb (Coghill, '29, p. 22, and figs. 9, 10).

Such primitive motor neurones were found even in older larvae which had begun to walk. It may be, then, that the motor neurones of *Amblystoma*, as a result of their original integrating function and of their central location in the spinal cord, are submitted to the same developmental physiological factors as are all the other correlation and commissural neurones and to no other. We know that even in the chick these latter neurones are exclusively controlled by intrinsic and intracentral factors and not at all influenced by peripheral factors (p. 476). Generally speaking, the interpretation would be this: groups of neurones which are located in a central position around the central canal are exclusively submitted to intraspinal factors. Groups of neurones, which have migrated away from the center (spinal ganglia, anterior and posterior horns, if present) would come under the control of the peripheral fields.

As a consequence of our assumption we expect that the motor neurones of the limb region of chick embryos never pass through a stage of double function but are from the beginning specialized, and localized peripherally. Indeed, the investigations of Ramón y Cajal (1890, '06, '08), who studied in detail the early development of spinal cord in chick em-

bryos, are in agreement with this. The motor neurones assume their typical form and location in the ventral horn from the fourth day (fig. 8, in Cajal, '29, p. 34). His many detailed data concerning the structure of the primary neurones and their axones and dendrites give no indication of a suspected integrative nature of these cells, and it is definitely stated: "Le cylindre axe ne se ramifie pas, pénétrant directement dans la racine antérieure" ('29, p. 138).

Both explanations, though differing in the developmental physiological details, are based on the assumption that the presence of the horns in the spinal cord of the chick and their absence in *Amblystoma* is the basic reason for the difference in reaction in the two forms. Such an interpretation would allow us to fit the different results obtained in *Amblystoma* and in higher forms into the general conception of the determination of the nervous system, as outlined above (p. 478):

We maintain that the major share of its determination and of its growth control is submitted to intracentral factors. The peripheral fields as an additional factor do take part in the growth control of their own centers. However, their role is restricted in a double sense: they control only quantitative characters, and this only above a certain threshold (a large number of neurones is formed in cases of complete lack of peripheral fields), and their influence is limited to spinal ganglia in all forms and to horns, if such structures are present.

SUMMARY

In order to study the influence of the peripheral fields on the development of the nervous system, the right wing bud was extirpated in 72-hour chick embryos. Nine operated specimens, 8 and 9 days old, were examined histologically. The results are as follows:

1. In addition to the wing, a more or less considerable amount of pectoral muscles had been removed in the operation.
2. A normal nerve plexus is formed on the operated side; but the distribution of the peripheral nerves, distally to the

plexus, is abnormal. All the muscles and rudiments of muscles which are present, are innervated. The skin of the operated region is abundantly supplied with nerves.

3. The spinal ganglia of the wing level of the operated side show a hypoplasia in volume of 37 per cent to 54 per cent on the average.

4. The right half of the spinal cord is smaller than the left side. Cell counts were performed separately for the different regions shown in figure 4. A marked cellular hypoplasia was found in the anterior horn (lateral motor group) and in the posterior horn. The mesial motor group, supplying the axial trunk muscles and the median part of the cord are not affected.

5. The problem of the mechanism of the embryonic correlation between the peripheral fields and the central nervous system was approached by comparing the quantitative variations in both systems. It was found that the number of cells in the lateral motor group varies in close quantitative relation, almost in a linear function, to the actual loss of the mass of muscles in the operated region. The loss of muscles ranges from 96 per cent to 31 per cent, the hypoplasia in lateral motor neurones ranges from 61 per cent to 22 per cent correspondingly. This fact suggests the idea, that each peripheral field controls the quantitative development of its own nerve center. The reactions of the other centers (spinal ganglia, mesial motor cells) support this assumption.

6. The stimuli going from the peripheral fields to their nerve centers are probably transmitted centripetally by the nerve fibers.

7. Even in cases of highest loss of muscles still a considerable number of motor neurones is formed. Also the spinal ganglia are well developed after a high decrease of the sensory fields. We conclude from this fact, that the peripheral fields are only additional factors, regulating the quantitative development of their fields and cooperating with intracentral mechanisms.

8. The results obtained in these experiments are in full agreement with those described by M. Shorey. Examinations of the nervous systems in cases of congenital abrachia in mammals and man justify the assumption, that in these higher forms the reaction of the nervous system to peripheral changes is exactly the same as in chick embryos. The results are partly in disagreement with those obtained by Detwiler in urodeles (*Amblystoma*). Two possible ways of explaining this discrepancy are discussed.

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