

CORRELATED ANATOMICAL AND PHYSIOLOGICAL STUDIES OF THE GROWTH OF THE NERVOUS SYSTEM OF AMPHIBIA

I. THE AFFERENT SYSTEM OF THE TRUNK OF AMBLYSTOMA

G. E. COGHILL

Department of Anatomy, University of Kansas

SIXTY FIGURES

The investigations upon the basis of which this paper is written have been in progress for several years. My purpose in the work and my general plan of study of the relation between the development of particular structures of the nervous system and the behavior of the embryo have been stated in several publications. In brief, my effort has been to analyze the function of the receptor system, to determine whether the development of movements is in a regular order or haphazard, to discover the exact relation of the nature of the stimulus to reaction, and to study the relation of behavior to the processes of growth and differentiation throughout the nervous system. Through such studies it has been expected that new light may be thrown upon the function of particular parts of the nervous system, the causal factors in behavior, elementary processes in the action of the nervous system and possibly upon fundamental problems of growth. That knowledge from this source may prove of interest to psychology also, is perhaps not too much to anticipate.

My earlier results were read, in part, before the American Society of Zoologists and the American Association of Anatomists in their Chicago meetings of 1907, and published a little later in the *Anatomical Record* (July, 1908) and in this *Journal* (June, 1909). These communications described the movements

of embryos of *Diemyctylus torosus* from the earliest responses to tactile stimulation up to the time when the embryo can swim, and showed that there are distinct types of movement which develop in a regular order of sequence till they culminate in locomotion. Later results from studies chiefly upon *Amblystoma* were presented before the American Association of Anatomists in the Cleveland meeting of 1912 and before the American Philosophical Society in Philadelphia, April, 1913. The part of the former report that related particularly to the motor column of the spinal cord was published in this Journal, April, 1913; the report before the American Philosophical Society is abstracted in *Science*, May 9, 1913 (p. 722). In these communications it has been emphasized that the earlier responses of the embryos under consideration are determined by the nature of the primary, or first reflex arc that becomes demonstrable by histological methods, that the progressive development of this arc determines the order of development of somatic movements and that the final common path of the reflexes stimulated from the various fields as the embryo develops becomes the nervous mechanism of locomotion.

These earlier presentations of my work have been made as preliminary to a more exhaustive treatment of the growth processes in the nervous system in their relation to behavior, and it is, accordingly, my purpose to make this communication one of a series which will treat in a more complete manner the particular phases of the problem in hand. As the first of such a series, this paper deals with the development of the sensory system of the trunk in its relation to the behavior of embryos of *Amblystoma* up to the swimming stage. The species used have been *A. punctatum* and *A. opacum*, but the latter only rarely.

I. THE ANATOMICAL PART

Embryos have been selected for this study according to the physiological standards described in my earlier papers, namely: (1) Embryos that move in response to an electric current but not in response to tactile stimulation with a hair or similar non-conducting and chemically inactive structure, designated as non-motile stage; (2) Embryos taken very soon after there is perceptible movement in response to tactile stimulation, designated early-flexure stage; (3) Embryos that move the trunk into a coiled condition, designated the coiled-reaction stage; (4) Embryos that move to the right and left simultaneously in different parts in a sinuous fashion, designated the S-reaction stage; (5) Embryos that have just acquired the power of locomotion through the serial S-reaction movements, designated the early-swimming stage.

For this study of the sensory system the S-reaction stage has been omitted from the series. This omission has been made because the important differences between the S-reaction and the early-swimming embryos relate to the commissural and motor systems rather than to the sensory system, and do not offer any distinctive contribution to the phase of the problem which this paper treats.

The histological methods employed in the preparation of the material for study are noted in connection with the descriptions of the several figures.

1. THE GIANT GANGLION CELLS

Since there are no perceptible dorsal roots from the fundaments of the spinal ganglia during the period under consideration, the giant ganglion, or Rohon-Beard cells obviously constitute the sensory system of the trunk at this time. It is with these cells, therefore, that this paper preeminently deals.

This series of neurones, which appears very early in the dorsal portion of the spinal cord of aquatic vertebrates, has been the subject of study on the part of anatomists from an early period

of comparative neurology, chiefly from the purely morphological point of view. Questions of homology, however, have no critical consideration in this paper. It is the relation of the giant ganglion cells to a functioning and at the same time differentiating nervous system that is here the center of interest. It is not the intention, however, to ignore the anatomical results of earlier authors on the subject, and a consideration of their results will be given farther on in the paper from the point of view of my own findings, both anatomical and physiological.

The following anatomical observations have been made by exhaustive study with the oil immersion lens systems (Zeiss and Bausch and Lomb) of serial sections of fifty-nine embryos selected from a collection of over two hundred such series because of their special adaptability to this phase of the problem. Of this number, thirteen are of the non-motile stage; seventeen, of the early-flexure stage; fourteen, of the coil-stage; ten, of the early-swimming stage. As representative of these neurones in the embryos of the non-motile stage over two hundred of the giant ganglion cells and fibers have been studied minutely in their various relations; of the early-flexure stage, about the same number; of the coil-reaction stage, approximately one hundred; and about an equal number of the early-swimming stage.

a. The distribution of the cells

The distribution of the giant ganglion cells has been studied exhaustively in one type specimen of each of the four stages of development, and their positions have been charted in graphic projections on the vertical plane as figures 56 to 59 of this paper. Exact counts have been made of the cells in these four cases only, but my study of numerous embryos convinces me that the number of the cells and their manner of distribution is fairly constant for each stage and that these features of the system, with minor qualifications as noted farther on, are typically represented in the figures cited.

The cells charted in the figures 56 to 59 are on one side of the median plane only, and, therefore, represent presumably about

one-half the number of the whole system in the spinal cord. By actual count there are on one side of the non-motile embryo 210 of these cells; in the embryo of the early-flexure stage, 222; in the embryo of the coil stage, 197; in the embryo of the early-swimming embryo, 253.

Except for the lower number in the coil stage there would appear in this series a gradual and progressive increase in the number of cells during the period under investigation. Indeed, such an increase in clearly differentiated cells probably occurs, for it is my judgment that the reduced number observed in the embryo of the coiled-reaction stage is due to the fact that the staining of this specimen is not well adapted to the differentiation of these cells from others around them. This is a misfortune from the point of view of this paper, and it should be mentioned that the four typical embryos were not selected for the projection of this system of neurones alone. They were selected upon more general considerations and the projections were made for the general purposes of the more comprehensive study that is in progress. The giant ganglion cells have simply been sketched into the projected outlines of the nervous and muscular systems which are being used for other phases of the work.

In the distribution according to regions it is found that within the range of the first eighteen post-auditory myotomes, which may be regarded as constituting the trunk, there are 149 giant-ganglion cells in the non-motile embryo; 150, in the early-flexure embryo; 128, in the coiled specimen; 176, in the embryo of the swimming stage. Caudad of this there are 61 in the non-motile; 72, in the early-flexure; 69, in the coil stage; 77, in the early-swimming stage.

Too great meaning should not be attached to the numerical details of these counts for, while the cells were counted with great care, the specimens were not prepared with a view to equivalent differential staining of these elements in all cases. It happens, however, that the youngest and oldest members of the series received essentially the same fixation and staining, and may, therefore, be regarded as of equal value for making satisfactory counts. The numbers of cells found, in these two specimens may

be regarded as trustworthy and of positive value. The other two specimens may be regarded as corroborative evidence with reference to the count and as valuable illustrations of the changes that are taking place in the distribution of the giant ganglion cells, particularly in the tail region, during this period of growth.

It is obvious from a glance at the graphic projections that there is in the youngest embryo of the series a quite regular distribution of the giant ganglion cells throughout the trunk region, excepting through the level of the first three or four myotomes. In the caudal region, on the other hand, they are thickly congregated throughout the extent of the segmented mesoderm and then more scattered, caudad. In comparison with this latter feature, the embryo of the early-flexure stage shows a more general scattering of the neurones through the cord of the lengthening tail bud, and this same process of distribution is seen in the two later stages. In the embryo of the coiled-stage the distribution is quite regular through the cord caudad to the level of the twenty-ninth myotome. This is the case, also, in the embryo of the early-swimming stage. Caudad of this in the coiled stage the cells are widely scattered through the level of the segmented mesoderm. In the embryo of the swimming stage, on the other hand, the cells are not distributed through the entire extent of the segmented mesoderm.

It seems obvious from this study of the numbers and positions of the giant ganglion cells that there can be little if any proliferation of cells belonging to this series during the period under consideration, and that the distribution of the cells through the caudal portion of the cord is brought about chiefly or wholly through mechanical processes allied with the differentiation of the mesoderm, and after the cells of the system have become differentiated into neuroblasts and neurones. The slight increase in numbers from 210 to 253 on one side should be regarded as due, not to proliferation of cells of the group, but to a progressive differentiation which enables the observer to recognize more of the cells with certainty. More detailed evidence on this point will be given in following paragraphs.

Another noteworthy feature in the distribution of the giant ganglion cells is conspicuous in these projections, namely, that the cells diminish in numbers rapidly from the level of about the fourth post-auditory myotome rostrad and assume a more ventral position in the spinal cord, while in the more caudal regions, also, there is a marked shifting of the cells ventrad. This dorso-ventral position of the cells in the rostral region might be explained as due to the proliferation of other cells dorsally, in relation to the development of the nuclei of the dorsal funiculi and allied structures; but no such explanation can apply to the ventral position of the cells in the caudal parts of the cord. In the latter region the ventral position of the cells is distinctly allied with the position of mesoderm, as the following description of cross sections will show. In fact, when the entire spinal cord is taken into account, the dorso-ventral position of the giant ganglion cells shows a more distinct correlation with the position of the mesoderm than it does with any structures in the cord. In following the further descriptions, one should keep this point in mind.

b. The giant ganglion cells as a sensory column

For the purpose of illustrating the relations of the giant ganglion cells within the spinal cord as a sensory column and the processes of differentiation that can be recognized in the system during this period of development figures 28 to 54 have been made with the aid of a drawing apparatus at a magnification of four hundred diameters (reduced to two hundred in the figures). This series of drawings is intended for use in future descriptions of the growth of the spinal cord and with this in view the other structures in the sections have been drawn with as much care as has been given to the giant ganglion cells. No detailed description of the other features of the cord will be given here, but a cursory examination of the figures will enable one to appreciate somewhat the setting of the functional sensory column in the midst of a mass of rapidly proliferating and differentiating cells which belong to other groups.

As introductory to the study of cross-section drawings of smaller magnification it will be helpful to examine figure 27, which is drawn at a magnification of 940 diameters. It is taken from the level between fifth and sixth myotomes of an embryo of the early-swimming stage. The unipolar condition of the giant ganglion cell in the latero-dorsal region is clearly brought out in relation to the sensory tract and the peripheral fiber. The latter projects out into the myoseptum. In the latero-ventral region motor neurones may be recognized, and, farther dorsad, a group of commissural or associative neuroblasts. There is no perceptible commissure at this level. In the latero-ventral angle of the cord is seen the motor tract, which is differentiated from the sensory tract by its more open or vesicular condition. This is probably due to its being composed of larger fibers than those of the sensory tract. The sensory tract is composed of a thin sheet of fibers situated immediately against the external limiting membrane of the cord and extending from the dorsal border of the motor tract to the process of the giant ganglion cell. Reference to figure 27a will show the dendritic nature of the peripheral process of the giant ganglion cells, since the contents of the perikaryon, the yolk spherules, are found distributed out into the fiber a considerable distance beyond the limits of the cord. Many more pronounced illustrations of this condition are found in my preparations.

Figures 28 to 54, now to be described, are taken from the same specimens from which the projections of figures 56 to 59 were made, and should be studied in connection with these projections. They should, also, be studied in groups according to the following considerations: figures 28 to 31 are from the level of the third myotome of the several specimens; figures 32 to 35, from the level of the eighth myotome; figures 36 to 39, from the level of the thirteenth myotome; figures 40 to 43 from the level of the eighteenth myotome; figures 44 to 47, from the level of the twenty-third myotome; figures 48 to 50 from the level of the twenty-eighth myotome of the three oldest embryos; figures 51 to 53, from the level of the thirty-third myotome of the same specimens; figure 54, from the level of the unsegmented

mesoderm immediately caudad of the thirty-seventh myotome of the oldest specimen. Figure 51, however, is in reality taken from the level of the unsegmented mesoderm immediately caudad of the thirty-second myotome. Grouped according to specimens, figures 28, 32, 36, 40 and 44 represent the embryo of the non-motile stage; figures 29, 33, 37, 41, 45, 48, and 51, the early-flexure stage; figures 30, 34, 38, 42, 46, 49, 52, the coil-reaction stage; figures 31, 35, 39, 43, 47, 50, 53 and 54, the early-swimming stage.

(1) *At the level of the third myotome.* In figure 28, which is taken from the level of the third myotome of the non-motile embryo two giant ganglion cells are found in the most lateral portion of the cord midway from its dorsal to its ventral limits. These cells are filled with yolk spherules (not represented in the figures) as are all the cells of this group in this stage. A granular pigmentation outlines the margins of the cells with their ventrally projecting processes. In contrast with the surrounding cells, the nuclear plasma of the giant ganglion cells takes on a faint tinge of the color used for counterstain of the cytoplasmic elements and the chromatin has a finer structure. In the latter part of the period under consideration the nuclei of motor and commissural cells take on these same characteristics in a considerable degree, but in this early period of development the giant ganglion cells stand out as clearly the most differentiated cells of the spinal cord. The processes of the giant ganglion cells at this level in the youngest embryo are directed ventral for a short distance, but it is impossible to detect long fibers here or the presence of longitudinal fibers of the system, either in transverse or longitudinal sections.

In the corresponding level of the early-flexure stage, represented in figure 29, the giant ganglion cell appears in a little more dorsal position. Although no fiber tract can be seen in relation with these cells in cross sections here, longitudinal sections show spindle-shaped processes projecting from the giant ganglion cells cephalad and caudad, forming a continuous column without a differentiated fiber tract. Such a fiber tract becomes perceptible,

however, at this level in the coiled-reaction stage (fig. 30). It is made up of a few fibers that are scattered along for some distance ventrad from the cells and immediately against the external limiting membrane of the cord. These fibers are barely perceptible in cross-section. The more dorsally situated have a perceptible inclination ventrad when viewed in the cross section of the cord and the ventrally projecting processes of the giant ganglion cells are in close relation with them. The more ventrally situated fibers, however, are cut in distinct cross section and form a differentiated longitudinal fiber tract.

At the level of the third myotome in the early-swimming embryo the sensory tract has extended farther ventrad. In fact it reaches almost or quite to the motor tract (*VT*) which occupies the most latero-ventral portion of the cord. In its dorso-ventral extension, however, the sensory tract is interrupted here and there by the neuroblasts of other groups. The giant ganglion cells in this case also lie dorsally of the tract and send their processes into its dorsal portion.

(2) *At the level of the eighth myotome.* In the non-motile embryo, at the level of the eighth myotome, the giant ganglion cells are situated in the dorso-lateral portion of the cord (fig. 32). In the cross-sections there are still no perceptible fibers associated with them. In suitable plane of section, however, a few fibers are demonstrable in a position just ventral of the cells. The direction of these fibers is rostrad and ventrad from the cells and they are obviously attenuated processes from these cells. Although not perceptible in transverse section their position has been sketched in on figure 32.

As compared with the non-motile embryo, that of the early-flexure stage shows a marked development of the sensory tract at this level (fig. 33). The characteristic process of the giant ganglion cell is here seen projecting ventrad towards the tract, the fibers of which are scattered along against the limiting membrane to near the motor tract. Study of sections adjacent to the one figured shows that the fibers are distributed sparsely through the region and that cells push in between them and

against the limiting membrane of the cord. In the section from which figure 33 was taken only the fibers figured nearest the giant ganglion cell could be detected. Those pictured farther ventrad in the figure were sketched in from nearby sections of the same series.

In the embryo of the coiled-reaction stage (fig. 34) the sensory tract is perceptibly strengthened as compared with that in figure 33. It appears as a continuous sheet of fibers from the motor tract dorsad to the giant ganglion cells, which are in this stage, also, located dorso-laterally. Just dorsad of the motor tract the sensory tract narrows down and disappears. In the early-swimming embryo (fig. 35) the sensory tract is difficult to outline clearly in this particular section on account of the neuroblasts which have invaded its territory, but in the opposite side of the cord in this section and in near-by sections of the series the sensory tract shows the same general relations as in figure 34, except that it is more strongly developed in the older embryo. The process of the giant ganglion cell in the figure reaches to the dorsal border of the sensory tract.

(3) *At the level of the thirteenth myotome.* At the level of the thirteenth myotome the giant ganglion cells still occupy a dorso-lateral position in the cord of all four embryos (figs. 36, 37, 38, 39), and their processes extend towards or into the tract. In transverse section no distinct fiber tract can be detected at this level in the non-motile embryo, but in favorable planes of section spindle-shaped and attenuated processes can be traced from the giant ganglion cells into an indefinite tract of short fibers. Its position is indicated in figure 36. In the embryo of the early-flexure stage (fig. 37) a giant ganglion cell appears in the lateral region of the cord, midway from the dorsal to the ventral borders. No sensory tract can be clearly differentiated in transverse section. In embryos of the coiled-reaction stage also no sensory tract can be detected in transverse sections. In favorable planes of section, however, there is unmistakable evidence of a small tract of fibers in the position indicated in figure 38.

In the early-swimming embryo the sensory tract appears clearly in cross section as shown in figure 39. It extends here from just dorsad of the motor tract to the ventral projections of the giant ganglion cells.

(4) *At the level of the eighteenth myotome.* At the level of the eighteenth myotome in the non-motile embryo the spinal cord frays out dorsally into neural crest structures (fig. 40). Scattered from the dorso-lateral angle of the cord ventrad to the level of the dorsal margin of the myotome are several of the giant ganglion cells, which are conspicuously differentiated from the other cells of the vicinity. In longitudinal sections cells in this position are found to have a definite orientation with their long axis longitudinal in the cord. Almost the entire cord, therefore, at this level is sensory. Some of the ganglion cells are in proximity to the skin, while others are quite as intimately related to the myotomes. These features of the sensory column will be brought out in detail in the latter part of the description.

Comparison of figure 41, which is at the corresponding level of the embryo of the early-flexure stage, with figure 40 shows a great change in the general features of the cord and in the position of the giant ganglion cells. The sensory column here occupies the dorsal half of the cord. No sensory fibers can be detected in it by the study of cross sections, but in longitudinal sections, a distinct fiber tract appears at the ventral end of the ganglion cells. Its position is sketched in the figure. The fibers of this tract must be short, however, for if they had great length they would be found farther ventrad in accordance with the relations in the more rostral portions of the cord.

In the corresponding level of the coiled-reaction stage there is still no perceptible fiber tract, but, again, in suitable plane there appears a sensory tract which is more clearly differentiated than the motor tract at the same level (fig. 42). In the embryo of the swimming stage the sensory column still occupies the dorsal half of the cord, and a giant ganglion cell is seen far ventrad (fig. 43). The sensory tract here is more clearly differentiated than in the younger embryo.

(5) *At the level of the twenty-third myotome.* In considering figure 44 one should note that, owing to the ventral curvature of the caudal region (fig. 56), this section is somewhat oblique and that the dorso-ventral dimension is therefore magnified somewhat out of proportion to the width. The extreme ventral position of the giant ganglion cells in the caudal region, and their proximity to the myotomes as well as to the skin is demonstrated here. Only two such cells appear in this figure but adjacent sections show that their distribution is general from the latero-dorsal angle to the latero-ventral angle throughout the caudal portion of the cord in this age. A comparison of figures 56, 40, 44, 19, 20, 21 and 22 will help to establish a clear idea of the exact relations in this part of the embryo.

In this level of the embryo of the early-reaction stage the ganglion cells are still found in the dorsal half of the cord with their processes extending ventrad. While no fibers can be seen here in cross section, there is in longitudinal section a perceptible tract that is made up of spindle-shaped processes of the ganglion cells. The sensory column is essentially of this same composition in the coiled-reaction and early-swimming embryos (figs. 46, 47), that is to say, it is made up of spindle-shaped processes of the cells and a few fibers that can be detected only in longitudinal section.

(6) *The sensory column at the level of the twenty-eighth myotome.* The non-motile embryo drops out of consideration here since it has only twenty-three well differentiated myotomes. In the early-flexure stage at this level the myotomes have assumed a ventral position relative to the spinal cord and the giant ganglion cells are accordingly found far ventrad in immediate proximity to the myotomes. Other sections show them distributed dorsally from this level to the dorso-lateral angle of the cord, though none appears in that region in the figure (48). The same features of distribution begin to appear, also, in the embryo of the coiled-reaction stage (fig. 49), but in the early-swimming stage the sensory column appears again in the dorsal half of the cord (fig. 50). Longitudinal sections show that in all these cases

the cells are oriented with their long axis longitudinal in the cord.

(7) *At the level of the thirty-third myotome.* Figure 51, as stated above, is drawn through the unsegmented mesoderm just caudad of the thirty-second myotome in the embryo of the early-flexure stage. The ventral half of the cord here is purely epithelial in structure and the myotomes are situated ventrally with reference to the more differentiated part of the cord. The ganglion cells are closely related to the mesoderm as well as to the skin.

In the embryo of the coiled-reaction stage (fig. 52) the sensory column consists of spindle-shaped cells occupying the dorsal half of the cord, with their long axis longitudinally oriented. The dorsal part of the cord here frays out into neural crest elements. In the embryo of the early-swimming stage (fig. 53) the attenuation of the spinal cord at the level of the thirty-third myotome is noteworthy. The sensory column in this stage, also, occupies the dorsal portion of the cord. The myotomes here extend well dorsad, but in figure 54, which is taken from the level of the unsegmented mesoderm just caudad of the thirty-seventh myotome, the mesoderm is found far ventrad. The cord here is much wider than it is five myotomes cephalad. In fact, a lateral distention of the spinal cord at the tip appears to be typical, and in many cases, particularly in the younger embryos, there is a perceptible tendency for the central canal to form ventricular evaginations. This condition may simply be the result of a stress upon the sides of the spinal cord exerted by the mesoderm in its growth, for, as shown in later descriptions, there is a region of firm attachment between the neural tube and the mesoderm in the caudal region during the early periods of development.

(8) *Generalization upon the basis of these descriptions.* The foregoing description of the sensory column at arbitrarily selected levels has been employed as a means of presenting briefly the results of exhaustive study of serial sections in various planes, and it is hoped, demonstrates sufficiently the basis for certain generalizations concerning the nature and differentiation of this

part of the spinal cord during the period under investigation. These generalizations may be stated as follows:

1. The giant ganglion cells and their processes constitute the sensory column of the cord and are the source of all the fibers of the sensory tract. (In the rostral region of the tract of the older stages there may be axones of the descending trigeminal tract, but this question will be critically considered in another paper).

2. In the more caudal portion of the several ages the column consists of bipolar cells oriented longitudinally in the cord.

3. In the youngest embryo the same condition prevails in the most rostral portion of the column, but in the older stages the fibers seem to incline more ventrad.

4. The axones of the cells are directed cephalad into the tract and those arising from more caudally situated neurones assume a ventral position with reference to the axones of cells situated farther cephalad, the tract during this time being a thin sheet of fibers immediately against the external limiting membrane of the cord, intercepted here and there in its dorso-ventral extent by neuroblasts of other groups.

5. The cells of the column have dendritic processes directed caudad, so that the tract is adapted to conduction rostrad.

6. There is progressive differentiation in the column, consisting chiefly in the growth of axones cephalad.

7. At the beginning of the period the cells of the column are already differentiated into neuroblasts or functional neurones, that is to say, proliferation of cells probably does not occur in this group during the period under consideration.

8. The growing, terminal portion of the cord throughout the extent of the unsegmented mesoderm at least, in so far as it is differentiated from an epithelial condition, is purely sensory, and the sensory column of the region occupies the whole lateral portion of the cord.

9. Throughout the extent of the trunk the neurones have acquired their typical position and orientation in the cord and, at least in the medial region, have established a sensory tract of

fibers a considerable time before the embryo can respond to tactile stimulation.

10. The dorso-ventral distribution of the cells of the column is definitely correlated with the position of the mesoderm.

c. The giant ganglion cells as peripheral nerves

Certain results of my physiological experiments, which will be described in the physiological part of this paper, have required, for their explanation, a critical study of the peripheral relations of the giant ganglion cells, since the dendritic process of these cells constitute the afferent peripheral system of the trunk during the period under investigation.

For the illustration of these relations drawings have been made with the Bausch and Lomb drawing apparatus at a magnification of 940 diameters and reduced to 470 in figures 1 to 27 and 27a.

(1) *The relation of the giant ganglion cells to the skin.* The relations of these cells to the skin is illustrated particularly in figures 8, 15 to 21, 24 and 25.

Figure 8 is taken from a series of horizontal sections, but at this level of the cord the section is obliquely transverse with the dorsal portion pitched caudad. It represents a ganglion cell fiber in the space between the last two myotomes in a non-motile embryo. Here a large fiber, with a broad conical base coming out of the cord, at the latero-dorsal angle, reaches directly to the skin. Its area of attachment is upon the thickened region of the skin that characteristically projects in between the myotomes. There is evidence of its branching immediately beneath the skin and having at least two terminals.

Figures 15 to 17 are drawn from three successive sections of a transverse series. They represent a fiber in the myoseptum between the thirteenth and fourteenth myotomes of an embryo of the non-motile stage. The particularly significant relations here are between the fiber and the myotome as the fiber leaves the cord, the intimate contact with myotomes as the fiber passes between them and the pseudopodial branching of the fiber im-

mediately beneath the skin. Filaments from this region reach to the skin; others are directed towards the myotome.

Another direct connection of the giant ganglion cell with the skin in a non-motile embryo is illustrated in figure 18. This fiber occurs between the ninth and tenth myotomes. As in the case of figure 8, the fiber connects with the projection of the skin into the space between the myotomes.

Figures 19 to 21 are intended to show the intimate relation that holds between the skin and the spinal cord in the caudal region of the non-motile embryo, and the place that the giant ganglion cells hold in this region. These figures are taken from the level of the unsegmented mesoderm. In figure 19 the skin is in immediate contact with the spinal cord. In the region of the most intimate contact, where there is no perceptible boundary between the two structures, is a small mass of densely fibrillated substance. This, upon being traced caudad, is found to be continuous with the giant ganglion cell shown in figure 20. Dorsad of this region of most intimate attachment is another attachment of smaller extent and less intensive adhesion. Still more dorsad is an attachment which consists of filaments between the two structures. That there is here strong adhesion between the skin and spinal cord is evidenced by the rent that appears between the outer and inner layers of cells in the skin. There has been from some source a pull upon the outer layer of epithelial cells, tending to separate the skin from underlying structures, but the adhesion of the inner layer of epithelial cells to the spinal cord has proved stronger than that to the outer layer of epithelial cells. On the opposite side of the cord, also, in this section is the same evidence of adhesion and pull. In this side, however, the skin is partially pulled away from the spinal cord so as to tear out some of the fibrillar structures. These retain their connections in the cord at one end while they are attached to the skin at the other.

In the next section caudad from that of figure 19 the more dorsal and smaller of the adhesions in this figure gives place to fibrillar strands between the skin and spinal cord, and pigment

granules appear in the connecting filaments. Some of these granules appear to be suspended in distended portions of the filament-like particles in a slender pseudopodium. They are near the spinal cord and have the same characteristics as the pigment granules that appear in the peripheral portions of the cord. This relation suggests that these filaments are outgrowths of the cells of the spinal cord and that the larger adhesions may be secondary and not primary arrangements of the parts.

The giant ganglion cell of figure 20 has already been mentioned as sending its axone into the area of adhesion just described. From the basal portion of its process arises a small filament which is conical at its base and the internal structures of which merge into those of the cell. Other small filaments, apparently of epithelial origin, occur just caudad of this (the section is inclined from the dorsal side cephalad and ventrad). Farther cephalad is one of the characteristic connectives between the spinal cord and the skin. In this is a small yolk spherule. This inclusion has important bearing on the nature of the filament, for yolk is exclusively intracellular in its early embryonic relations in these animals. Therefore, since there are no mesenchymal cells in the vicinity to which the filament can belong, it must be either an integral part of a cell of the spinal cord or skin, or a syncytial connective between cells of the cord and skin. Further evidence of the cytoplasmic nature of these connectives will appear in the following paragraph.

Figure 21 illustrates a region of contact between the spinal cord and the skin in which the adhesion of the giant ganglion cell to the skin is accidentally shown. The lower part of the figure is directed caudad, so that the process of the giant ganglion cell (the most caudal of a group of three) must be regarded as an ascending process. Here again the rent between the outer and inner layers of the epithelia of the skin shows evidence of a pull having been exerted upon the outer surface of the skin. This probably occurred in the manipulation of the cut sections while they were being adjusted upon the slide. The adhesion of the fiber of the giant ganglion cell to the skin is shown by its relation

to the rent portions of the cord, it being pulled away from the outer parts of the cord along with the external limiting membrane. In this case also there are on both sides of the area of adhesion filamentous connectives between the skin and spinal cord, and in some of them yolk spherules are found. It is of particular interest here to note, further, that there are filamentous connectives across the rent of the skin, between the epithelial cells of one layer and those of the other. These connectives have the same structural and staining characteristics as have the connectives between the skin and spinal cord. If our interpretation of the cause of the rent in the tissue is correct, these connectives across the rent in the skin were fixed by the solution in their natural relations and afterwards torn from their normal setting in the epithelial cells. In other words, they may be regarded as intracellular structures that have been torn from their natural relations after fixation. In structure and staining reactions, then, the connectives between the spinal cord and skin have the appearance of cytoplasm.

Such adhesions as have been described between the skin and the spinal cord occur, during the period under investigation, only near the end of the tail bud, and particularly in the younger stages.

Figure 24 illustrates the manner of distribution of the fibers of the giant ganglion cells beneath the skin in the embryo of early-swimming stage. Mesenchyme cells are abundant in the vicinity of this fiber but its course is largely free from contact with them. Comparison of this with figures 8, 17 and 18 will give some idea of the changes that have taken place in the peripheral relations of these fibers in the transition from the non-motile to the early-flexure stage. In the later stage the individual fiber has wider extension or distribution than in the earlier condition, although in the latter there is connection with the skin on the part of these fibers throughout the extent of the segmented mesoderm; while in the more caudal region, as has just been shown, the relation between the giant ganglion cells and the skin in regions of adhesions is of such a nature as to admit of

stimulation of the cells of the sensory column through the skin. In figures 23 and 25, taken from farther cephalad in the embryos of the early-flexure stage, are shown the relations that such fibers as are illustrated in figure 24 assume with mesenchyme cells beneath the skin.

(2) *The relation of the giant ganglion cells to the myotomes.* Figures 1, 3, 4, 7, 10, 14 and 26 have been selected to illustrate the relation which the ganglion cells hold to the muscular system.

Figure 1 shows this relation in the embryo of the non-motile stage, at the level of the sixth myotome. This fiber projects from a giant ganglion cell through the dorso-lateral border of the cord and applies itself immediately to the caudal end of the myotome. Through the region of this contact it sends spinous processes in between the cells of the myotome. This is essentially the relation seen in figure 15, only from a different point of view. The relation of the fiber with the myotome is just as intimate as is the relation of any of the cutaneous fibers to the skin. The fiber is not simply passing across the surface of the myotome in close contact, but it has clearly differentiated processes that pierce the myotome. Similar relations are shown in figure 3. This contact also is with the end of the myotome. The peripheral fiber is here clearly seen to be a process from the same cell that sends its axone into the sensory tract.

In figure 4 appears an important bit of evidence on these fibers to the muscular system. Here at the level of the twelfth myotome, a fiber projects latero-dorsad till it emerges from the cord, then, instead of taking a short course through an open field to the skin, it swerves abruptly ventrad to the border of the myotome, upon which it spreads out in a disc-like terminal. It is difficult to conceive of any reason for a skin-sensory fiber to behave in this manner.

Figures 10 and 11 are taken from successive sections passing between the levels of the fifth and sixth myotomes cephalad of the unsegmented mesoderm in a non-motile embryo. Here the myotomes are closely pressed against the spinal cord, and the nerve fiber emerges from the cord at the dorsal border of the myotome. In both the sections the expansion of this fiber upon the

myotome and its projections in between the myotome cells are clearly shown. In figure 11 the relation of the terminal as it applies itself closely over a yolk spherule, partially encircling it, is typical of these endings. In this figure also the process from the same cell can be seen ascending into the sensory tract.

Figures 7, 12 and 13, taken from an embryo of the non-motile stage, illustrate the terminations of these fibers upon the muscles by smaller branches. In figure 7 there is a terminal upon one myotome and branches out towards the other; in figure 13, there are terminals upon both myotomes. Figure 12 is introduced because it represents one of the clearest cases of the relation of these finer branches of the nerve fibers to the myotomes. Within the cord, it should be noted, the process of the cell projects towards the myotome directly. Immediately after emerging through the external limiting membrane it divides into a more dorsal and a more ventral division. The destination of the dorsal division is not certain, although it apparently sends filaments into the myotome. The ventral division, however, sends a process directly against the myotome, where it spreads in both directions and pierces the myotome by fine, spinous processes. There can be no doubt about the origin and nature of this fiber and the only reasonable interpretation is that it, in part, terminates in the myotome as indicated in the figure.

Figure 14 is taken from the level between the second and third myotomes cephalad of the unsegmented mesoderm, in an embryo of the non-motile stage. It shows how the myotome at this level is closely pressed against the spinal cord so that a mere line marks the boundary between the structures. At the dorsal border of this contact a giant ganglion cell fiber emerges from the cord against the myotome at its end. As it passes across the end of the cells it sends the characteristic spinous processes into the myotome. Farther out it spreads out into an amoeboid film, which is applied to the myotome at one edge and extends out towards the skin at the other.

In figure 26 is shown the only case which has been observed where, by chance, the nerve fiber to the myotome has been disconnected, in part, from the myotome so as to show clearly the

nature of the terminals. The section from which this figure is taken passes between the sixth and seventh myotomes of an embryo of the coiled-reaction stage. The fiber here represented passes latero-dorsad from the border of the cord to a mesenchyme cell, where it branches and sends one division directly against the end of the myotome. At the end of the fiber it sends out claw-like processes into the myotome. But the most striking part of the structure is the series of spinous projections which beset the fiber through the last part of its course. These processes are clearly continuous with the substance of the fiber itself, and, by shifting the focal plane, one can clearly determine that they project outward from the fiber and upward in the preparation towards the observer. In this way they can be traced out into fibrillar structures that reach the limits of vision with ordinary oil immersion lens systems. To complete the picture, however, one must appreciate that the adjacent section of the series shows that the myotome, seen here only in contact with the end of the fiber, is shifted over the position of this fiber; so that these projections are clearly seen to be the terminal arrangement of the fiber upon the surface of the myotome. The spines along the border of the fiber are of the same sort as those at the end, but have been lifted away from their normal relation with the myotome. This preparation completes the picture of such conditions as are partially shown in figures 7, 10, 11, 12, 13 and 14.

The intimate contact between the spinal cord and the myotomes has already been mentioned. This relation is seen in its most complicated form in the caudal region of embryos of the non-motile stage, and is pictured in figure 22. This section is from a horizontal series but, with reference to the spinal cord in this region, it is directed obliquely from dorsad, cephalad and ventrad. The relation between the spinal cord and the myotome is here essentially the same as that figured between the skin and the cord in figure 19, only it is even more extensive. In many regions of this contact there is no perceptible border line between the cord and the myotome and the structures of one seem to pass over among those of the other and become indistinguishable from them. In the most dorsal portion of the area of adhesion is

found a giant ganglion cell which sends its process out into one of these fused region. In other words, this ganglion cell holds the same relation to the myotome as does that of figure 21 to the skin. Figures 21 and 22 are taken from the region of the unsegmented mesoderm of the same embryo.

In conclusion upon this phase of the work it may be confidently stated that the giant ganglion cells are muscle-sensory as well as skin-sensory in function. In fact the study as a whole gives me the impression that the muscle-sensory elements are more highly differentiated in the youngest stage studied than are the skin-sensory elements, but of course there is no means of determining this mathematically. In the youngest stage of this series both systems are established in their terminal relations with the peripheral organs.

(3) *The innervation of both skin and muscle by the same neurone.* Since the neurones of the sensory column innervate both muscular and cutaneous organs a question arises concerning the possibility of differentiating two sets of neurones in the system, the one innervating the skin and the other supplying the muscular system. The proposition of undertaking to make such an analysis of the system, however, is met with the unmistakable evidence that a single cell of this group, in many cases, terminates both in a myotome and in the skin. Several figures have been made to illustrate this relation.

Figure 2 is taken from the section adjacent to that of figure 1, and shows the continuation of the fiber, which has terminals in the myotome in figure 1, on out to the skin. Figure 6, in like manner, is from the section adjacent to that of figure 5. A composite of the two figures would give unmistakable evidence of one fiber branching and sending one process to the skin and the other to a myotome. In figure 9 this relation appears in one and the same section. Here the branching occurs near the skin and the branch that goes to the myotome has the characteristic ending described above in connection with specific treatment of the relations with the myotomes. Figure 8 is from the section adjacent to that of figure 9 and shows another branch of the same neurone terminating in the skin. The fiber of figures 15,

16 and 17, taken from successive sections also has the appearance of innervating the myotome at its base where it leaves the cord in figure 15, while it sends terminals to the skin in figure 17. In figure 23 there is a clear case of a large fiber from a giant ganglion cell sending a branch directly into the myotome, as it passes across the ends of the cells, and continuing on out to the skin. In figure 25 the giant ganglion cell fiber can be seen to pass through a groove in the end of the myotome and from this position to send a characteristic spinous process in among the cells of the myotome. The fiber in its further course branches out to the skin.

(4) *The number of peripheral fibers.* No special attempt has been made to count the number of peripheral fibers in the giant ganglion cell system, but some data on this point, extracted from my records of the general study, may be of interest.

The total number of fibers that were noted as adapted to special study and for written records was 186 in the thirteen embryos of the non-motile stage, and the largest number for any one specimen was 50. For seventeen embryos of the early-flexure stage the corresponding numbers are 185 and 57. In fourteen embryos of the coiled-reaction stage the total is 87 and the maximum in a single specimen, 28. For ten specimens of the swimming stage, the numbers are 115 and 36.

Further examination of my notes on the study of the giant ganglion cells and their fibers shows that of the 186 peripheral fibers studied specially in the non-motile embryos, and of the 185 studied specially in the embryos of the non-flexure stages, 136 in each case pass out over or in near relation to the ends of the myotomes. In the non-motile embryos, according to my judgment in studying from section to section 38 fibers were traced to the skin, 34 were regarded as terminating in the myotomes, and 20 had the appearance of ending both in the skin and in the myotomes. In the earlier embryos practically all the fibers observed had intimate relation to the ends of the myotome. In the older embryos occasional fibers, particularly in the more rostral regions, were observed to pass out directly to the skin over the middle part of myotomes.

Although the numbers tabulated above are not the results of a specific effort to count the fibers of this system, yet my impression, based upon my knowledge of the nature of all the material studied and the methods of study employed, is that it may be correctly inferred from them that there is no appreciable increase in the number of peripheral fibers in the transition from the non-motile to the early-flexure stage, that almost all the fibers in the younger stages pass out over or in close relation to the ends of the myotomes, and that, in the older stages, there are more fibers passing out to the skin over the middle portions of the myotomes.

In considering the numbers of fibers observed in the older embryos it must be kept in mind that there occurs during this period a rapid development of other structures that obscure the finer relations of the fibers. There results from this factor a much greater difficulty in following the fibers or detecting their presence and, to my mind, this explains why the maximum number of fibers recorded for any one individual of the two older stages is smaller than the maximum number found in the two younger stages.

(5) *Summary.* A summary of the peripheral relations of the giant ganglion cells may be outlined as follows:

1. The giant ganglion cells innervate both skin and muscle.
2. A single neurone of the system may innervate both skin and muscle.
3. These relations with the skin and myotomes become established throughout the extent of the segmented mesoderm some time before the embryo responds to tactile stimulation.
4. In the level of the unsegmented mesoderm the spinal cord and the giant ganglion cells have strong adhesions both with the skin and mesoderm, the substance of the cord in restricted regions becoming indistinguishable from the skin on the one hand and from the mesoderm on the other.
5. While there is no positive proof that there is an increase in the number of fibers to the skin during the period under investigation, there is clear evidence of a progressive differentiation and growth of the fibers, particularly in the extension and elaboration of the sub-epithelial plexus so that a single fiber acquires a

wider distribution, and in the association of the fibers with mesenchymal cells.

6. The great majority of the fibers of this system pass out of the cord into intimate relation with the ends of the myotomes. There may be in the later stages an increasing number that pass out to the skin over the middle portion of the myotomes or relatively free from them.

2. THE SPINAL GANGLIA AND THE LATERAL LINE ORGANS

That the anlagen of the spinal ganglia have no perceptible dorsal roots during this time has already been mentioned. Their condition in embryos of the early-swimming stage may be illustrated by the section through the fourth ganglion in figure 27. The cells here are closely crowded together into a compact group with definite border, but there are no clearly differentiated neurones among them. Comparison of the members of the series that appear in an embryo of this age shows that the anlagen of the ganglia which are situated in what may be termed the cervical and lumbar regions are larger than those in the mid-trunk region and farther caudad. This is obviously in anticipation of the sensory innervation of the limbs.

With view to a description of the lateral line system in a future paper the primordia of the lateral line organs of the head have been projected upon figures 56 to 59 along with the primordia of the organs in the trunk. Only a brief description of the distribution of these primordia in the trunk, however, will be given here, since a knowledge of their distribution and various experimental data have made it possible to eliminate the consideration of the lateral line organs from the fundamental physiological problems that constitute the real occasion for the anatomical part of the paper.

The appearance of the primordia as seen in section through the skin is represented in figure 27, which shows one of the structures at the level of the fifth or sixth myotome in an embryo of the early-swimming stage. The primordia of the youngest embryos of the series are not so clearly differentiated.

In the embryo of the non-motile stage no lateral line primordia occur caudad of the third myotome. Lying over the second myotome laterally, and extending partially over the first and third, is a broad primordium (fig. 56, *Po. LL. 2*), which lies immediately dorsad of an ectodermal thickening allied with the visceral system. In the embryo of the early-flexure stage (fig. 57) there are two such primordia, one (*Po. LL*) situated over the first myotome, and a long primordium (*LL*) extending over the second, third, fourth and fifth myotomes. The latter is constricted in its rostral portion, with apparently the tendency to separate into two primordia. In the embryo of the coiled-reaction stage the smaller primordium of the last stage seems to have coalesced with another which lay near the caudal border of the auditory vesicle, to form a single large primordium which extends from the auditory vesicle to near the second myotome. Farther caudad there are two primordia instead of one, as in the younger stage. The more rostral of the two (fig. 58) is short and lies over the third and fourth myotomes. The other extends through the level of the seventh, eighth, ninth and tenth myotomes.

As compared with the last embryo the oldest of the series (fig. 59) shows a considerable differentiation of the system of primordia. There is one large primordium over the first myotome, in addition to the one slightly cephalad and extending over the auditory vesicle. Ventrad of the second myoseptum is a small primordium (*Inf. LL*) which is situated just behind the external gills. It presumably represents the inferior line. In the position of the lateral line proper there are four distinct, short primordia scattered along over the eighth to twelfth myotomes, and a long one extending continuously through the thirteenth to seventeenth myotomes. In this embryo a dorsal group of primordia also appears, over the fourth, fifth, sixth and seventh myotomes, near the level of their dorsal border.

II. THE PHYSIOLOGICAL PART

In the treatment of the physiological part of this paper it is necessary, first of all, to show that the results of the various experiments which are to be discussed have to do with the afferent system of the trunk as opposed to that of the head, since the scope of this paper is explicitly limited to the problems of the sensory system of the trunk.

As already noted in the anatomical part, the definitive spinal nerves have no place in this problem since there are no dorsal spinal roots during the period under investigation. There is, however, more or less overlapping of the sensory field of the giant ganglion cells by the lateral line nerve and possibly by the cutaneous component of the vagus, and this community of area in distribution of the nerves from the two regions requires particular analysis. The anatomical details of this relation are left for a later consideration of the cranial system of nerves; but the distribution of the lateral line primordia has been given in the anatomical part of this paper, and the distribution of the general cutaneous component of the vagus has been described in my earlier studies on the cranial nerves of larval *Amblystoma* ('02).

Upon the basis of anatomical facts from these sources, one is able, by the simple experiment of transecting an embryo at about the level of the second myotome, to convince himself that the cranial nerves mentioned do not play any distinctive part in the reactions to stimulation upon the trunk, for the trunk of an embryo that has been transected in the manner indicated exhibits all the peculiarities of irritability that characterize the normal embryo. Indeed, there is not in all my experimental work upon the subject any evidence that the lateral line system during this period of development influences behavior in any way, or that there is any difference between the cranial nerves and giant ganglion cells as regards cutaneous irritability. There is no occasion, therefore, to question the validity of the experimental evidence to be presented in this paper concerning the receptive functions of the giant ganglion cells as the afferent system of the trunk.

1. THE REFLEX MECHANISM

In my earlier paper on *Diemyctylus* ('09) it was shown that trunk movements in response to stimulation upon the caudal portion of the trunk as well as to stimulation upon the head are initiated in the rostral portion of the musculature and that they progress from this region caudad as a wave of contraction through the myotomes of one side. While it is difficult or impossible to detect this characteristic of movement in amphibian embryos that develop rapidly and move quickly, the same cephalo-caudal progression of contraction clearly occurs in *Amblystoma* as has been described for *Diemyctylus*.

This feature of behavior is not only explained but made necessary by the fact that the motor innervation of the myotomes is by collaterals of neurones that constitute a continuous motor column in the spinal cord of the embryo, as shown in my paper on this subject ('13). My anatomical studies establish such a motor tract and column in the latero-ventral portion of the spinal cord. Numerous of my experiments corroborate these anatomical findings. By piercing the embryo, for instance, or by cutting it through from the ventral side so as to sever the ventral part of the cord while the dorsal portion is left intact, cephalo-caudal conduction, as evidenced by muscular contraction, has been intercepted without interfering with conduction caudo-cephalad past the lesion. In like manner, by inflicting a cut into the dorsal part of the cord, conduction caudo-cephalad has been intercepted without interference with the conduction cephalo-caudad past the lesion. In the former case, the muscle wave affects only the part cephalad of the lesion when the stimulus is caudad of the lesion; in the latter case, the entire trunk contracts in response to a stimulus that is applied cephalad of the lesion while no response at all can be elicited by stimulation caudad of the lesion. These experiments, although they do not establish the exact dorso-ventral extension of the tracts, certainly furnish ample physiological corroboration of my anatomical results, namely, that the most ventral part of the cord is motor and that the dorsal

portion is sensory. Furthermore, that the sensory and motor tracts of the same side are physiologically distinct and separate structures throughout the greater part of their extent is proved by the fact that the caudal piece of embryos that have been transected at certain levels, varying with the age, have no power of response to stimulation, either by tactile or chemical means. In fact, under normal conditions, the sensory and motor tracts of the same side seem to be absolutely isolated from one another physiologically, for reaction to a stimulus on one side of the embryo is typically followed by a contraction in the muscles of the opposite side, as my earlier work on *Diemyctylus* showed and as my later experiments on *Amblystoma* confirm. The explanation for the apparent exceptions to this rule in response is mentioned later on in connection with the proprioceptive functions of the giant ganglion cells.

The correlation of anatomical and physiological evidence, therefore, gives ample justification for interpreting the reflex mechanism of the trunk of these embryos as made up of a dorsal afferent system consisting of the giant ganglion cells, of a ventral motor system composed of a continuous, conducting column of neurones which innervate the muscles, and of an associative system, which, in the rostral portion of the cord, connects the afferent system of one side with the motor system of the other.

2. THE RECEPTIVE FUNCTIONS OF THE GIANT GANGLION CELLS

a. The interoceptive field

There is no evidence that the giant ganglion cells have an interoceptive field of stimulation or that the embryo of the age under consideration is influenced through any medium by its entodermal surfaces. The mouth is not formed till long after the period and the entodermal surfaces are not accessible to the typical stimuli of later life. The cloacal aperture of the archenteron is, of course established earlier, and it is conceivable that in the use of soluble substances for stimulating agents, these might diffuse into the cavity at a very slow rate and in exceedingly

minute quantities, but there is no suggestion of an influence from this source upon behavior, or of any nervous connection that could conduct stimulation from the field of the entoderm in the trunk to the spinal cord.

b. The exteroceptive field

The exteroceptive field of the giant ganglion cells must be considered with reference to tactile stimulation and chemical action.

(1) *Irritability to tactile stimuli.* From the later treatment of the action of soluble substances upon the skin it will be understood that tests for tactile irritability must be made with instruments that are chemically inert in water. As such a stimulating instrument, a hair, conveniently mounted in a holder, has been used in all my experiments, a method described in my earlier papers and adopted by Hooker ('11) to differentiate between tactile stimulation of the nerves and direct stimulation of the muscles.

The anatomical part of this paper shows that the cutaneous fibers from the giant ganglion cells are established in their typical relations with the skin in the non-motile embryo. Accordingly, it is found that when response begins, that is to say, in the early-flexure stage, irritability to tactile stimulation appears regularly over the trunk without any perceptible differentiation in cephalocaudal levels. In embryos of the early-flexure stage such sub-epithelial fibers as are shown in figure 24 are common, and, as is well known from the work of various authors which my own observations confirm, the fibers of some of the giant ganglion cells turn ventrad around the lateral border of the myotomes and pass far towards the ventral surface of the animal. Such structures as these account for a comparatively regular irritability over the entire surface of the trunk and tail bud, even to its tip. And as the dorsal and ventral fins expand into thin laminae these are irritable to their very margins.

In the very early-flexure stage response is irregular and uncertain when the stimulus is applied as a light touch to a single spot in the

skin, whereas the threshold of stimulation at such a time is much lower when the tip of the hair is moved gently over the surface of the skin. This is a conspicuous feature in the behavior of these embryos in the early period and is obviously to be accounted for upon the principle of summation of subminimal stimuli or alliance between reflexes. In the movement of the hair over the surface of the skin several giant ganglion cells, such as illustrated in figure 18, would be excited, or several endings of a single neurone such as that illustrated in figure 24. In case the stimulus excites more than one neurone, summation would seem to occur in the associative center; in case there is excitation only of several endings of a single neurone the summation phenomena could be referred only to the individual ganglion cell. It is reasonable to infer, however, that the movement of the hair upon the skin in the direction of the longitudinal axis of the embryo would stimulate numerous endings of several neurones, and that, therefore, there are exhibited here in this primitive reflex mechanism both the summation stimuli in the giant ganglion cells and the alliance between afferent stimuli in the associative center. There is nothing in this experiment upon which positively to base a differentiation between summation in the peripheral neurone and alliance of reflexes in the associative center; but since an experiment to be described later demonstrates conclusively the phenomena of antagonism between reflexes in these embryos there is strong presumption in favor of the idea that there is here effected an alliance between neurones that have been peripherally stimulated at intervals, for as the hair moves over the skin there is extension of both time and area of stimulation.

(2) *The action of hydrochloric acid as a stimulating agent.* Sheldon's work upon the reaction of the dogfish to chemical stimuli ('09) called my attention to the desirability of extending my studies on amphibian embryos to their reaction to substances in solution, with a view to correlating the action of chemical stimuli with definite structural elements in the nervous system. During several seasons, therefore, my attention has been given in part to experimentation upon this phase of the problem.

Preliminary experiments showed that response could be elicited by the use of the various substances to which the dogfish reacts in Sheldon's experiments, and that the action of the various inorganic acids is essentially the same so far as response is concerned. To simplify the experimentation, therefore, hydrochloric acid was adopted as a type of chemical stimuli. According to Sheldon's procedure a normal solution of the acid was prepared by titration against a gram-molecule solution of an alkali. From this stock of normal hydrochloric acid the various dilutions used as stimulating agents were made.

My first experiments consisted in spraying the solution against the embryo. As a means of applying such a stimulus with precision, pipettes were made from pieces of glass tubing, upon one end of which a very thin-walled bulb was blown while the other end was drawn out into a very fine capillary aperture. With the acid enough methylene blue was mixed to give it a perceptible color, so that the diffusion of the substance through the water could be followed under the microscope (the Zeiss binocular being used in these experiments). By this method a very fine jet of acid could be applied to restricted areas of the skin as comparatively localized stimuli.

It was necessary, of course, in the application of this method, to eliminate methylene blue and the mechanical impact of the spray as factors in stimulation. This was done by spraying the embryos with pure water and with a solution of methylene blue in pure water. Such check experiments showed that the mechanical impact of the spray and the methylene blue content of the solution were negligible factors so far as stimulation is concerned.

Embryos selected according to my typical physiological stages as determined by reactions to tactile stimulation were tested with various dilutions of the hydrochloric acid. Embryos of the non-motile stage, as determined by tactile stimulation, gave no response to the acid spray, while embryos that responded to tactile stimulation responded also to the acid stimulation when sprayed with dilutions as great as $n/400$. With stronger solutions the reactions were prompt and vigorous, while with greater dilutions

a latency period frequently followed stimulation. In embryos of the early-flexure stage this period was noted in some instances as being ten to twenty-five seconds in duration.

In embryos of the early-flexure stage reaction followed stimulation on the caudal portion of the trunk as well as on the head, and the movements were of the same nature as if they had been stimulated by tactile means. Furthermore, in case of transection of embryos it is found that caudal portions that respond to tactile stimulation respond also to chemical stimulation, and caudal portions that do not respond to tactile stimulation do not respond to chemical stimulation. In other words, in development and in mutilations response to chemical stimulation comes and goes hand in hand with response to tactile stimulation, and the two forms of stimuli excite the same forms of response. These facts create a strong presumption against the idea that there are different mechanisms involved in the reactions to the different forms of stimulation.

By the use of a spray it is obviously impossible to determine the threshold of stimulation exactly, because of the increasing dilution of the acid as it diffuses through the water. To study this point more carefully and study comparatively the action of different concentrations of the acid, embryos were immersed in various solutions and their activity observed. The following experiment, which has been repeated with various modifications, may be accepted as representative of the results of this method of study.

Four *Amblystoma* embryos of the early-swimming stage were immersed in similar dishes containing the following solutions: (a) cistern water, from the stock used for growing the embryos in the laboratory; (b) distilled water which had been aerated by pouring from one dish to another repeatedly; (c) HCl n/2000; (d) HCl n/3000; (e) HCl n/4000. The distilled water used in diluting the normal solution of the acid had also been aerated in the manner mentioned.

At the expiration of five minutes the greatest activity was shown by the embryos in the dish with HCl n/2000. Two minutes later there was slight activity among the embryos in HCl n/4000,

although during the first ten minutes of the experiment the embryos in distilled water manifested as much disturbance as did those in HCl n/4000, and at times apparently more. Thirty minutes after the beginning of the experiment the embryos that were immersed in HCl n/3000 showed the greatest activity; those in distilled water were slightly active; those in HCl n/4000 were perfectly quiet. It was observed throughout the experiment that the movements of the embryos in the acid solutions were of a more convulsive nature than were the movements of those in distilled water. Two hours and five minutes after the beginning of the experiment the embryos in HCl n/3000 were still the most active; those in HCl n/2000 were totally inactive; those in HCl n/4000 and in distilled water were slightly active. During the whole experiment up to this point no movements had been observed among the embryos in the cistern water. At the expiration of four hours and twenty-five minutes after the immersion in the solutions, the specimens in HCl n/3000 and those in HCl n/4000 were more active than those in distilled water. An hour later the embryos in HCl n/4000 were still more active than those in distilled water.

During the progress of this experiment it became obvious that at least not all the reactions that were occurring in the various acid solutions could be regarded as due to a normal process of stimulation, for within three hours after the beginning of the experiment the four specimens that were immersed in HCl n/2000 showed marked shriveling of the margins of the fins and the skin had become pale. Upon the discovery of this destructive action, these four specimens were removed and two others introduced into the identical solution. These became completely inert to ordinary means of tactile stimulation within forty-five minutes after immersion in the solution. Further observations showed that the specimens that were immersed in HCl n/3000 for four hours had undergone various degrees of decline in irritability to tactile stimulation, and that one had wholly lost the power of response to such stimulation. Prolonged immersion in HCl n/4000 also proved to have a very perceptible injurious effect upon the skin of the embryos.

As a result of experiments along the line indicated above, the leading question in my experimentation with acid took this form: can a concentration of acid be found that will stimulate and not destroy the skin; and, if so, is there anything in the nature of the response that differentiates normal stimulation from destructive actions by substances in solution?

As a means of studying this aspect of the question the method of recording movements with a myograph was devised, and used according to the method described in connection with figure 60. Studies were made with this method upon the action of hydrochloric acid in as great dilution as $n/10,000$; with the result that degrees of concentration of the acid which are not adequate to stimulate the reflex mechanism were found to have a destructive action upon the skin perceptible under the microscope.

In figure 60, graphs *A*, *B* and *C* represent respectively the action of HCl $n/300$, $n/400$ and $n/500$ upon the behavior of embryos of *Rana catesbiana* of the advanced swimming stage. The solid line of each graph represents the composite of the activity of five specimens in the acid, while the broken line represents the composite of the activity of the same five specimens in pond water following the same kind of mechanical agitation in changing from dish to dish as occurred in the manipulation with acid. The reaction in water was in each case taken immediately before the reaction in acid.

In the three graphs on the action of acid there is a striking similarity; but the most noteworthy result of this method of study is that the composite of the activity of a number of specimens can be represented by a curve. Particularly is this impressive when one knows that, although these very embryos were removed from the acid immediately following the experiment (after an immersion of less than seventy-five seconds) they showed unmistakable evidence of the destructive action of the stimulating agent upon the skin. In addition, these graphs seem to show, particularly when the increasing normal activity as indicated by the broken line is taken into account, that the intensity of the response (the height of the curve) increases, while the duration of the response (the length of the curve) decreases

with the degree of concentration of the acid, that is to say, the intensity of the response tends to vary directly with the destructive action of the stimulating agent while the duration of the response tends to vary inversely with the destructive action.

Myograms made by this method on certain embryos under the action of HCl $n/1000$ showed no positive evidence of stimulation; yet immersion in the same solution for a period of fifteen minutes caused pronounced disintegration of the skin. Prolonged study with these quantitative methods has given a mass of unquestionable evidence in favor of the conclusion that the action of hydrochloric acid upon the skin of amphibian embryos cannot be regarded as a normal stimulation of nerve endings or sensory cells of any sort.

Various observations by other methods have confirmed this conclusion. If, for instance, as observed also independently by a student in my laboratory, Mr. M. W. Shipley, an embryo of *Amblystoma* is immersed in a dilution of HCl which does not excite movement, and is, after a brief period, transferred again to the original medium of pond water, it is, by this last immersion, excited to a long series of convulsive movements. This characteristic of the action of HCl on the skin was studied closely, in one case, upon 40 embryos of *Amblystoma* in the coiled-reaction stage. In this experiment each embryo was first observed for one minute in a dish of pond water and its movements noted. It was then placed in HCl $n/1000$ and observed for the same length of time. Immediately following this it was replaced in the pond water from which it was originally taken. Through the whole process every movement was recorded. As a result of this experiment upon forty embryos the following conclusions were drawn: (1) There was no great variation among the embryos in the degree of normal activity in pond water. (2) There was great variation among the embryos in irritability to the acid. (3) There was great variation among the individuals in their irritability to pond water after the bath in HCl, and there is a distinct correlation between irritability to the acid and the subsequent irritability to pond water. (4) There was, on the whole, much greater activity in the pond water following the bath in

acid than there was in the acid. According to the records of the experiment, there were five movements among forty embryos during the minute in the pond water originally, indicating the degree of normal activity. In the HCl $n/1000$ there were 60 movements during the same period, while there were 151 movements during the one minute of immersion in pond water after the bath in HCl $n/1000$. These data certainly prove that, while the acid excited response, it rendered the skin abnormal in some respect.

With view to determining the nature of the action of very dilute solutions of hydrochloric acid upon the skin of these embryos, specimens were selected which had not nearly reached the stage of earliest response and which exhibited the typical ciliary movement over the surface of the skin. In these embryos the reaction of the skin alone was studied without the intervention of nervous or muscular phenomena.

Embryos of this age, when immersed in pure water in which fine granulated carmine is suspended, keep the surface of the body clear of this substance indefinitely. In such a preparation the carmine particles may be seen under the microscope in a perpetual stream over the surface flowing cephalo-caudad and off at the caudad end of the animal. If, however, the embryo is immersed in HCl $n/1000$ in which finely pulverized carmine is suspended, the particles begin to adhere to the surface of the skin in less than two minutes. The cilia beat vigorously among the accumulating carmine particles but fail to dislodge them so long as the embryo remains in acid. Immersion in a stronger solution of the acid produces this effect more quickly and in a short time causes the exudation of globules of adhesive substance on the surface of the ectodermal cells. Prolonged immersion intensifies this action till the complete disruption of the cells occurs. And when this disruption occurs it is most pronounced in the regions where the particles of carmine first adhere in the dilute acid solution. Parker ('12) places the threshold of stimulation by hydrochloric acid in the mouth of man at $n/1000$. This being correct, the ectodermal cell of the amphibian embryo which has no nerve supply responds directly to as great a dilution of the acid as does the

highly differentiated and innervated surfaces in the oral cavity of man. But this is not all, a distinctly perceptible reaction of the ectoderm cells of these embryos has been observed in HCl n/10,000.

From these and from numerous other experiments with hydrochloric acid as a stimulating agent, the following conclusions have been drawn:

1. The ciliated cutaneous epithelial cells of young *Amblystoma* embryos react directly to exceedingly dilute solution of hydrochloric acid without the intervention of a nervous system or nervous connection of any kind.

2. No degree of concentration of the acid can be found which will excite muscular response without causing destructive processes in the skin.

3. There is a distinct correlation between the nature of the muscular response and the injurious action of the acid which is used as a stimulating agent.

4. There is no evidence of a normal irritability of the skin to acid.

5. If there should prove to be such a normal irritability, it must necessarily act upon the same reflex mechanism as that through which reaction to tactile stimulation takes place.

c. The proprioceptive field

The anatomical part of this paper has dealt in detail with the endings of the giant ganglion cells upon the myotomes. That such endings are in fact sensory is demonstrable experimentally.

It has been explained in various connections that, during a certain period of development, particularly during that period which is characterized by the coiled-reaction, these embryos move almost constantly away from the side touched. When the embryo reaches the swimming stage, however, the movements become more irregular in direction relative to the side stimulated, and with further development every suggestion of this law of response that prevailed in the earlier period disappears. If, however, an embryo of the advanced swimming stage, which has

lost the characteristic crossed reflex, be immersed in a solution of curare, the extent of the movement in response to tactile stimulation gradually becomes reduced, and the forms of movement go out of the behavior in the reverse order to that according to which they appeared, that is to say, the effective swimming movement gives place to feeble S-reactions; these, to coiled-reactions; these, to feeble flexures; till eventually only the slightest head movement occurs, and finally responses of all kinds cease. Now, during the later period of the decline in motility, when behavior has reverted to the earlier type, the regularity of crossed response reappears, and through long series of responses the movements will be constantly away from the side stimulated. The most obvious inference to be drawn from this recurrence in the form of behavior is that the progressive paralysis of the motor nerve endings by curare eliminates more and more the stimulations of the muscle sensory endings of the giant ganglion cells, and the associative center or motor column, one or both, are left to the almost exclusive stimulation from localized cutaneous areas.

These results from experiments with curare are confirmed by another simple form of experiment, namely, the transection of swimming embryos at about the level of the pectoral limb bud, so as to leave in the head piece just enough muscle to give a perceptible contraction when observed under the microscope. The head piece of such a transected embryo, it is found, through long series of reactions approaching a hundred in rapid succession, contracts the muscles constantly on the side opposite the stimulus. Here, again, the associative and motor centers have been severed from the influence of the greater part of the trunk, and the dominating factor of the trunk as compared with the cranial field of stimulation is the muscle system.

From such experiments as these, it seems necessary to conclude that the giant ganglion cells have a proprioceptive field of stimulation through their endings on the myotomes, and that this field of stimulation has a profound influence over the behavior of the animal when it responds to localized cutaneous stimulation. This primitive reflex mechanism, therefore, exhibits the phenomena of

antagonism between reflexes after the manner of the mammalian reflex arc.

In my paper upon *Diemyctylus* ('09) attention was called to the secondary movements which occur in the behavior of these embryos, that is to say, movements that frequently occur immediately after the initial response or before it is completed. These movements might be accounted for upon the hypothesis that the motor or associative cells act rhythmically to a single stimulus, only for the fact that the secondary movement sometimes exceeds the initial movement in extent. Although there may be a tendency for these cells to act in rhythm, there must be something besides the rhythmic activity to account for the acceleration of movement. In the light of the anatomical and physiological demonstration of a proprioceptive field of stimulation it is more reasonable to suppose that stimuli from this field, through imperfect alliance with stimuli from the cutaneous field, arouse the secondary movements. We may recognize in these movements, therefore, the phenomena of alliance between reflexes. The movements, in and of themselves, have the characteristics of the reflex after-discharge of the mammalian reflex arc, and, indeed, regardless of the above interpretation of their immediate cause, they may be perfectly analogous to the reflex after-discharge, since the latter response of the mammalian reflex arc may involve both the rhythmic action of neurones and excitation from the proprioceptive field.

The anatomical part of the paper brought out the fact that a single giant ganglion cell may innervate both skin and muscle. Some of these cells are apparently distributed exclusively to the skin; others may be distributed exclusively to the muscles; but some certainly go both to skin and muscle. In the consideration of the interaction of stimuli from the skin on the one hand and from the muscles on the other, it is necessary, therefore, to assume that there is no physiological differentiation between the stimuli from the different sources. Exteroceptive and proprioceptive stimuli might become allied, or the one reinforce the other, within one and the same neurone.

These facts lead further to the conclusion that the impulses from the proprioceptive field follow the same conduction paths as do those which originate in the skin. This means that action in the muscular system of one side tends to excite contraction in the muscles of the opposite side. This relation, indeed, may be the paramount factor in adaptation that determined the integration of the nervous system into physiologically distinct longitudinal paths on the same side and crossed paths only in the cephalic portion between the afferent path of one side and the efferent path of the other, for through such a mechanism one act of the swimming movement would stimulate the next with the result of serial contractions which effect locomotion. The question as to whether proprioceptive or exteroceptive stimulation was the primary concern of the giant ganglion cells resolves itself, of course, into pure conjecture; but in ontogenesis these two functions are obviously merged into a common action through one and the same mechanism.

III. DISCUSSION OF RESULTS

The literature upon the subject of the giant ganglion cells is very extensive and no effort will be made here to review it in detail. Comparatively recent critiques upon the literature have been offered by Dahlgren ('97), Van Gehuchten ('97), Harrison ('01) and others, and since the contributions on the subject are almost exclusively morphological with little or no reference to the related physiological problems, and since the investigations have been made upon the widely divergent forms with more or less disagreement among the morphologists, it is only in the way of corroboration of my own anatomical findings that any help has been drawn from the literature upon my specific problem of correlation of function and structure in the particular animals in hand. My investigations were not undertaken with the idea of discovering new things in anatomy; they were undertaken with a view to correlating specific structure, in particular animals, with known physiological characteristics of those animals. My physiological experiments therefore, and not the observation of others, have been my guide and my check.

As might be expected, my observations add little to what has been well known concerning the form and orientation of the giant ganglion cells within the spinal cord. These features of the cell have been exhaustively treated by Beard ('92, '96), Dahlgren ('97), Studnička ('95), Tagliani ('95), Sargent ('98), Johnston ('00), Harrison ('01) and others. Some authors, including Johnston, regard the cell process that is directed caudad in the cord as a neurite, and therefore interpret the column as physiologically descending as well as ascending. This may be true in the animals studied by these investigators but there is no evidence of descending impulses in the column of the giant ganglion cells in *Amblystoma* embryos used in my work, and, in some cases, the peripheral fiber has been observed to arise from the descending process of the cell.

The lateral position of the cells in the more rostral portion of the cord and their occurrence well forward in the medulla have been observed by Johnston in *Catostomus*. He observes also that in *Catostomus* and *Coregonus* the nuclei are differentiated from the surrounding nuclei in their staining reaction, being colored red while the surrounding nuclei are colored green with the Ehrlich-Biondi triple stain. With the use of orange G, Lyons blue and erythrosin as cytoplasmic stains my preparations show the nuclei of the giant ganglion cells tinged slightly with these colors, in this manner differentiated from the nuclei about them.

My results add to the knowledge of these cells as regards their central relations chiefly in demonstrating their organization into an afferent conduction path which, through the greater part of its extent if not through it all, is physiologically distinct from the motor tract of the same side. My findings show that this column of cells is an integral part of a reflex mechanism which has the essential characteristic of the typical reflex arc of higher vertebrates.

Upon the peripheral relations of the giant ganglion cells there has been some difference of opinion. Studnička ('95) regards the peripheral processes as motor upon the basis of his observation that they terminate in the myotomes in *Rana* and *Bufo*. Tag-

liani and Beard (in his earlier paper) are quoted by other authors as being of the same opinion with Studnička. More recent observers, however, discredit this interpretation and fail to find the endings of the fibers in the myotomes. The general interpretation, which has been insisted upon in recent years, that the dorsal part of the cord is sensory, probably created a presumption in the minds of later observers against the idea that these fibers end in the myotomes, for such endings had been interpreted as motor. My anatomical preparations, however, seem to be unequivocal on this point, and my physiological results are equally positive concerning the existence of a functional proprioceptive field of stimulation. The harmony between my anatomical and physiological observations adds weight to my conclusion that the giant ganglion cells are muscle sensory as well as skin sensory in function.

That the same neurone may carry impulses from the skin and the muscle as my anatomical findings indicate is also against the presumption of current anatomy and physiology. In the consideration of this point, however, it should be borne in mind that the giant ganglion cell is obviously a primitive structure. This seems to be conceded by all morphologists who have studied the subject. In my opinion, the giant ganglion cells represent the afferent element of the nervous mechanism of the earliest chordates that propelled themselves in locomotion by means of a mesodermal muscular system. In such an ancestral form, as in the ontogenetic stage of development now under consideration, physiological differentiation in the synaptic centers between cutaneous and muscle sensory impulses could be of little, if any, significance. The paramount feature of adaptation of the reflex mechanism of these embryos is immediately centered in locomotion and not in differential sensory functions. From the point of view of the efficiency of this mechanism as it actually works in the life of amphibian embryos there is no reason apparent why impulses should not be carried from the skin and the muscle to the spinal cord through the same neurone.

With reference to the subepithelial structures of the giant ganglion cells, the repetition of Wintrebert's ('04) experiments

by Hooker ('11) is of interest. Hooker's experiments were made to test the property of the skin as a conductor of impulses longitudinally in the trunk. Incidentally they lend credence to the idea, if they do not certainly demonstrate, that the subepithelial terminals of the giant ganglion cells do not form a conducting syncytium in or beneath the skin. This is in harmony with my experiments in which severing of the dorsal portion of the cord made it impossible to stimulate response to a light touch applied caudad of the lesion. There is obviously here no cutaneous or subcutaneous structure which can conduct stimuli longitudinally in the trunk for any considerable distance.

My experience with hydrochloric acid as a stimulating agent have important bearings upon the work of Parker ('12), Sheldon ('09), Cole ('10) and others upon fishes and Amphibia with reference to a general or "common chemical sense" in the skin. These authors hold the view that there is a special set of receptors which are normally irritable to various substances in solution. My conclusions do not harmonize with this view so far as amphibian embryos are concerned. Moreover, a comparison of my results in detail with those of Parker, Sheldon and Cole convinces me that the burden of proof is still upon them as regards the nervous irritability of the skin to chemical stimuli in fishes and amphibians generally; for every essential characteristic of response which they describe can be duplicated in amphibian embryos which are known to be responding to a violent disruptive action of the stimulating chemical agent upon the epithelium of the skin.

To understand how the response of fishes and amphibians to acid, or other substances in solution, may be caused by the destructive action of the stimulating agent, it is only necessary to recall that the deeper cells of the cutaneous epithelium of all vertebrates are permanently embryonic; and that the general cutaneous nerve fibers end among these embryonic cells as the terminals of giant ganglion cells end upon the deeper layer of epithelial cells of the amphibian embryo. My experiments upon young, ciliated embryos with HCl $n/1000$ and $n/10,000$ show that the cutaneous epithelial cells of the embryo, regardless of nerve endings, are beyond comparison with the skin of fishes in sen-

sitiveness to the action of acid. They would, in fact, explode almost instantaneously upon immersion in such concentrations of acid as were used by Parker and Sheldon in their experiments upon fishes. It is my opinion that the deeper embryonic cells of the skin of adult fishes and amphibians would act in the same way if exposed directly to the acid. In their normal condition, however, they are bound down and protected by a thick, less sensitive and more impervious layer of cells. Under such condition the acid must cause exceedingly violent mechanical disturbances beneath a comparatively passive exterior; while the less sensitive outer layer of cells protect the deeper cells during processes of repair when the destructive action ceases.

Sheldon's experiments in which he found that areas of the skin which have become fatigued to tactile stimulation are still sensitive to various chemical agents, and that areas that have been stimulated by chemical agents are thereafter for a time insensitive to tactile stimulation, are exactly in line with my observation of embryos immersed in HCl $n/2000$, $n/3000$ and $n/4000$. His experiments mean to me that the chemical agent has not fatigued but destroyed the sensitive portions of the skin to such an extent as to render it inert to normal stimulation; while the "fatigue" to tactile stimulation is merely adaptation, just as my ear is now so adapted to the sound of the clock that the tick does not affect my motor system. These experiments of Sheldon's, to my mind, illustrate exactly the difference between the effect of normal stimulation and destructive action by stimulating agents.

In like manner, Cole's experiments with cocaine upon frogs which were stimulated with chlorides are not conclusive on the point for which the author is contending. In interpreting this experiment it must be considered that the action of the chloride is much more extensive and affects many more cells at once than does any of the single acts of pinching or pricking which Cole employed to test the effect of the cocainization upon the receptors to tactile stimuli. Furthermore chemical action has a much more violent and destructive effect upon protoplasm than does mechanical stress and strain, as may be experienced, for instance,

in the manipulation of growth cultures of microorganisms. In short, in testing the effect of cocaine upon the irritability to the chloride, Cole applied stimuli which in both extent and intensity are beyond comparison with the stimuli which he used to test the effect of cocaine upon the irritability to tactile stimuli. Furthermore, Cole's observation that abrasions in the skin increase the irritability to acid, or shorten the reaction time, is also in harmony with the hypothesis that the action of the chloride is destructive and not a normal physiological stimulus.

When the epithelial cell itself, without the intervention of any nervous structure, is sensitive to as high a dilution of hydrochloric acid as are the taste organs of man (Parker ('12) estimates this at $n/1000$), it is not surprising that the oral surfaces of the shark should be sensitive to $n/75$ (Sheldon); nor does a special set of receptors seem necessary to enable *Ammocoetes* to respond to $n/40$ (Parker); or *Ameiurus*, to $n/2$ (Parker) when applied to the skin of the trunk. Such concentrations of acids as this are beyond comparison with the degree of concentration which will act destructively upon epithelial cells. Such strengths of chemical stimulation certainly have no place in the normal environment of the animals upon which they were used experimentally, and the idea that there is a special set of receptors, or nerves (Sheldon), to take account of such stimuli even in these animals should have absolutely unequivocal and uncontradictory evidence in its favor before it is adopted as a general biological principle.

LITERATURE CITED

- BEARD, J. 1892 The transient ganglion cells in Raja. *Anat. Anz.*, Bd. 7.
 1896 The history of a transient nervous apparatus in certain Ichthyopsida. *Zool. Jahrb., Abt. f. Morphol.*, Bd. 9.
- COGHILL, G. E. 1902 The cranial nerves of *Amblystoma tigrinum*. *Jour. Comp. Neur.*, vol. 12.
 1908 The development of the swimming movement in amphibian embryos. *Anat. Rec.*, vol. 2.
 1909 The reaction to tactile stimuli and the development of the swimming movement in embryos of *Diemyctylus torosus*, Eschscholtz. *Jour. Comp. Neur.*, vol. 19.
 1913a The primary ventral roots and the somatic motor column of *Amblystoma*. *Jour. Comp. Neur.*, vol. 23.
 1913b The correlation of structural development and function in the growth of the vertebrate nervous system. *Science*, vol. 37.
- COLE, LAWRENCE W. 1910 Reactions of frogs to chlorides of ammonia, potassium, sodium and lithium. *Jour. Comp. Neur.*, vol. 20.
- DAHLGREN, U. 1897 The giant ganglion cells in the spinal cord of the order Heterosomata Cope. *Anat. Anzeiger*, Bd. 13.
- HARRISON, ROSS GRANVILLE 1901 Ueber die Histogenese des peripheren Nervensystems bei *Salmo salar*. *Archiv f. mikr. Anatomie*, Bd., 57.
- HOOKE, DAVENPORT 1911 The development and function of voluntary and cardiac muscle in embryos without nerves. *Jour. Exp. Zool.*, vol. 11.
- JOHNSTON, J. B. 1900 The giant ganglion cells of *Catostomus* and *Coregonus*. *Jour. Comp. Neur.*, vol. 10.
- PARKER, GEORGE HOWARD 1912 The relation of smell, taste, and the common chemical sense in vertebrates. *Jour. Acad. of Natural Sciences of Philadelphia*, September 7, 1912.
- SHELDON, RALPH EDWARD 1909 The reactions of the dogfish to chemical stimuli. *Jour. Comp. Neur.*, vol. 19.
- STUDNÍČKA, F. K. 1895 Ein Beitrag zur vergleichenden Histologie und Histogenese des Rückenmarkes. *Sitzungsberichte der konigl. böhmischen Gesellschaft der Wissenschaften. Mathematisch-naturwissenschaftliche Classe*, 1895.
- TAGLIANI, GIULIO 1895 Ueber die Riesennervenzellen im Rückenmarke von *Solea impar*. *Anat. Anz.*, Bd. 15.
- VAN GEHUCHTEN, A. 1897 Contribution à l'étude des cellules dorsales (Hinterzellen) de la moelle épinière des vertébrés inférieurs. *Bull. Acad. Belg.*, T. 34.
- WINTREBERT, M. P. 1904 Sur l'existence d'une irritabilité excitomotrice primitive, indépendante des voies nerveuses chez les embryons ciliés des Batraciens. *Comptes Rendus de la Soc. de Biol.*, vol. 57.

ABBREVIATIONS

<i>Ad.</i> , areas of adhesion between spinal cord and skin or mesoderm	<i>Mes.</i> , mesenchyme
<i>APDC.</i> , ascending process of the giant ganglion cell	<i>Ms.</i> , muscle-sensory ending of giant ganglion cell
<i>Aud. V.</i> , auditory vesicle	<i>Opt. St.</i> , optic stalk, cut at the surface of the brain
<i>C.</i> , notochord	<i>Po. LL.</i> , postauditory lateral line primordium
<i>CC.</i> , central canal of the spinal cord	<i>Pr. LL.</i> , preauditory lateral line primordium
<i>Com.</i> , commissural cell	<i>R. V, VII, etc.</i> , positions of the roots of the corresponding cranial nerves
<i>DC.</i> , giant ganglion cell	<i>S.</i> , spinal cord
<i>DCF.</i> , peripheral fiber from the giant ganglion cell	<i>SG.</i> , anlage of the spinal ganglion
<i>Dor. LL.</i> , primordium of the dorsal division of the lateral line	<i>Sub. LL.</i> , suborbital lateral line primordium
<i>DT.</i> , sensory, or dorso-lateral tract, arising from giant ganglion cells	<i>Sup. LL.</i> , supraorbital lateral line primordium
<i>Ec. Th.</i> , ectodermal thickenings connected with the visceral pouches	<i>VC.</i> , motor cells, the cells of origin of the motor, or ventro-lateral tract
<i>Inf. LL.</i> , primordium of the inferior or ventral division of the lateral line	<i>VT.</i> , motor, or ventro-lateral tract
<i>LL.</i> , primordium of the lateral line	<i>Y.</i> , yolk spherule
<i>M.</i> , myotome	<i>Z.</i> , Cytoplasmic connectives between the spinal cord and the skin.
<i>Md. LL.</i> , primordium of the mandibular line	



Fig. 1 From an embryo of non-motile stage, level of sixth myotome. Fixation in corrosive acetic mixture; stained with Boemer's hematoxylin and acidulated orange G; transverse plane; $10\ \mu$. Figures 1 to 27 are all magnified 470 diameters.

Fig. 2 From the same specimen, level of sixth myotome.

Fig. 3 Same specimen, level between eighth and ninth myotomes.

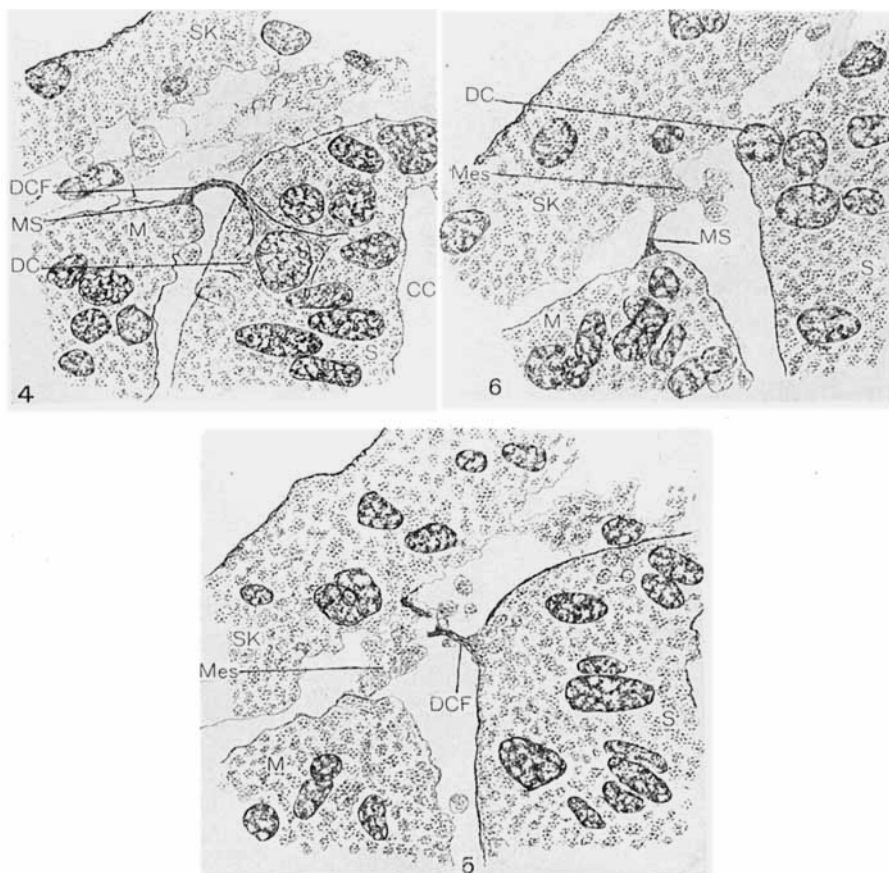


Fig. 4 Same specimen, level of the twelfth myotome.

Fig. 5 Same specimen, level of caudal end of fifteenth myotome.

Fig. 6 Same specimen, level of caudal end of fifteenth myotome.

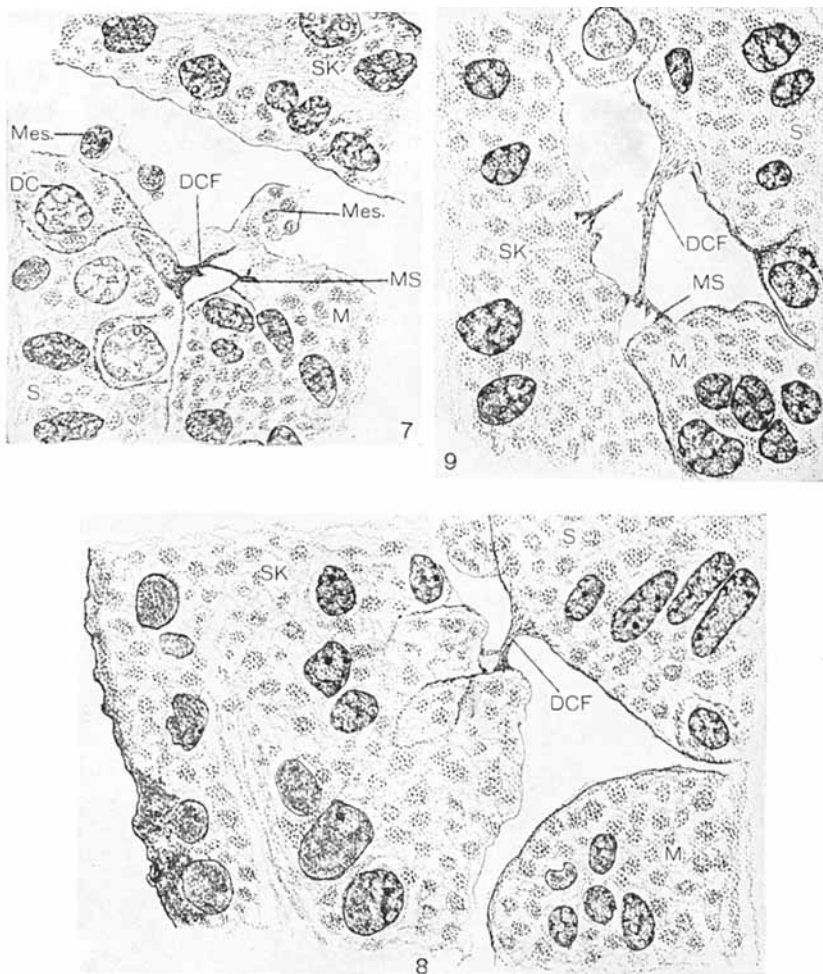


Fig. 7 From an embryo of the non-motile stage, between the eighteenth and nineteenth myotomes. Fixation, Van Gehuchten's fluid (alcohol, chloroform, acetic acid); stained with erythrosin and toluidin blue; horizontal plane, 5μ .

Fig. 8. From an embryo of the non-motile stage, between the last two myotomes cephalad of the unsegmented mesoderm; fixation and staining like the last; transverse plane, 10μ .

Fig. 9 Same specimen, from section adjacent to the last.

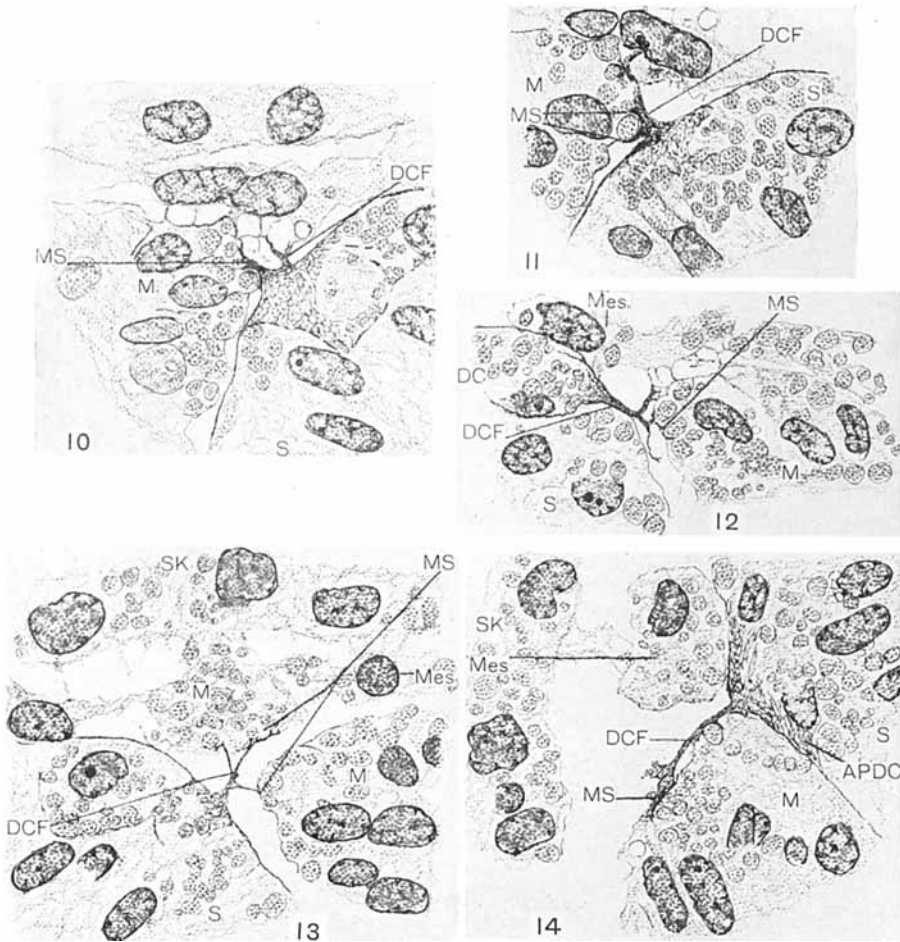


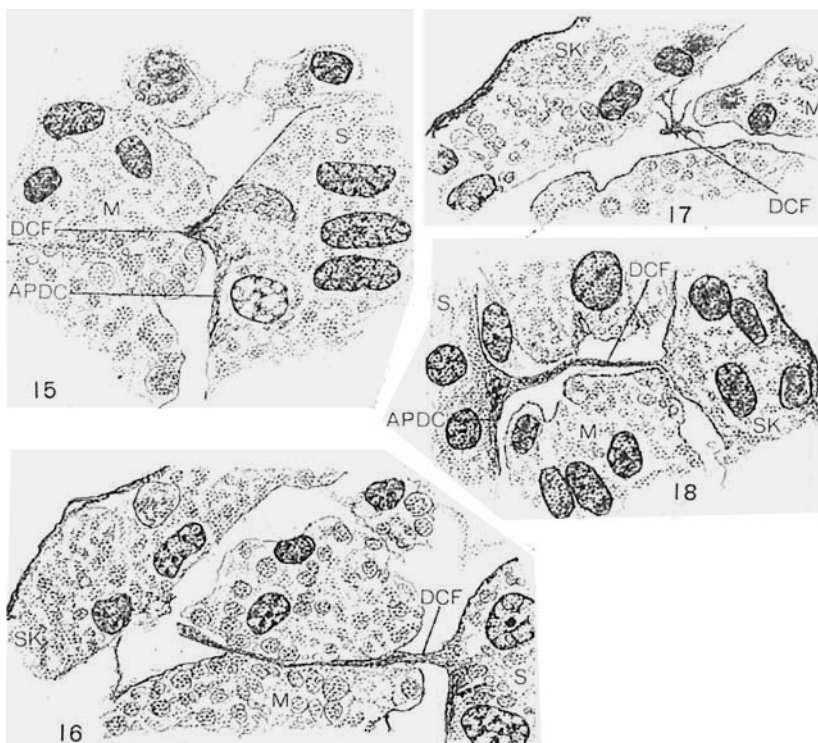
Fig. 10 From an embryo of the same age, same fixation and same staining as the last; transverse plane; 10μ . At the level of the fifth and sixth myotomes cephalad of the unsegmented mesoderm.

Fig. 11 From a section adjacent to the last.

Fig. 12 From the same embryo; two sections removed from the last.

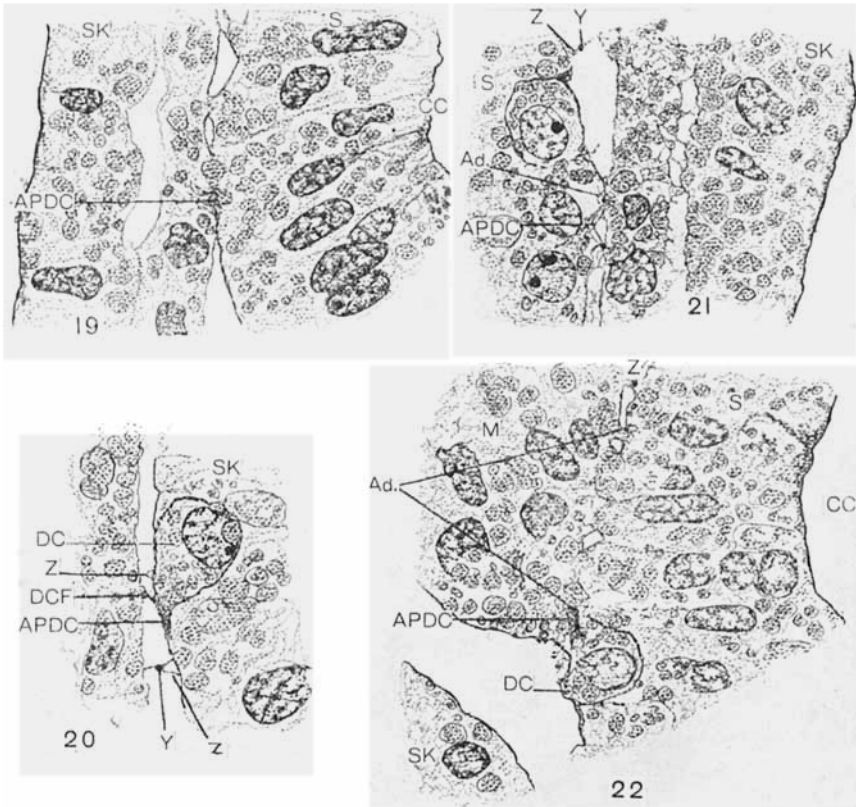
Fig. 13 From the same section as that of figure 11.

Fig. 14 From the same embryo, between the second and third myotomes cephalad of the unsegmented mesoderm.



Figs. 15, 16, 17 From an embryo of the non-motile stage, between the thirteenth and fourteenth myotomes. Same fixation and staining as figure 7. Transverse plane; 5μ .

Fig. 18 From a non-motile embryo, at the level between the ninth and tenth myotomes. Fixation in formalin-Zenker; Delafield's hematoxylin and acidulated orange G; horizontal plane; 10μ .



Figs. 19 to 22. From the same embryo as figure 7, in the level of the unsegmented mesoderm.

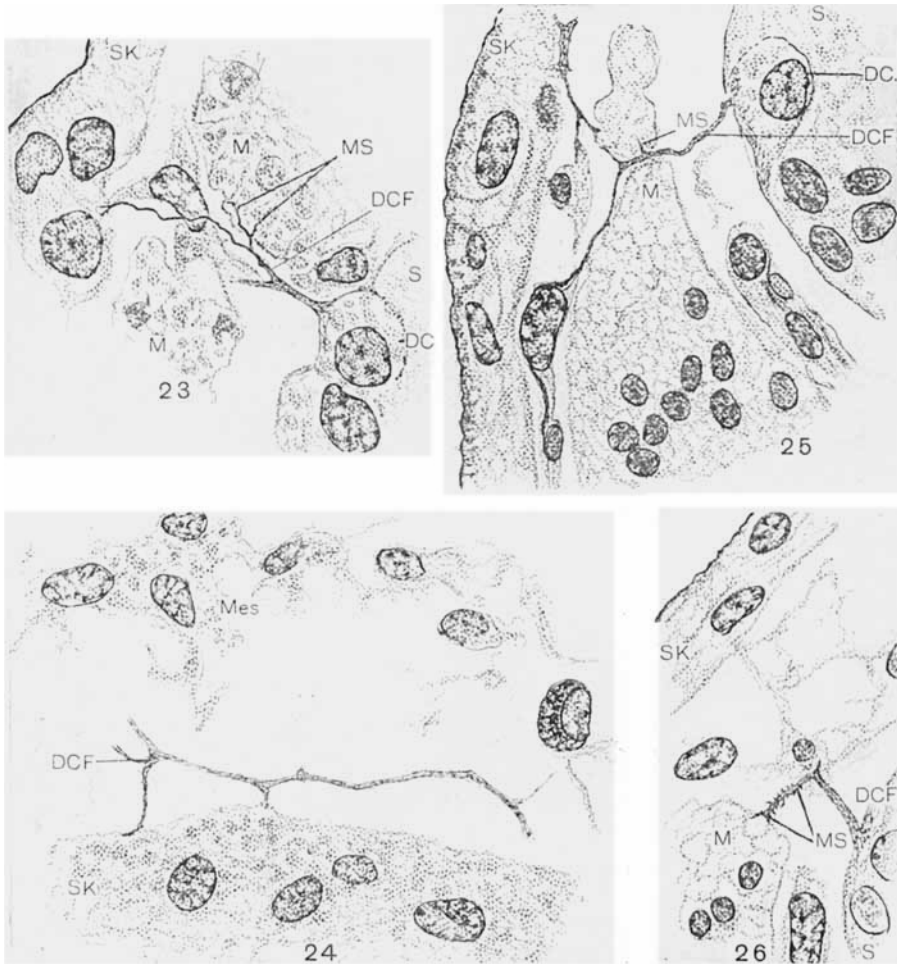


Fig. 23 From an embryo of the early-flexure stage, in the rostral portion of the trunk. Fixation in corrosive sublimate-acetic acid mixture; stained in alum carmine and Lyon's blue; horizontal plane; 10 μ .

Fig. 24 From an embryo of the early-flexure stage, at the level of the eighteenth myotome. Same fixation and staining as figure 7; horizontal plane; 7 μ .

Fig. 25 From an embryo of the coil reaction stage, at the level between the fourth and fifth myotomes. Fixation in corrosive sublimate-acetic acid mixture; stained in alum carmine and Lyon's blue; transverse plane; 10 μ .

Fig. 26 From the same specimen, at the level between the sixth and seventh myotomes.

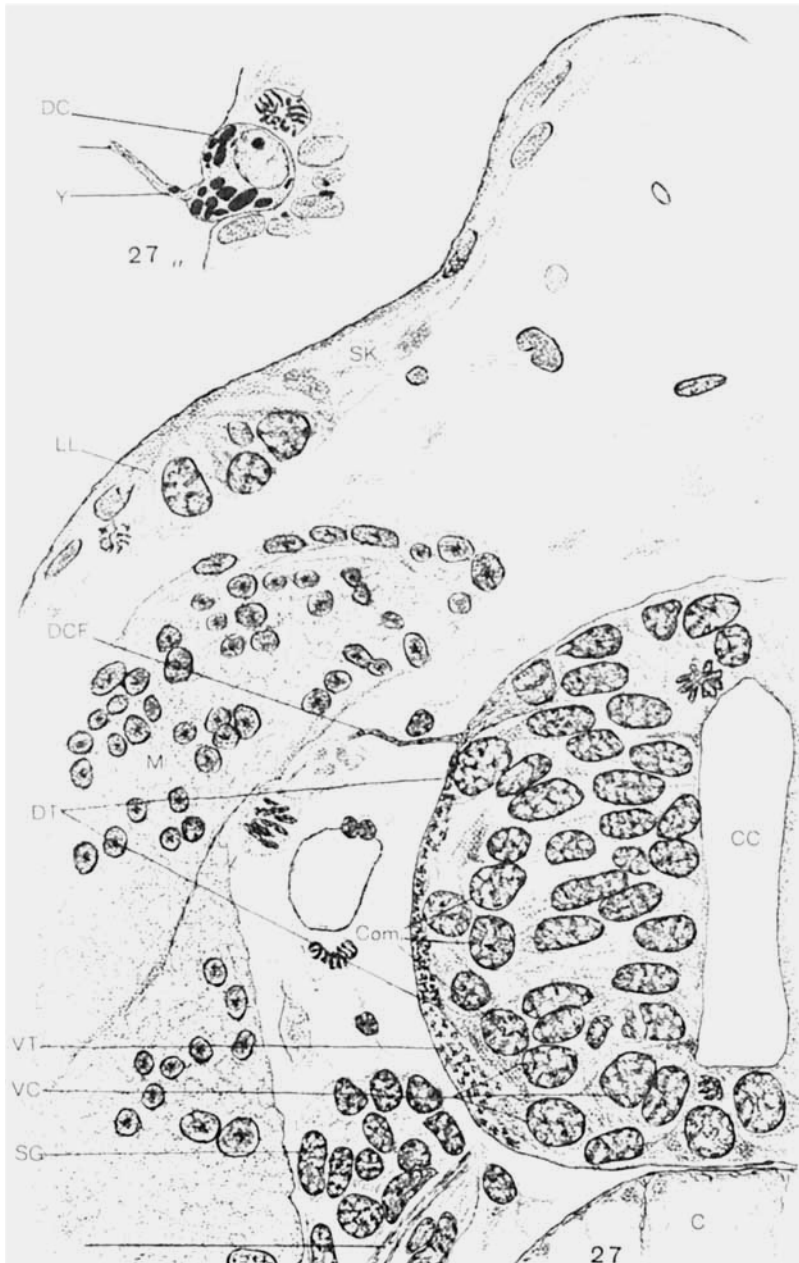


Fig. 27 From an embryo of the early-swimming stage, at the level between the fifth and sixth myotomes. Same treatment, plane and thickness as in case of figure 25. $\times 470$.

Fig. 27a From an embryo of the early-swimming stage; fixation in Zenker's solution; stained in iron hematoxylin; plane of section oblique vertical-horizontal; thickness, 7μ . $\times 470$.

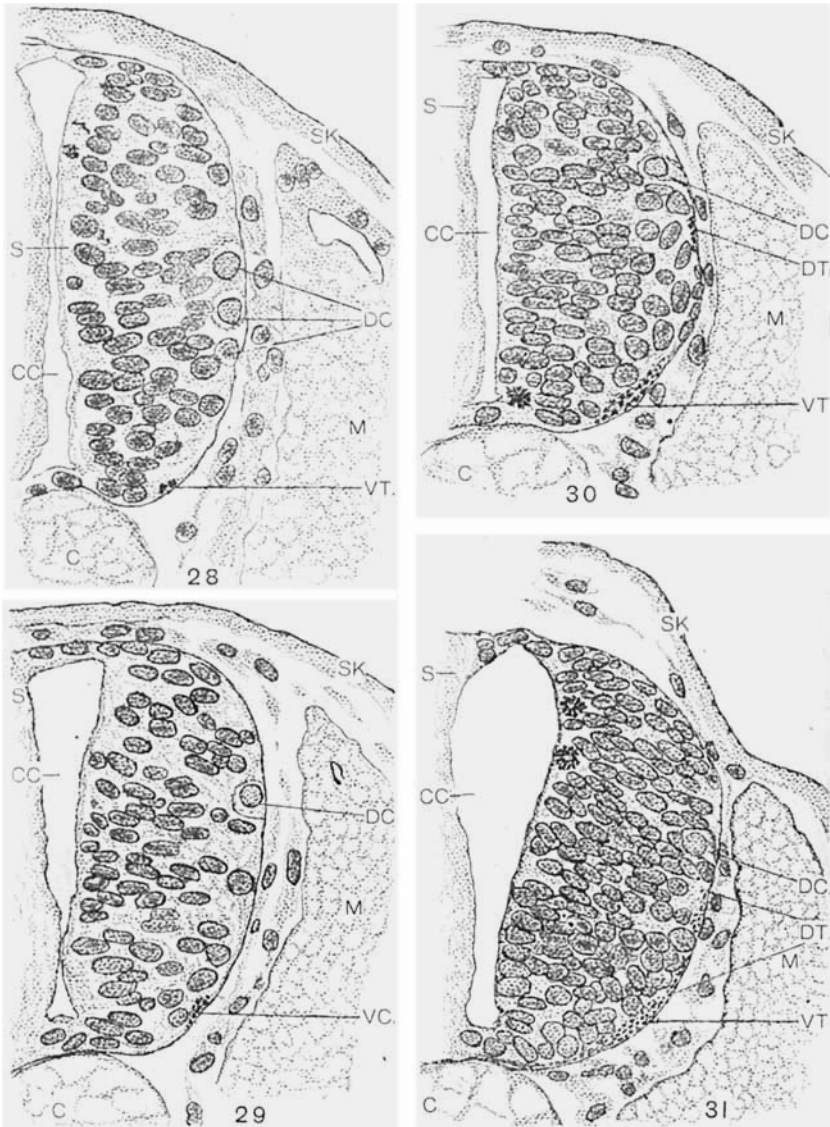
Figures 28 to 54 were drawn with the aid of the Bausch and Lomb drawing apparatus at a magnification of 400, reduced to 200 in the figures. They are taken from four embryos, designated in my series of preparations as 467, 473, 449 and 444. These embryos are taken as type specimens, respectively, of the four physiological stages that are described in the paper, namely, the non-motile stage; the early-flexure stage, the coiled stage, and the early-swimming stage. The methods of preparation of these four specimens were as follows:

467, Non-motile stage; fixation in corrosive-sublimate-acetic-acid mixture; stained in Delafield's hematoxylin and acidulated orange G; transverse plane; 10 μ .

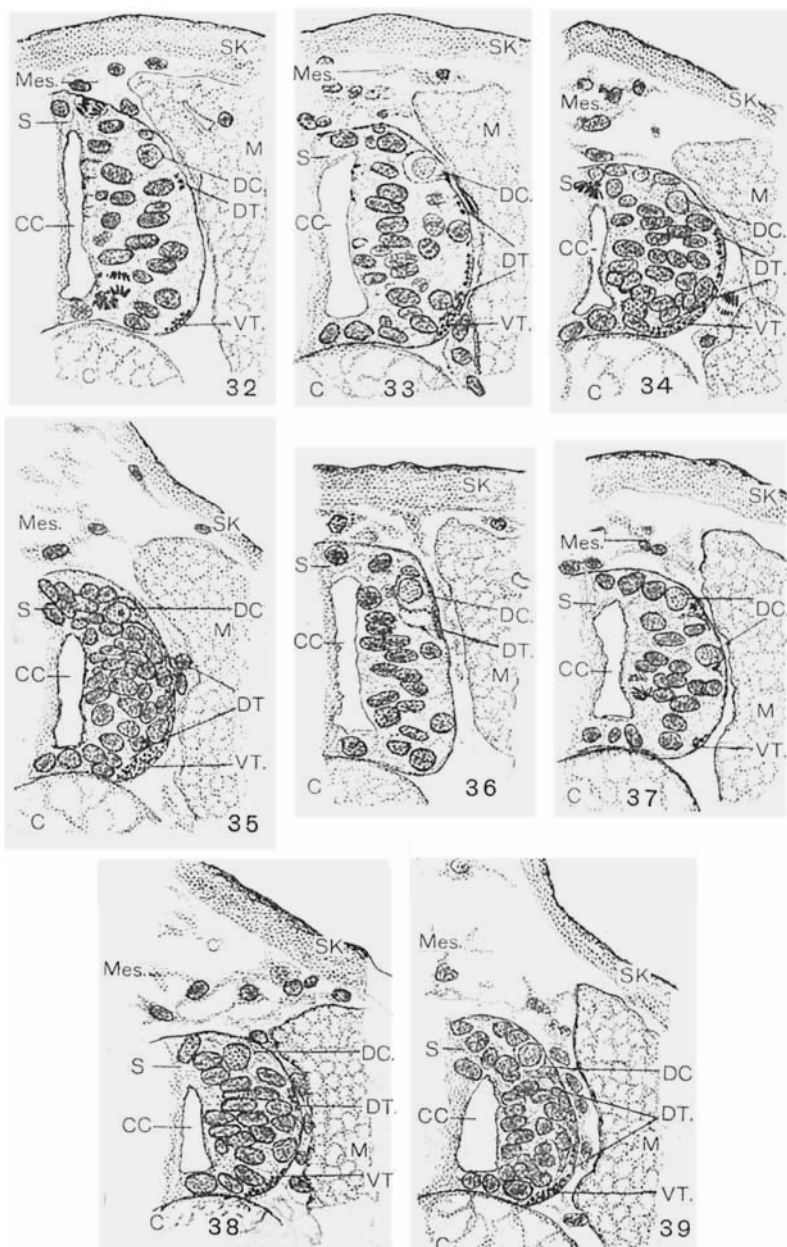
473, Early-flexure stage; fixation in corrosive sublimate-acetic acid mixture; stained in alum carmine and Lyon's blue; transverse plane; thickness, 10 μ .

449, Coil reaction stage; same fixation; stained in cochineal and Lyon's blue; transverse plane; thickness, 10 μ .

444, Early-swimming stage; same fixation; stained with Boemer's hematoxylin and acidulated orange G; transverse plane; thickness, 10 μ .

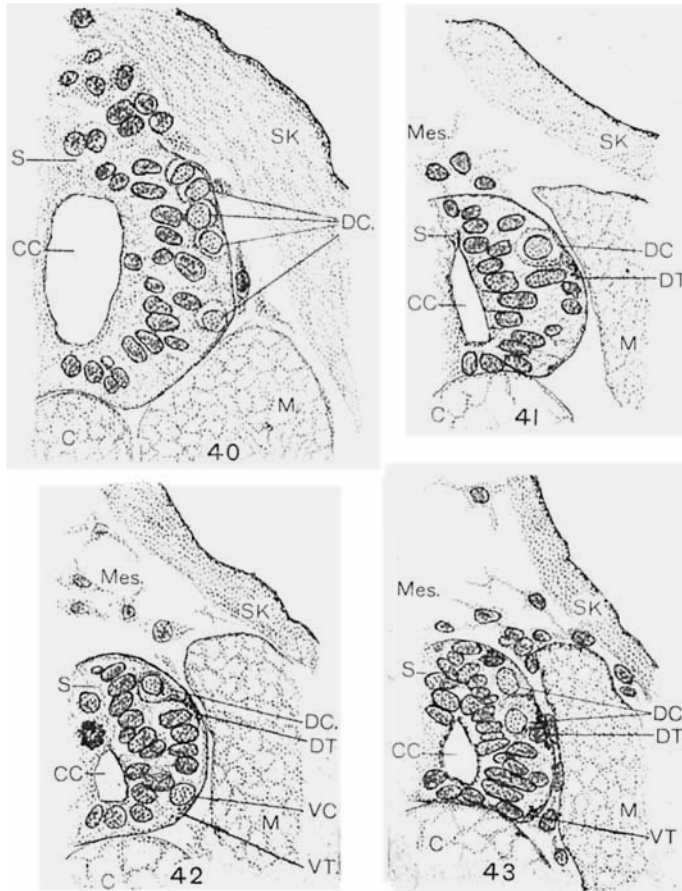


Figs. 28 to 31 From the level of the third myotome of Specimens 467, 473, 449 and 444, respectively.

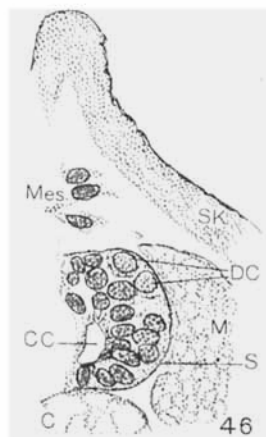
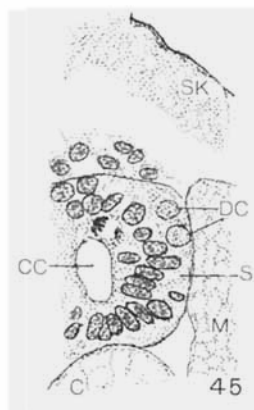
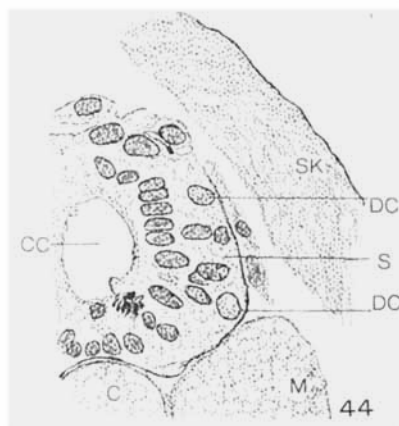


Figs. 32, 33, 34, 35 From the level of the eighth myotome of Specimens 467, 473, 449, and 444, respectively.

Figs. 36, 37, 38, 39 From the level of the thirteenth myotome 467, 473, 449 and 444, respectively:



Figs. 40, 41, 42, 43 From the level of the eighteenth myotome of the same specimens, 467, 473, 449 and 444, respectively.



Figs. 44, 45, 46, 47 From the level of the twenty-third myotome of the same specimens, 467, 473, 449 and 444, respectively.

Figs. 48, 49, 50 From the level of the twenty-eighth myotome of Specimens 473, 449 and 444, respectively.

Figs. 51, 52, 53 From the level of the thirty-third myotome of the Specimens 473, 449 and 444, respectively.

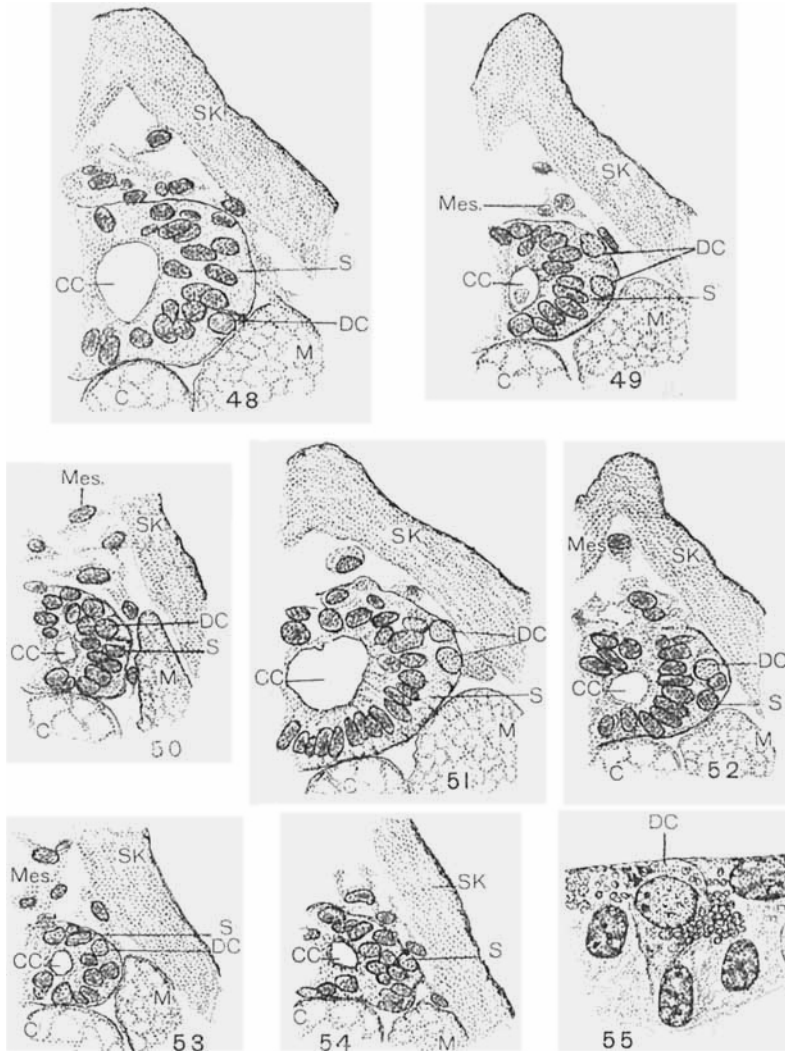


Fig. 54 From the level of the unsegmented mesoderm immediately caudal of the thirty-seventh myotome of Specimen 444.

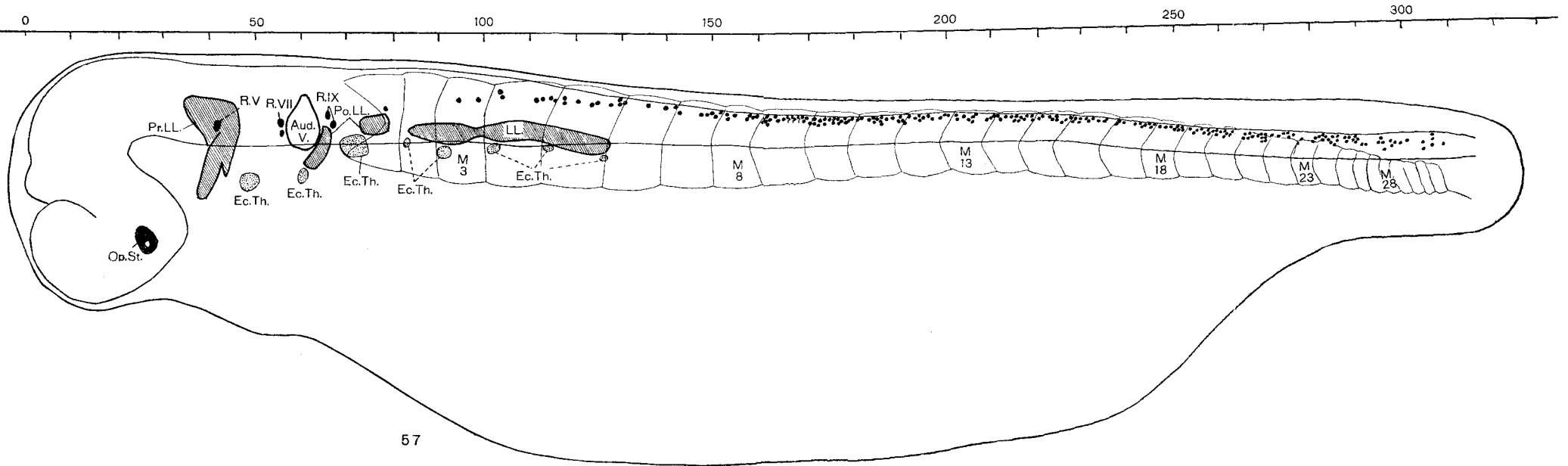
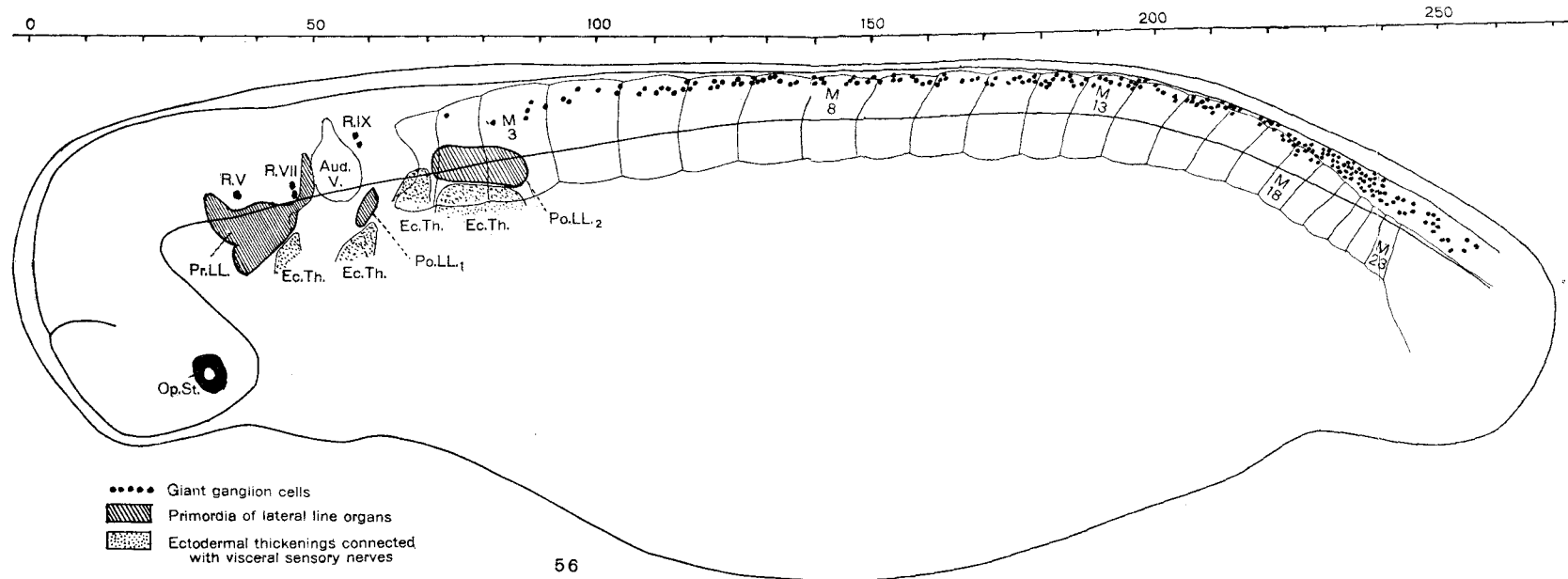
Fig. 55 From an embryo of the non-motile stage, at the level of the root of the glossopharyngeal nerve. Transverse plane. Fixations in Van Gechuchten's alcohol-chloroform-acetic-acid solution transverse plane; thickness, 10 μ .

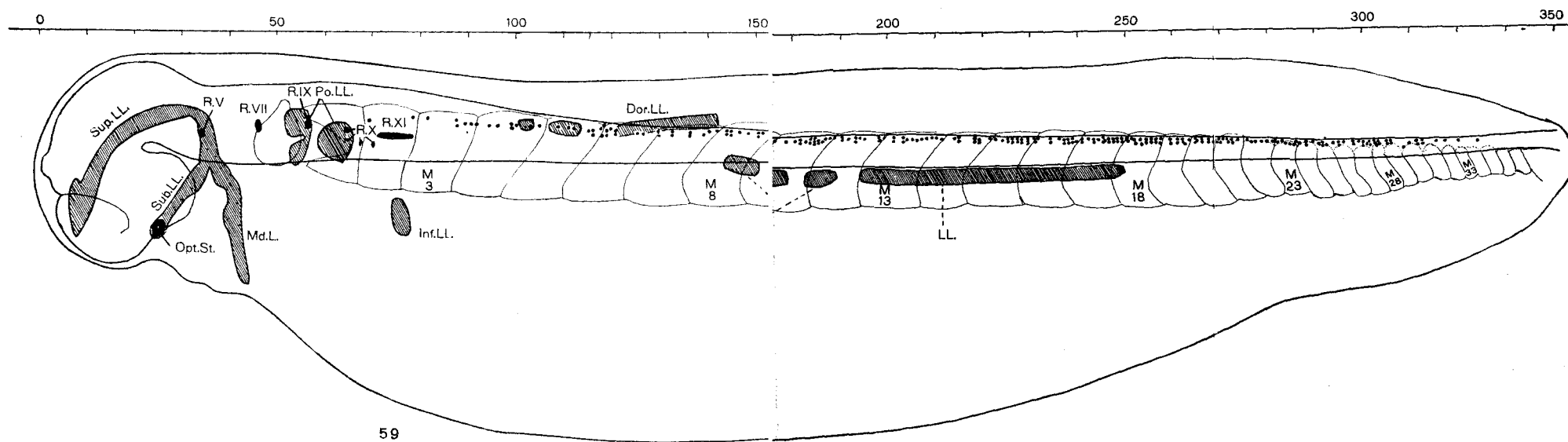
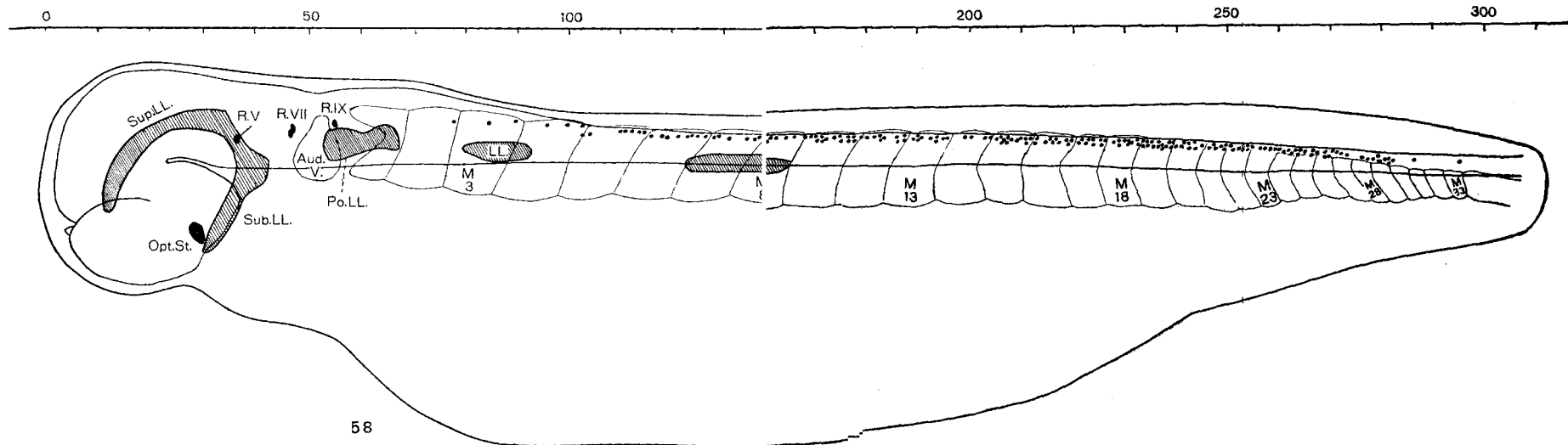
PLATES 1 AND 2

EXPLANATION OF FIGURES

56, 57, 58, 59 are graphic projections, made by the usual microscopical methods, at a magnification of 100 diameters (reduced in the figures to 40 diameters), from the Specimens 467, 473, 449 and 444, respectively. The methods of preparation of these specimens have been described in connection with the figures 28 to 54.

These figures show the projection of the central nervous system, the giant ganglion cells, the lateral line primordia and the ectodermal thickenings that are associated with the visceral pouches, in the four type specimens, 467, the non-motile stage, figure 56; 473, the early-flexure stage, figure 57; 449, the coil stage, figure 58; 444, the early-swimming stage, figure 59.





EXPLANATION OF FIGURE

60 Three graphs to show the activity of embryos of *Rana catesbeiana* to different dilutions of hydrochloric acid when immersed in the solution during a period of 65 seconds, as compared with their activity in water under otherwise similar conditions.

Each of these three graphs, *A*, *B* and *C*, represents the composite of the activity of five specimens. The scale on the horizontal axis indicates the time in seconds, calculated from the beginning of each experiment, when movement occurred. The scale on the vertical axis indicates in seconds the total duration of the activity of the five specimens during the successive seconds indicated on the horizontal scale. For example, in graph *A*, during the fifth second of the several experiments there was activity during a total time of about three and one-half seconds on the part of all five embryos. Again, in graph *A*, the same five embryos, during the same period of immersion in water (being handled by the same pipette and passed into the same kind of a dish as were employed in the experiments with acid) manifested perceptible activity only in the sixty-second second of the period, and there was a total activity of less than a second on the part of all five specimens.

The records upon the basis of which these graphs are constructed were made upon the rolling drum of a myograph. The recording arm of the instrument was controlled by an electro-magnet, through which the current was made and broken by the observer according to the movements of the embryos during the period. The time of the activity therefore as well as its duration was recorded for each individual specimen with a considerable degree of accuracy. The accuracy of the method depends, of course, upon the experience of the observer. In my records on the behavior of amphibian embryos are over two hundred such myograms, from which the thirty for these graphs have been selected because they were taken in rapid succession upon embryos of the same lot of eggs, and therefore represent embryos in very nearly the same age. In the activity in water, however, there is evidence of an increasing tendency to move spontaneously, the record being taken in the order of the graphs, *A*, *B*, *C*.

