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The Reactions of the Vertebrate Embryo to Stimulation and the Associated Changes in the Nervous System.

By

Stewart Paton

Baltimore.

With one text-figure and plates 23—25.

The observations recorded in the following pages are the results of a series of studies begun several years ago with the intention of describing, as briefly as is consistent with accuracy and clearness of statement, some of the more important of the earliest reactions of the embryo to stimulation, and then noting in a parallel column the synchronous morphological changes in the nervous system.

As every investigator knows, who has attempted to solve any of the problems involving a discussion of the reciprocal relations of structure and function, the difficulties, both real and fictitious, that interfere with such an undertaking, are manifold. In order to avoid the dangers of speculation the attempt has been made in common with Jennings (Behavior of the Lower Organisms, Columbia University, Biological Series 10, 1906) and other investigators, to study organisms as "masses of matter".

The present aim has been to try to describe some of the more striking phenomena that occur in the embryo at the time when the first cardiac beats and earliest responses to external stimulation begin, and then to determine in a general, but not specific way, how far these reactions are dependant upon the functional activity of a nervous system. This problem is essentially different from the far more difficult task of trying to determine the links between the two sets of facts. Every effort has been made to make use of a phraseology that will not convey to the mind of the reader the idea

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that functional events may, without qualification, be expressed by the same signs and symbols as those employed by the morphologist.

The last word upon these borderline questions will undoubtedly come from the physicist and chemist, but the work of preparing the way for them is still carried on in a field from which the student of morphology and histology may not yet be excluded.

As far as possible, in recording events and conditions, a form of parallelism has been followed in order to facilitate description and to avoid any implied acquiescence in purely theoretical considerations. It must be understood, however, that this form of parallelism is provisional and relative, and therefore quite distinct from that frequently adopted as a philosophical creed by many physiologists when discussing the relations of structure and function in the nervous system.

The attempt has also been made to describe the results of these observations as objectively as possible and appreciating the well merited sympathy expressed by BALFOUR, for the student of modern scientific literature, in the Preface to his classical work on the Elasmobranchs, to refer to the work of others only when the occasion seems to call for it, and therefore a sufficient reason exists to justify any additional tax being imposed upon the reader's attention and patience.

In Section 1 a description of some of the primitive physiological reactions of the embryo to its environment, to incident stimuli, and to metabolic processes is given, while in Section 2 there is a general outline of the development of the nervous system at certain epochs, followed by a discussion of what seems to be the more important histological characteristics of these periods; and finally the attempt is made in a few instances to indicate the bearing that these detached and isolated facts have to each other. In Section 3 the technical methods employed in the study of the tissues are described in detail.

Section 1.

The observations in the main have been conducted upon the embryos of *Amblystoma mexicanum*, *Salamandra maculata*, *Rana*, *Salmo fontinalis*, *Pristiurus melanostomus*, *Scyllium canicula* and *stellare*, *Torpedo marmorata* and *ocellata*, and upon a few specimens of *Amphioxus*. As the records of results obtained in the study of

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Pristiurus, *Scyllium*, *Torpedo* and *Lacerta* embryos are more complete than are those made upon the other species, the attention will be directed chiefly to the conditions existing in the three types of the Elasmobranchs, and the single representative of the Lacertidae.

There are two reasons why the results obtained have not been equally satisfactory in regard to all species studied. In the first place the investigations made upon the Elasmobranchs and *Lacerta* were begun at a time when I had profited greatly by the experience gained by studying other embryos. In the second place the ease with which the Elasmobranchs may, at a very early period in their development be removed from the case in which they are enclosed, and kept alive for considerable periods of time either in normal salt solution or in a $3^1/_2\,^0/_0$ solution, which approximates the specific gravity of sea water, was an important factor in facilitating observation.

In *Lacerta*, on account of the extreme difficulty of removing the embryo uninjured from the shell the importance of the physiological observations was much less than those connected with the study of the tissues. In Selachians, in order to reduce the source of possible error to a minimum, the attempt was frequently made to study the character, rhythm, and rapidity of the movements before the egg-case was opened; the egg being placed for this purpose in a glass vessel filled with sea-water of the same temperature as that contained in the aquarium from which the egg had been taken. Unfortunately, owing to the thickness of the egg capsule, it is only possible by this procedure to determine more or less roughly the character of the movements. Even with the exercise of the greatest caution in the removal of the embryo from the capsule there is at first nearly always a well marked alteration in the rhythm of the movements, due to a variety of causes, depending upon the physical and chemical differences that exist between the fluid contained within the capsule and that to which the embryo finds itself suddenly transferred. The difficulties are greatly increased if the attempt is made to remove the young Trout or Salmon embryo from its shell on account of the relative large size of the sac — and therefore these species are far less adapted to experiments of this nature than are the Selachians.

The first movements that occur in *Pristiurus* embryos in any way suggesting the possibility of muscular activity have been occasionally seen in embryos of 3,5 mm., but as a rule are easily re-

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cognizable and possess certain definite and distinguishing characteristics in those of 4 mm. in length. In several instances at the earlier stage (3,5 mm.) it has been noted that about 1 mm. from the head end of embryo there is a point of flexion at which jerky lateral movements take place. Occasionally the tail end, but to a less extent, is also deflected laterally at the same instant that the anterior part swings out from the median line. Several of these movements more or less rhythmic in character occur at intervals of one or two seconds and are followed by a pause of several seconds, and then the cycle begins again. Although the entire head, and as has been said, sometimes the tail, swing laterally so that at the end of the outward excursion the entire embryo on the side towards which the movement takes place forms a concave curve, it is probable that only the middle third of the body is actively concerned in the movement, the two ends playing only a passive part in the swing. The pivotal point at which this flexion seems to occur is practically just above the point of attachment of the embryo to the egg, at a point where the myotomes develope most rapidly, and where neurofibrils are found as early as the stage represented by embryos of 5mm.—6mm. in length, appearing in the syncytium or plasmodium formed by the primitive myoblasts or their antecedents.

An exceedingly important point in connection with these primitive movements is that, if each individual excursion of the body is compared with those taking place at a time when the reactions represent the active participation of the skeletal muscles, it becomes exceedingly difficult to determine the individual characteristics which distinguish the earlier from the later forms. The differences are merely those of degree, but not of kind. If the temperature of the salt solution is the same as that of the water from which the egg was taken these movements, even in small embryos, may continue for hours. The signs of approaching death are a loss of the rhythmic character of the movements which soon become almost continuous. At first the length of excursion is greater, giving the impression of hyper-abduction and hyper-adduction, at the same time becoming more abrupt and jerky in character. The ab- and adduction occurring in embryos between 3,5 mm. and 5 mm. and which under normal conditions are the only ones observed, in this preagonal stage are frequently accompanied by a dorsal flexion which at times becomes tonic. The first of these spasmodic contractions may gene-

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rally be abolished or inhibited by the action of cocain — a few drops of a 2% solution added to each 50 cc. of the fluid in which the embryo swims are quite sufficient for this purpose. Chloretone (acetonchloroform) seems to have a similar effect, but does not apparently weaken the heart's action to the same extent as does cocain. When the movements begin to assume the pronounced characteristics of the preagonal stage neither the former nor the latter seem to diminish their intensity.

The exact moment at which these movements begin unquestionably varies in different embryos. The same holds true for all muscular contractions including those of the heart. Generally in embryos (*Pristiurus*, *Scyllium*, *Torpedo*) which have attained a length of 4 mm. they are so well developed as to be easily recognized.

A *Scyllium* embryo, length 6 mm., lying in a small glass vessel containing salt solution was placed upon the stage of a microscope and the reactions studied with the aid of Zeiss ocular 2—obj. a_3. The bodily movements which at this period are very well marked occurred in distinct groups, three or four rapidly succeeding each other were followed by a pause. The outward swing of the head and tail seemed to be due to a vigorous impulsion or rather an active muscular contraction taking place in the middle third of the embryo. The return excursion to the middle line however, was slower and gave the impression of being merely a passive movement, similar to the manner in which an india-rubber band flies back into place after the tension has been relaxed.

In an embryo of the same species, length 7 mm., the character of the return swing resembled that of the outward excursion, and after prolonged observation the inference that it was an active, and not merely a passive movement, seemed to be justified. In the earliest stages when these movements occur it is noticeable that the excursions of the head are greater than those of the tail. This may be in part the result of the attachment of the embryo and in part referable to the fact that the myotome, which is more fully developed in the cranial than it is in the caudal part of the body is beginning to function. If a needle is carefully slipped under the head or the tail of an embryo of 8 mm.—9 mm. in length and one end is gently raised, the length of the excursions of the other are changed without being completely inhibited.

In *Pristiurus* or *Scyllium* embryos (length 10 mm.) if the head is allowed to swing only in one direction, for example to the right,

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and then on its return to the middle line is prevented, by means of a needle whose point is covered with blotting paper, from passing outwards to the left, it will be noticed that on the next outward excursion to the right the distance covered by both head and tail is shorter than would be the case if the swing to the left had been uninterrupted.

In the diagram the line *A—B* represents the median line of the body, the curve *C—D* the outer limit of the unimpeded excursion to the right, while the dotted line *E—F* represents the extent of movement when the passage of head to the left is prevented. After careful study of these movements the impression is strengthened that the difference between the two outward excursions of the head to the right cannot be explained simply as the result of the body gaining greater impetus in the swing when the movement to the left is unimpeded, and therefore as a result passing further out to the right on its return. The explanation of this phenomenon is to be sought for in the interference caused to the whole movement by an obstruction acting at the opposite end of the body. Generally as the embryo grows the interference with the spontaneous movements of one end is associated less and less with corresponding disturbances in those of the other. At first the general radiation of impulses causing contractions is seriously impaired by the presence of any opposition, even if acting upon a very remote point. Gradually the extent and strength of the movements described become more marked, depending to some degree upon a variety of physical conditions in addition to those associated with the growth of the embryo. The temperature of the fluids in which the embryo is placed, as well as the differences in chemical constitution undoubtedly have a decided influence although unfortunately no exact data in reference to these points may be given.

It is hardly necessary to mention that the tendency shown by the embryo, even in its earliest stages of development to lie upon its belly, as does the adult fish, is simply the result of the natural position upon the egg. In embryos of 13 mm. or 14 mm., as soon as the attachment which holds them in position is severed, they fall at once upon their side, but occasionally display a tendency to right themselves when swimming. These early attemps while in motion

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to pass from the lateral position to that maintained by the adult fish are probably the result of the shape of the body combined with the propulsion through the water and are not to be attributed to the direct influence exerted by the nervous system. Even embryos of 20 mm. or more in length rest upon their side when not forging ahead. It is impossible to determine the time when the preservation of a stable equilibrium may be said to become a function of the nervous system. In every instance the change that takes place between movements, obviously the result of purely chemical and physical processes in the organism, and those reflecting the modifying and coordinating action produced by the interposition of a nervous system is exceedingly gradual and no distinguishing characteristics mark the advent of this domination.

Gradually as the embryo increases in length the movements become more and more complicated. Already in the case of *Pristiurus*, *Scyllium* and *Torpedo* at 15 mm. a rotatory motion appears and at 20 mm. this has become a true screw movement which adds considerably to the rapidity of the progression. At 25 mm. the embryo is almost constantly engaged in twisting and untwisting the long narrow body and tail.

Trout embryos of 15 mm. are capable of swimming once or twice around a dish of 5 cm. in diameter, but in all the fish examined definite progression is possible long before this stage is reached; thus for example at 9 or 10 mm. the embryo is quite capable, when detached, of forging slowly ahead.

When we come to consider the reactions that follow an incident stimulus, such as the touch or prick of a needle, the difficulties of accurately observing and interpreting the phenomena are very great. The results themselves are often apparently conflicting and consequently any attempt to interpret even those that seem to be the most favorable and positive should be made with the greatest caution.

In *Scyllium* embryos of 7—8 mm. it is frequently observed, that when the tail, head or body is gently touched with a needle whose point is guarded by a piece of blotting paper, that a slight but positive increase occurs in the number as well as in the extent of the movements. If the head is prevented from swinging, by gently pressing the needle still covered with paper against it, the lashing of the tail becomes more pronounced. On the other hand at this stage it is still very difficult to determine with accuracy whether when the surface is pricked with an extremely fine and sharp needle

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the character of the movements made by the embryo is materially altered.

An interesting phenomenon, and one of the earliest reactions following an impinging stimulus may be observed in Trout, if the sac of the embryo after removal from the shell is gently pricked or squeezed with the forceps. Instantaneously all the movements of the young fish become greatly exaggerated and persist for several seconds to be renewed immediately when the stimulus is again applied. I first noticed this reaction several years ago but have never discovered a satisfactory explanation for its occurrence. Possibly it may be due to transmission of an impulse by means of the fluid, which fills and distends the sac, to the skeletal muscles. No neurofibrils are to be found in the wall of the sac at this period. As far as I know this reaction, or rather series of reactions, following stimulation of the sac has not been described.

In very young embryos gentle pressure over the cardiac area causes a slight change in the rhythm and rapidity of the bodily movements, a reaction that may be set down to the interference with the circulation, and not to any sensory anomaly.

The first premonition conveying to the observer the distinct impression that the movements following direct stimulation may be the result of the dominating and coordinating influence of the nervous system occurs at the time when the neurofibrils begin to develope in the lateral line organs. This period is represented in Selachians of from 9—10 mm. in length and has been also noted at early stages in trout embryos.

One of the earliest responses to stimulation to appear, almost coincidently with that associated with the development of the lateral line organs, is obtained by pricking the belly of the fish. The immediate change in position following stimulation, or the alteration in the rhythm of the swing of the head and tail indicate the presence of genuine reflex activities. In studying these reactions it has been considered that a definite change in position of the embryo, or an alteration in the number and character of the movements, if following immediately and constantly upon an incident stimulus were evidence of the beginning functional activity of so-called reflex arcs and centres. The difficulties connected with any attempt to localize the areas on the body, the stimulation of which are immediately followed by reflex movements, are so great as to make an accurate investigation impossible. The difference however

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that does exist between the sensitiveness of head and body is, until a comparatively late stage in the life of the embryo, considerable. The epidermal layer of the former may be gently rubbed or pricked without any visible response, a condition that is in marked contrast to the series of reflex activities initiated by stimulating the latter.

The eye seems to be quite insensible to tactile stimuli in all the stages examined.

In *Pristiurus*, *Scyllium* and *Torpedo* in contrast to Trout and Salmon the spontaneous movements of the lower jaw are absent. In the two latter they may be easily seen in embryos of 16 mm. or 17 mm. while the gills move rhythmically and rapidly as early as 13 mm.

The difficulty of determining the effect of light as a stimulus is very great. In a number of instances I have observed that if *Pristiurus* embryos (12—14 mm.) after removal from the egg case were placed in a glass dish containing 3% salt solution and bright sun light was then suddenly thrown upon them by means of a mirror that either one of two things happened. If the embryo prior to this stimulation was resting quietly on the bottom of the dish an attempt was at once made to swim; but if movements were already being executed at the moment when the incident light stimulus fell upon the body a change in their rhythm and number took place. It was not determined accurately whether one part of the surface of the body was more sensitive to the action of light rays than was another. This general apparent sensitiveness to the action of light rays may really be due in part at least to the heat rays. Without further study it would not be justifiable to compare these primitive reactions to those which develope later on and are undoubtedly the result of the functional activity of the retina.

The prompt and unmistakable responses to thermal stimuli appear at an early stage — for example energetic wrigglings occur in trout (14 mm.) when touched with a warm needle, and the same is equally true of the selachians.

The study of the cardiac movements was particularly interesting, for it was observed that with the exercise of care in removal of embryo from the shell that the heart was generally seen to be beating regularly and with considerable force in selachians only 5 mm. long. In two or three instances I have noted regular unmistakable cardiac activity in embryos measuring 4 mm.

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The following table gives a general idea of the rapidity of the heart beats at different periods in the life of the embryo.

Length of embryo		Number of beats per minute after removal from egg-case and embryo placed in 3% salt solution
Pristiurus	5 mm	16—20
	10 »	27—29
	14 »	36—38
	17 »	43—45

This table with only very slight modifications is applicable to all the different species of Selachians I have studied.

In Trout and Salmon on the other hand the rate of cardiac pulsation is considerably greater; for example at 10 mm. it is 25 or 28 and at 15 mm. it has increased to 75 or 80 per minute.

I have frequently noticed that when the Selachian embryo is first brought into the dish containing salt solution that the rapidity of the heart's action is less than it is after an interval of half a minute. This change may be the result of shock.

Unfortunately I have not studied with any degree of accuracy the effect of changes of temperature of the salt solution upon the heart, but even to the casual observer there cannot be any doubt that comparatively slight changes in the temperature of the fluid medium in which the embryo swims modify very materially the rate and rhythm of the cardiac cycle.

Section 2.

a) General Description of Nervous System.

In order to avoid repetition the general appearances of the nervous system of an embryo of *Torpedo ocellata* of 7 mm. in length will be described and the discussion of the condition noted in the earlier stages postponed until considering the questions relating to the finer histological structure (Section 3). By following this plan it is hoped that the reader will get as concise and clear an idea, as the actual facts permit, of the general relation existing between the physiological reactions that have already been described and the correlative changes occurring in the central and peripheral nervous system.

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Prior to the period about to be described neither the peripheral nor central nervous system may be said to contain any fully differentiated nerve tracts, and yet, as will be shown later on, paths do exist by which impulses may be received and transmitted.

When the embryos have reached a length of from 7—8 mm. the development and differentiation of the various elements in the central as well as peripheral nervous system proceed with remarkable rapidity, and I know of no phenomena more striking than the sudden change that occurs in the appearance of the nervous system in Selachian embryos between the length of 5 and 8 mm. In the former stage, as will be shown later on, the merest rudiments of a nervous system exist, so primitive and detached that from the morphological standpoint one is at first inclined to regard them as negligible elements. Then a marvellous change begins and almost instantaneously in the brain, spiral cord, and ganglia as well as in the periphery, countless neurofibrils running either in nerve bundles or each one quite free, seem suddenly to be called into existence.

In a well stained series of sections through a *Torpedo* embryo of 7 mm. in length the following conditions may be noted.

No neurofibrils are seen in the first few sections of this series extending through the mid-brain, then as one passes a few coarse comma-like filaments begin to make their appearance in the periphery and, by the time a point in the series has been reached corresponding to the plane just above the eyes, a single bundle of fibrils may easily be made out extending from the dorsal almost to the ventral end of that part of the mid-brain contained in the section. In each plane, it may easily be seen that these primitive bundles are frequently made up of two or three fibrils which seem to spring in the majority, if not all instances, from cells lying tangentially and the only ones to be found in this zone. A number of black dots representing undoubtedly the cross sections of fibrils running longitudinally are noticed in the periphery. The fibrils connecting the cranial with the caudally situated portions of the brain become more numerous as one approaches the point of origin of the fifth nerve while the bundles lying along in the marginal veil zone (Randschleier) diminish rapidly in number. In sagittal sections a few fibrils may be noted marking the beginning of the Oculomotorius. The process of neurofibrillation in the Trigeminus has already progressed considerably. A few fibrils coming

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from a group of cells lying ventrally and in the periphery of the brain pass backwards in the marginal veil, until they reach a point just behind which the Trigeminus fibres first make their appearance. This nerve even at this early stage presents interesting features. A collection of pear-shaped cells lying in the outer margin of the medulla are connected with fibril bundles uniting them with the mass of the Trigeminus ganglion. In each section, in this area one meets with two or three of these cells, while within the ganglia itself large numbers of rather thick deeply stained fibrils, all practically running in the same direction towards the periphery on the one side and the medulla upon the other, are plainly visible. This observation does not confirm the affirmation of Klinkhardt (Kopfganglien und Sinneslinien der Selachier. in: Jena. Zeit. Naturw. 40. Bd. 1905) to the effect that at this period the ganglion as far as nervous connections are concerned, is isolated from the medulla. Not only do central connections exist but the ganglion is also already intimately united with the inner surface of the epidermal layer by neurofibrils which sometimes seem to form a loose plexus. Often, however, the individual filaments end abruptly and blindly without penetrating further between the nuclei of this layer.

It is not at all improbable, as Guthke (Ganglien und Nerven des Kopfes von *Torpedo ocellata*. in: Jena. Zeit. Naturw. 42. Bd. 1906) has pointed out, that nuclei derived from the ectoderm enter the ganglion thus forming, genuine connections with the periphery.

The majority of fibrils are found in the ganglion in those sections where it has attained its greatest dimensions, but only a comparatively few appear in the branch to the ciliary ganglion.

The branch described both by van Wijhe and Guthke as running perpendicularly to the course of the R. ophthalmicus profundus and connecting the ciliary ganglion with the epidermis does not yet contain any fibrils, although numerous protoplasmic strands may be seen uniting the ganglionic mass with the periphery. A few rather coarse deeply stained fibrils may, at this early stage, be detected in the R. ophthalmicus profundus and are the first to appear in the cross sections as we pass from the head to the tail. They curve inwards behind the eye to a point in the mesenchyme where they can not be traced any further. Thus it will be seen that the whole tract of the Nervus ophthalmicus profundus is already practically differentiated into fibrils.

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Guthke's description of this nerve in *Torpedo* embryos of 11 mm. in length (Stadium 2) correspond to the conditions observed at a very much earlier period when the tissues have been stained by a method capable of picking out the neurofibrils. The difference between the stages represented by the embryos described in the present paper and those mentioned by Guthke is even greater than at first appears. The lengths of the embryos upon which my observations were conducted were determined prior to fixation, while Guthke's measurements were made after the material had been fixed in sublimate and subsequently preserved in alcohol, so that there must be a difference of at least 5 mm. between my embryo of 7 mm. in length in which this nerve was clearly descernible and the Stadium 2 of Guthke representing the conditions in embryos of 12 mm. It has frequently been noted that embryos of this size shrink at least 1 mm. during the process of fixation so that an embryo of 11 mm. in length if measured in alcohol may be said to be at least 12 mm. long when alive.

The Ramus ophthalmicus superficialis of the Facial is not yet differentiated.

Several sections, each one 5 μ in thickness, intervene between the point at which the last fibril bundles forming the Trigeminus root are seen entering the medulla, and that where those passing into the Facial first make their appearance. In this interval within the medulla a few bundles of fibrils in each section are seen in the marginal- veil zone running in the direction of the anterior commissure; while the cross sections of others connecting more remote centres are also visible in this same location. When the Acustico-facial ganglion comes into view large numbers of fibrils are already seen creeping in among the nuclei but are not yet visible in the epidermis at the points where those two structures are in contact. All points of the ganglion, those lying cranially as well as caudally, contain a comparatively larger number of fibrils than does the Trigeminus ganglion.

After a break of ten or twelve sections, bundles of fibrils are seen entering the Glossopharyngeus. The connections between the ganglion and medulla are already numerous, a fact that is also opposed to the observations of Guthke who refers to the comparative meagreness of the connections existing between these two masses. Not unfrequently thick bundles may be followed, extending

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quite through the ganglion to a point just below the epidermal thickening. It is quite noticeable that around the group of large pear-shape cells lying in the latero-ventral part of the medulla even more fibrils are differentiated than in the Acustico-facial area. The intimate connections consisting of neurofibril bundles that unite all the ganglionic masses, beginning with the Trigeminus and including the Vagus, are a very striking feature as is also the splitting up of the coarse fibril bundles into exceedingly fine filaments about the latero-ventral group of cells from the Trigeminus through all the sections to the caudal Vagus area.

Even after passing the Glossopharyngeus this arrangement of fibrils in the medulla remains the same; three or four sections intervening before the first roots of the Vagus are seen. The latter are not so closely surrounded by cells as are the Glossopharyngeal fibres, and may be easily traced to the inner border of the epidermal thickening. As in the case of all the other epidermal thickenings it is impossible to discover any fibrils among the nuclei of this layer. The cranial bundles coming from the Vagus may be followed as they pass ventrally, frequently close under the epidermis with which they seem, except at the points above mentioned, to have no connection. The caudal branches sweep forward in a curve, until they reach that part of the mesenchyme lying between the œsophagus and epidermal plaque where they end abruptly. In no instance have I been able at this stage to detect the presence of neurofibrils coming from the direction of the central nervous system, either penetrating among the nuclei lining the alimentary tract nor among those of the heart. Near the caudal end of the Vagus area in the medulla we begin to meet with fibrils which pass outwards, at a point slightly dorsal to the exit of the ventral roots, originally described by Balfour as "the questionable anterior roots of the Vagus on debatable border ground".

Some of the fibrils in these bundles come from the large latero-ventral group of cells, that from Trigeminus area on is a prominent landmark, but others undoubtedly come from the direction of the anterior commissure. For the present I shall not discuss further their significance or origin nor their bearing upon the question as to whether they are to be regarded as forming a part of the Hypoglossus or are the first of the ventral roots. As Dohrn has suggested they may be designated as occipito-spinal nerves. These bundles pass directly outwards and on reaching

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the inner border of the cranial end of the myotome split up, some filaments passing dorsally, others ventrally, but only very occasionally do any seem to penetrate between the nuclei. Beginning with the sections where the ventral roots become prominent several important topographical changes are noticeable. In the first place the fibril bundles which might possibly be said to belong to the Vagus have diminished greatly in numbers so that only a few straggling filaments remain to mark their point of exit from the cord. The second striking feature is the corresponding decrease in the number of neurofibrils connected with the prominent latero-ventral group of cells to which reference has frequently been made. The comparatively few fibrils which still continue to leave the medulla at the spot corresponding to the exit of the true Vagus roots are now directed obliquely dorsal, instead of bending sharply and passing outwards at right angles to the medulla, as was the case in the sections through the areas lying above or in front of this this plane. Probably these filaments belong to the Vagus and are not the dorsal roots of spinal nerves. Gradually as the cranial portions of the spinal ganglia come into the field the contrast between the ventral horns with their countless fibrils and the dorsal part of the cord containing scarcely a single fibril is very great. Within the cranial portions of the spinal ganglia it is still impossible to detect the presence of any neurofibrils but as one passes caudally these gradually augment in number as the accumulation of cells increases, until the total number of fibril bundles contained in these structures may be said roughly to equal those in the ventral roots. The spinal ganglia bundles form with those from the ventral roots a plexus just in front of the ganglia, from which point one may follow the fibrils creeping along the inner border of the myotome almost to its ventral end. There is a marked disproportion in number between those in the ventral as compared with the few that leave the dorsal part of the ganglia.

An exceedingly important point is in reference to the number of fibrils passing to the myotome. These are generally finer filaments given off from the main trunk of the Ramus nervi spinalis ventralis, which bend sharply at right angles as they approach the inner surface of the myotome, where, at this stage, they generally end abruptly, and only in rare instances, in cross sections, are they seen to penetrate among the nuclei. The entire volume of the ventral roots is apparently proportional to the development of the

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myotome. Where this latter structure is in a more advanced stage of differentiation there the number of fibrils forming the bundles is the greatest. This proportionate relationship becomes very apparent as we pass caudally; practically to the end of the cord neurofibrils may be seen, but they diminish greatly in number as the myotome becomes of less structural as well as functional importance.

As would be expected the Sympathetic does not yet exist as a differentiated system, but in certain localities there are connections between cells that may represent undifferentiated tracts.

In considering the development of the nervous system and the relation it bears to the primitive movements of the embryo it is important to refer briefly to the conditions present at this period in the myotome. The nuclei in cross sections are relatively small, many are still round, and only a comparatively few have an oblong shape. The boundaries between cells in the great majority of instances are very indistinctly outlined, and in many places a fusion between the processes has apparently taken place giving rise to a definite syncytium corresponding to the description given by Godlewsky, Marceau and others, as characteristic of the early stages in the development of the skeletal muscles in Vertebrates. The place where the first neurofibrils from the ventral root bundles begin to creep along the inner surface of the myotome is a particularly favorable spot to study the syncytium or plasmodium formed by the fusion of the myoblastic cells. At this stage there is no evidence obtained from the study of cross sections of the existence of myofibrils. The structure of the myotome may be said to consist as yet merely in cells with variously shaped nuclei, and at the different points of fusion of an intervening mass of protoplasm.

In frontal sections the conditions are more easily studied. The elongated nuclei of the myoblast are easily recognisable, each containing two or more deeply stained nucleoli. Where the connections between the myomeres occur, longitudinally arranged faintly stained filaments may be made out that may possibly be primitve myofibrils.

In contrast to the arrangement of the fibrils noted in studying transverse sections I have observed that the neurofibrils in the preparations cut longitudinally frequently pass into the myotome, but no definite ending can be made out.

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Passing to the stage represented by a *Torpedo* embryo of 14 mm. in length very great changes are noted. Within the brain and medulla the number of fibrils has very greatly increased. As a rule deeply stained bundles skirt the outer edge of the sections through the central nervous system, forming long commissural tracts, not only between the two halves of the medulla, but between the cranial nerves of the opposite side. Only in exceptionable cases do fibrils seem to bring the cells in the deeper layers or along the central canal into connection with those in the outer layers. That the differentiation into fibrils takes place much more rapidly in the external zones of the central nervous system than it does towards the median line is a very striking fact. In the more frontal portions of the central nervous system the differentiation has scarcely begun. A few scattered neurofibrils in the outer zone are practically the only evidences that indicate the beginning of this process; a condition in marked contrast to the progress already made in the medulla.

When we study the individual cranial nerves we are at once struck by the rapidity with which the process of differentiation has progressed. The Ophthalmicus profundus, ending free in the mesochyme, in the sections in front of the plane of the eye stands out very sharply as it contains numerous bundles of deeply stained fibrils which may be traced with ease, as the nerve arches back of the eye, into the mesocephalic ganglion (Ciliary ganglion). This latter structure is a very prominent feature and although it contains many neurofibrils and is in close contact for several sections with the so-called Ciliary field no fibrils are seen to enter this epithelial thickening. I also failed to detect any fibrils in the Ramus dorsalis ganglii mesocephalici. Although it is an easy matter to make out that undifferentiated strands of protoplasm connect the Ciliary field and ganglion I have not been able to discover that any differentiation into neurofibrils takes place in this tract in *Torpedo*. Dr. R. Gast has called my attention to the fact that in sections of *Mustelus* stained by the same method this connecting tract between the Ciliary ganglion and periphery is well differentiated into neurofibrils even at the early stage represented by embryos of 7,5 mm. in length. In the plane just in front of the point where the Ganglion trigemini comes into the field a bundle of deeply stained fibrils becomes visible in the epidermal thickening in the region supplied by the Superficial ophthalmic, and is an important object in the area through

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all the sections in which the Trigeminus ganglion appears. The fibrils entering the periphery to form this nerve lie in the same plane as does the upper portion of the Facial ganglion. Both the Trigeminus and Acustico-facial ganglia now contain countless fibrils. The connections with the ventro-lateral group of cells in both instances have, as compared with the earlier stages, been greatly re-enforced. In the Facial nuclei, more markedly than in the case of the Trigeminus, the differentiation of both cells and fibrils has extended inwards from the periphery towards the central canal, and many of these elements are surrounded by a diffuse neuroreticulum. Beginning with the more caudally situated portions of the medullary centre of the Trigeminus the number of neurofibrils appearing in the dorsal part of the medulla, as well as those fringing the whole of the outer border of the lateral zone increases rapidly, while the neurofibrillation taking place about two groups of cells in the marginal veil zone, dorsal to the prominent ventro-lateral group, has already become a prominent characteristic of sections through this area. Both the Ramus mandibularis as well as Ramus maxillaris are very well supplied with fibrils. In each of these nerves, although the neurofibrils may readily be found in contact with the inner epithelial layer, they do not seem to penetrate it at this stage. Thick bundles of fibrils enter the Acustico-facial ganglion from all the lateral parts of the medulla. Those coming from the extreme ventral or dorsal areas bend sharply at their point of exit as they pass into the ganglionic mass.

The Buccal branch of the Facial may be seen in close contact with the medulla sending off many fibrils which creep in between the epidermal nuclei, sometimes ending abruptly just before they reach the most external layer of cells, while others in the deeper layers frequently unite to form a definite network. The Ramus hyoideus with its two branches post- and praetrematicus is easily recognizable, its neurofibrils at this period still seem to end quite free in the mesenchyme. The Acustic branch from the ganglion has already attained considerable proportions and filaments from it may already be detected penetrating the wall of the ear. The manner in which these auditory fibrils end does not differ in any particular from that observed in connection with the terminations in the epidermis of the other nerves already described. I have not been able to satisfy myself that the Ramus posttrematicus is, as Guthke affirms, so free from nuclei as to make this condition more or less of a

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prominent characteristic of the development of the nerve. In this respect it does not differ essentially from a number of other nerves, for at several points in its course one may see elongated nuclei lying close to the nerve although there are one or two stretches where it lies quite free in the mesenchymatous tissue.

In the Glossopharyngeal the process of neurofibrillation has made considerable advance as compared with the earlier stage. Many bundles unite the ganglion with the medulla and the individual branches are well differentiated. The post- and praetrematic branches contain, on a rough estimate, about an equal number of fibrils.

When the Vagus appears in the field of the cross-sections the arrangement of fibrils within the medulla is practically identical with that of the Glossopharyngeal area. In both cases the large ventro-lateral group of cells is surrounded by many fibrils uniting to form bundles and entering the ganglia as roots. The total number of such bundles coming from the dorsal part of the medulla is markedly diminished as compared with the Trigeminus and Facial centres. In the Vagus at this period, in contrast to the Glossopharyngeal, neurofibrils may be detected at practically all the points where the former nerve is in contact with the epidermal thickening. This fact has an important bearing upon the development of the lateral-line organs. All branches of the Vagus including the Ramus intestinalis are richly supplied with neurofibrils but only in rare instances do they enter the organs which they ultimately supply.

The Spinal nerves are in an advanced stage of neurofibrillation, both ventral and dorsal roots are very prominent in the field as well as the plexus formed by their union, just ventral to the spinal ganglia. From this plexus a large recurrent branch is given off which supplies the dorsal part of the myotome. The ventral branch follows the inner bord of the myotome almost to its ventral end. In its course it gives off comparatively few branches, and the majority of these enter the myotome. The communicating branches with the collection of cells forming the sympathetic are well developed, the bundles of fibrils frequently split up forming a network about the cells so that frequently in the plane of one section several cells united by a common network may be made out. The cell-clumps and the neurofibrils forming the sympathetic ganglia are already separated by quite an interval from the ventral branches of the spinal nerves. At the outer edge of these ganglia close to the inner border of the

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large bundle forming the ventral branch of the spinal nerves one frequently sees fibrils coming from the Sympathetic, dividing to form a T-shaped branch, one arm of which runs dorsally parallel to the spinal nerves, while the other is continued ventrally. An interesting fact is that the size of these filaments is the same as that of the main trunk. Their destination is not known. Only in rare instances may filaments be detected approaching the various organs which are supplied by the Sympathetic. In the present paper no attempt will be made to discuss in detail the various points connected with the development of the Sympathetic except to refer to the impression gained by the careful study of a large number of sections as not being antagonistic to the view that parts of the Sympathetic may even at this period be functionally active. It is however very apparent that from a morphological standpoint the development of this system has proceeded more rapidly than investigators have hitherto believed to be the case.

b) Histological Studies.

So much has been written concerning the earliest stages of the development of the nervous system, that there is no occasion for repeating what has already been said upon this subject. The appearances of the nervous system prior to the period represented by Selachian embryos of 4,5 mm. in length will therefore not be described. The details of the method used in preparing the material for study are given in Section 3.

The following conditions were observed in a series of sections taken from a *Pristiurus* embryo of 5 mm. in length in which it had been noted that the heart beat rhythmically but slowly, at the rate of 19 or 20 to the minute while passive lateral movements of the head and tail, the result of the abrupt jerky contractions of the body, so eminently characteristic of this stage of the development, were also present.

In the transverse sections of the spinal cord most of the nuclei were elongated and oval of from 10μ—12μ in length and 3μ—7μ in breadth, while a few only were circular. Some of the nuclei in the inner cell layer had begun to stain deeper than others and, as becomes evident later on this fact marks the beginning differentiation of the spongioblasts. Except in the species where the large cells of Beard begin to make their appearance no other distinguishing

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characteristics yet mark the process of cell differentiation within the cord. The germinative cells of His are, as recent investigations have shown, to be regarded simply as mitotic cells.

In the region of the ventral roots there is a marked bulging forward of the protoplasm and in many sections a "bridge" about 4μ—5μ in width similar to those shown by Kerr to exist in *Lepidosiren* (On some Points in the Early Development of Motor Nerve Trunks and Myotomes in *Lepidosiren paradoxa*. in: Trans. R. Soc. Edinburgh Vol. 41 1904) may be seen spanning the distance between the edge of the medullary substance and the inner border of myotome. One of these bridges is shown in Plate 23 Fig. 1. The structure of the protoplasm of which these strands are composed seems to be similar in all respects to that forming the general matrix and does not at any point, except at the places to be described later on, present evidence of a fibrillar structure. Less frequently strands of protoplasm are seen in other localities, as for example at the point where later in the development of the embryo processes of giant ganglion cells (Beard) emerge from the cord, or where the matrix surrounding the cells of the ganglionic masses is in contact with the periphery. Any attempt to determine the moment when these bridges appear and the manner in which they are formed necessitates the consideration of questions of fundamental importance.

Are these structures the product of a single cell or do several elements contribute protoplasm to span the interval between two given points as widely separated as the periphery and cord? Hensen's idea that these bridges are originally thrown across from one cell to another and then as the embryo grows these threads are pulled out to many times their original length, is an exceedingly ingenious and suggestive hypothesis but has not yet been proved. There cannot be any ground however for doubting the existence of these structures. The debatable point is merely in regard to the manner in which they are formed.

Neal (The Development of the Ventral Nerves in Selachii. in: Mark Annivers. Vol. New York 1904), in an interesting paper on the development of the ventral nerves in Selachians, says that he had been unable in any of his sections to show the existence of a protoplasmic connection "even of the most attenuated kind between the somite and the neural tube before the first neuraxon makes its exit from the neural tube". At first it was difficult for me to re-

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concile this statement with the results of my own observations, as well as those of other investigators who have repeatedly observed these bridges in Selachians. At the time when NEAL's investigations were conducted there was no method of staining which was capable of differentiating the component parts of the neuraxon, and it is not at all improbable that the structure which he had reason to believe was the growing end of a neuroblast was only an undifferentiated protoplasmic band or bridge. In the second place the method of fixation undoubtedly has something to do with the failure to detect the existence of these structures, which are much more easily demonstrable in sections fixed in corrosive-acetic or neutral formol, than they are in solutions containing picric acid. For the reasons mentioned the structures represented by NEAL as neuraxons cannot be accepted as such without further proof. Probably the long processes depicted by this investigator as being projected from medullary cells are in reality made up of two components: a short process and the long undifferentiated protoplasmic strand or bridge with which it is apparently fused so as to give, in specimens stained by certain methods, the appearance of a single long process. In Plate 23 Fig. 1 one of these bridges is represented, the proximal side of which has fused with the matrix of the cord while the distal is united with that of the myotome.

A similar condition is also depicted in Fig. 2. At later stages one may find connections present between the cord and group of cells, which eventually form the spinal ganglia, and between the latter and the periphery. Further there is abundant opportunity to study these plasmodesmata (HELD) in the region of the Cranial nerves, where undifferentiated links of protoplasm frequently unite the existing ganglionic masses either with the central nervous system or with the periphery. In the case of the Oculomotorius and Trochlearis the existence of these bridges is very problematic.

A number of investigators have already directed attention to the presence of these primitive protoplasmic bands in connection with the anterior nerve roots. In 1888 DOHRN, in describing the origin of the ventral roots in Selachians said that the first appearance of these structures was characterized by the extrusion of a homogeneous structureless protoplasmic effusion from the spinal cord. Among more recent observers it is only necessary to quote FRORIEP (Verh. Anat. Ges. 18. Vers. 1904 pag. 12) as reaffirming the existence of these structureless homogeneous bands uniting the cord and sur-

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rounding tissues, but few will be ready to admit with him that in all cases it is possible to determine with accuracy the individual connection of these protoplasmic spans with the intra-medullary cells.

The view recently expressed by Held (Verh. Anat. Ges. 20. Vers. 1906 pag. 185—202) to the effect that at the time when the first motor and sensory nerve roots become visible these plasma bridges already form such an intricate and extensive network as to baffle any attempt at this comparatively late period in development of determining the cells from which they originally sprang is more in accord with the results of the present series of observations. Five possible sources of origin have been assigned to these structures: 1) the cells of the neural canal; 2) those of the myotome; 3) both of these groups have been said to take part in their formation; 4) the cells of the mesenchyme and 5) those of the chorda. Although Held seems to think that the latter may contribute material for their formation, I have seen no evidence which would seem to support this view. Unfortunately it is impossible to pick out the individual cells which actually take part in the formation of these bridges so that the whole question is still open for further investigation. There are a number of reasons why it seems probable that these structures are sometimes of poly- and not of mono-cellular origin. There is no direct ocular evidence that only one cell is immediately concerned in their production, while in many sections, in the areas to be described the protoplasmic extrusions seem to be so extensive that the idea of considering them to be the products of a single cell is scarcely tenable.

In the section from which the drawing represented in Figure 3, Plate 23, was made it at once becomes apparent that the dimensions of the bridge have attained such magnitude, particularly in breadth, as to render it unlikely that this mass has during its development been thrown out from a single cell. There are two paths within the cord which I have closely studied, with the object of determining if possible the exact boundaries of the cells and the relation they bear to the surrounding matrix. The first is the triangle, already described as forming the base of the span that extends from the point of emergence of the ventral roots to the myotome, and the second is the rim surrounding the central canal which at certain stages and localities contains few if any nuclei. In both places it is impossible to determine with accuracy the exact cells

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which eject sufficient plasma not only to form, but to supply material in large enough quantity to permit of the growth of this portion of the matrix. A third example of the extreme difficulty of defining the cell boundaries is observed in connection with the development of the lateral line where neurofibrils appear at a very early date and where the bonds between the ganglion and rudimentary sense organs are so complex and numerous that there are countless paths which the fibrils may follow as soon as they once begin to be laid down. This whole question, connected with the origin and growth of these bridges, is so involved that certain phases of it deserve far more attention than they have yet received.

In passing a word of caution may be introduced in regard to the possibility which exists of mistaking these bridges or strands for the processes of neuroblasts. If sections are stained by methods which are incapable of differentiating the fibrils from the surrounding protoplasm, then it is quite impossible to say with any degree of accuracy whether the process of a given cell is or is not a true nerve. In the very interesting report of Harrison upon his experiments on the development of the peripheral nerves, he says that in one instance, after transplantation of the ganglionic crest with the cord, sheathless nerve fibres ran freely through the peritoneal cavity. As all true nerves are now known to contain neurofibrils it seems to me inadvisable to refer to any long process as a nerve, until by the use of a differential stain one has determined whether the so-called nerve is anything more than the undifferentiated protoplasm thrown out from a cell. I am very far, however, from denying that a nerve cell may not under certain circumstances throw out a process of very considerable length, and in fact there seems to me to be evidence that such a condition does exist in the place that corresponds to what Held calls the primary nuclear free stretch of the nerve. The greatest caution should be observed in assuming that mere length of process, without positive knowledge regarding the nature of the structures contained in it, is in any sense to be considered a criterion as to whether a cellular prolongation is or is not to be called a nerve. Even admitting that there is some justification from a physiological standpoint in referring to an undifferentiated tract of protoplasm as a nerve, it will be seen that the term if used in this broad sense would give rise to endless confusion, and it would at once become necessary to define its application in each individual case.

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It is not difficult to find outside of the nervous system conditions that are analogous to those occurring within the neural canal. In studying the histogenesis of the retina, WEYSE & BURGESS (Amer. Natural. Vol. 40 1906) have called attention to the peculiar manner in which the reticular layers are formed from the cytoplasm without any extrusion of nuclear substance, while the rods and cones seem to develope from threads extending between the pigment layer and the external limiting membrane.

In the development of the skeletal as well as cardiac muscles a fusion of the cells quite analogous to that observed in the nervous system has been described by GODLEWSKI (Arch. Mikr. Anat. 60. Bd. 1902 pag. 111) whose results have in the main been recently confirmed by MARCEAU (Ann. Sc. N. [8] Tome 19 1904). On account of this remarkable similarity of the two processes it is worth while recalling GODLEWSKI's account of the changes as they occur in the myotome. Referring to the disposition of the cells in the more cranially situated portions of the myotome in a rabbit embryo of 11 days, he says, that in this region the cell boundaries tend gradually to become more and more indistinct. A cell syncytium is formed, which increases in extent not as in the ordinary manner by the growth of the cell body, nor by nuclear division, but by the fusion of cells possessing similar structural characteristics.

It is not my purpose in the present paper to attempt to describe more in detail the various points where a so-called syncytium or extrusion of protoplasm is formed, but merely to direct attention to the importance of studying the origin and development of these protoplasmic effusions on account of the relation they bear to the subsequent fully differentiated tracts for the conduction of nervous impulses. After studying carefully sections fixed and stained by a great variety of methods, I am satisfied that these apparent extrusions, quite free from nuclei, cannot be the result of an imperfect technique. This was the idea which suggested itself to my mind when these areas first attracted my attention ten years ago in studying the histogenesis of the cerebral cortex in the pig. At that time the true significance of this condition was overlooked and these apparent early connections between the cells were considered to be mere artefacts and, consequently, were studied very superficially. The importance of the subject was again presented in a very striking manner when it was noticed that, at the time the first physiological reactions for external stimulation began to appear in the

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embryo, many points of contact between the periphery and central nervous system were already distinctly outlined by these undifferentiated tracts. To HENSEN undoubtedly belongs the credit for having years ago called attention to the importance of these connections, and recently this investigator has repeated and amplified his original descriptions (Die Entwicklungsmechanik der Nervenbahnen im Embryo der Säugethiere, Kiel u. Leipzig 1903). KERR, as already indicated, has confirmed some of these observations in *Lepidosiren*. The very decided view expressed by HENSEN however against the possibility of connections existing between nerve and connective tissue cells does not seem to be justifiable particularly for the later stages in the development of the embryo. The question is still an open one, for at the time when the mesenchyme begins to creep in between the myotome and neural canal it is extremely difficult to determine the relations between the intruding tissue and the established connections that have bridged the interval between the two structures. At various points, at more advanced stages, one gets the impression from the manner in which mesenchymatous cells cluster about the primary bridges or plasmodesmata that the possibility of material being thrown out from the former to supply the substance necessary for the expansion and development of the latter, may be a possibility. KERR has described the manner in which the protoplasmic mass forming the rudimentary nerve spreads out over the inner surface of the myotome and admits the possibility of its covering the whole of the inner surface. I have frequently observed a similar condition but it has been practically impossible to determine accurately the cells from which this mass may be ejected. This fact alone should justify extreme caution in expressing a definite opinion as to the number or identity of the elements concerned in its production. Even if it should be proven that the primary bond of union between myotome and cord is the product of a single cell, this does not in the least preclude the possibility of other cells contributing material to permit of its expansion and growth along the inner surface of the myotome. Prior to the appearance of the neurofibrils the nuclei within the cord are very sharply outlined but here also it is quite impossible to determine the limits of the cell boundaries. The whole mass seems to be welded together by the intervening strands of protoplasm which are continuous, not only with the substance forming the neural base of the bridge at the point of exit of the ventral roots, but also with the material enter-

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ing the latter and the narrow band (marginal veil) along the outer edge of the cord. The inter-nuclear strands are also apparently united along the margin of the central canal by a band whose varying width depends upon the proximity of the nuclei to the canal. The matrix in all parts seems either to be homogeneous and finely granular, or in preparations fixed in sublimate-acetic and stained by hæmatoxylin-eosin, to be very faintly fibrillar, giving the appearance indicated by Hensen (Die Entwicklungsmechanik etc., Fig. 58 a and 58 b) as well as other observers. At present no attempt will be made to decide which one of these two structural forms represents the true characteristics of the matrix. This primitive fibrillation has apparently no connection with the development of the neurofibrils. The very faint primary striation is observed at the point where the true neurofibrils develope as well as between the nuclei surrounding the central canal and only in very rare instances has even a suggestion of this form of structure been found in the bridges. The primitive neurofibrils are coarse, deeply stained structures appearing primarily in a locality where more than in any other place the ground substance, even after sublimate fixation, seems to be granular in character, while the more delicate and attenuated filaments only become visible at later stages. (Compare Figs. 1, 2, 3, 7, 9 with 11, Plate 23 and with Fig. 22, Plate 25.)

In embryos of from 5 mm. to 5,5 mm. a change has taken place, and either within the myotome or the substance of the bridges connected with the ventral roots, coarse deeply stained, slightly tortuous fibrils begin to appear. These vary in length from 5μ to 15μ and lie quite free in the surrounding protoplasm. In sections which have been carefully differentiated it is impossible to show that they are connected with any cell. The apparent independence of these primitive neurofibrils in the ventral roots from cells is one of their distinguishing characteristics, but in the large cells of Beard a different arrangement exists. There the fibrils appear in the apical process of the cell close to its nucleus (compare Fig. 2 with Fig. 12, Plate 23). Unfortunately at present, one cannot speak with any degree of certainty regarding the exact relation of the primitive fibrils in the ventral root area to the adjacent nuclei as there is no method of fixing and staining material which gives both a sharp delineation of cell boundaries and a clear picture of the neurofibrils. If sections of the cord of Selachian embryos of from 4 mm.—5 mm. in length, fixed and stained by any of the me-

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thods most favorable for the determination of cell boundaries, be carefully studied, it is apparent that in certain areas, particulary at the point where the ventral roots develope, there are undifferentiated portions of protoplasm. The same is true of the marginal tract (Randschleier). The affirmation frequently made that these areas are completely filled up by the processes of neuroblasts, with the addition of the intermediate supporting tissue derived from spongioblasts, lacks confirmation. There is no evidence that at this period any of the products of these latter elements have yet reached even the inner border of the group of neuroblasts lying in the antero-lateral region of the cord.

The description of the intimate connections so far reported to exist between the primary ventral root fibrils and cells cannot be said to be convincing, and they are demonstrable only in those instances where the methods of fixation and staining fail to differentiate sharply between the protoplasm extruded from a cell to form a process and the neurofibrils. The sections where these apparent connections are most clearly shown are those in which silver is precipitated in comparatively large quantities and where only a slight differentiation of the tissues, even under the most favorable conditions, is possible. Such a method for example is the one recently employed by Ramón y Cajal. A careful comparative study of the results obtained by the use of this method and the one described in Section 3 shows that the differentiation of the tissue obtained by the latter is far greater than by the former. The excessive shrinkage that occurs when the former is used is an additional drawback in studying the finer structure of the nervous system.

The precipitation of silver taking place at the ends of the neuraxone in preparations stained by Ramón y Cajal, and described by this investigator as bulbous ends (Wachsthumskeule) are clearly artefacts, and show the unreliability of his method when used as a means for studying the histology of the nervous system. The swollen ends of the processes represented in the illustrations accompanying his latest contribution (Anat. Anz. 30. Bd. pag. 113—144 1907 Fig. 11 [*c*] and Fig. 12) are obviously artificial products. Within the processes the individual fibrils are glued together by the excessive deposit of silver, while the distal ends are surrounded or encapsulated by an imperfectly stained coating of protoplasm in which they are embedded. The apparent independence of the neurofibrils is well shown in my Fig. 7, Plate 23.

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In Fig. 3, Plate 23 it may very readily be seen that the fibrils lying in the apex of the neuroblast (*a*) are distinctly differentiated from the envelope of protoplasm which forms the process. No such envelope surrounds the fibrils lying within the bridge (*c*). By studying the sections in front of and behind the plane of section, represented in this drawing, it becomes obvious that the bundle of fibrils whose thicker ends rests at the point (*c*) in the bridge is not connected with those in the cell (*a*). In imperfectly differentiated specimens artificial links are frequently formed by the silver deposit between the ends of two such bundles which histologically are quite distinct, and in this way silhouettes of cells with elongated processes are often obtained.

Ramón y Cajal first called attention to the development of the primitive neurofibrils at some distance from the cells with which they were supposed to be connected and, as a possible explanation of the phenomenon he suggested that as the parts of the protoplasm furthest from the nucleus were the oldest, therefore they were the first to be differentiated. Naturally the correctness of the inference depends upon the proof that the fibrils observed are definitely connected with individual cells. Methods capable of differentiating the tissues and not giving merely silhouettes, the result of deposition of silver, have so far failed to show the existence of these links. If however in spite of this fact the suggestion is made that the primary fibrils, lying for example in the myotome in embryos of 5 mm. in length, are merely the terminal portions of processes belonging to intramedullary cells, then the difficulty arises of explaining how it is that these fibrils lie in a territory into which the processes have not yet penetrated. If the affirmation is made that these fibrils, lying at a considerable distance from the ventral horns, are in reality connected with the unstained intramedullary tracts I am not prepared to definitely accept nor to deny the validity of the objection.

Should incontrovertible evidence be furnished demonstrating the continuity between the medullary cell processes and these extramedulary fibrils it would not materially conflict with any of the other facts observed. It is necessary to remember however, that no reliable method of staining has yet been employed that is capable of demonstrating the presence of processes in the vicinity of the distal ends of these primitive filaments.

In Fig. 5, Plate 23 the degree of differentiation that exists between the fibrils and the protoplasm of the processes is represented.

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Occasionally one notices a cell within the cord throwing out a process which tapers rapidly as it approaches the base of the bridge, and either before or soon after entering this structure loses its identity in the surrounding mass of protoplasm. In a later stage, represented by *Torpedo* embryos of 7 mm. in length, the relation of the fibrils to the cell processes and nuclei may be studied to advantage. A representation of these relations is given in Fig. 5. Two nuclei are visible with very faintly stained processes (*b*) and (*d*) which may be followed in the direction of the ventral roots (*c*). A small connecting branch (*e*) seems to unite the fibrils in one process with those in another. An important point to be noted is that no process distinct from the surrounding matrix can be distinguished at the inner pole of the nuclei. The thin tapering spiral processes capping the nucleus and running in the direction of the ventral roots are the only definite outlines indicating cell boundaries that exist in these sections. At (*a*) is a single fibril which has crept past the nucleus and entered the field where cell boundaries, if they exist, are indistinguishable. In Fig. 8 of the same Plate these conditions are even clearer, although the embryo from which the sections were prepared was only 6 mm. long. This difference in the length serves to emphasize the fact that the period marking the beginning of neurofibrillation as well as the rate at which it proceeds varies considerably even in embryos of the same species.

At (*c*) in Fig. 8 delicate fibrils may be seen lying either upon or at the side of the nucleus, while at (*D*) two coarse and several finer fibrils contained in the common envelope (*N*) diverge from the others, passing in the direction of the two nuclei at the extreme right in the drawing, to skirt the edge of the cord. Again (*b'*) two fibrils running quite parallel to each other are represented passing a nucleus in the outer layer and continuing their course as far as one in the second row.

In Fig. 9, Plate 23 there is a drawing representing a section of the cord with the ventral roots in one of the early stages of *Lacerta muralis*. The intense staining of the fibrils and the fact that they are of a more uniform thickness and generally coarser than is the case in Selachians is a prominent characteristic. These structural variations in different species deserve closer study than has yet been given to them.

The neurofibrils within the bridges and the triangular area in the cord begin to multiply quite rapidly (Figs. 8 and 9, Plate 23) and at the same time single fibrils or bundles begin to appear in

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the outer zone or marginal veil. At a very early period commissural bundles already connect both sides of the cord. Not infrequently single coarse fibrils, forming a very striking element in the field, sweep in a long curve from the triangular area at the base of the motor roots on one side across the ventral commissure and then bend inwards until they have reached the inner layer of cells. The probable significance of these structures will be referred to later on.

At the points at which neurofibrillation precedes most rapidly, namely within the ventral root bridges and the triangular area so often described, the process spreads inwards in the direction of the central canal. The single coarse fibrils already described split up at their distal ends into fine brush-like arrangements well shown at (*c*) and (*b*) in Fig. 8, Plate 23. The multiplication of fibrils by longitudinal division, a process similar to that described by Heidenhain, Apáthy, Maurer and others, may be observed in this area. The coarser and thicker bundles of neurofibrils are characteristic of these earlier stages of development. Later on the process of longitudinal division which occurs splits these strands up into finer filaments. The contrast in this respect between the early and later stages is well shown by comparing Figs. 3 and 7 with 11, Plate 23.

If the attention is directed to the process of differentiation in other parts of the cord, it will be seen that a few large deeply stained nuclei are met with lying along the central canal with processes extending towards the outer cell layers but never showing any evidence of fibrillation. These structures are undoubtedly spongioblasts and may readily be distinguished from all other elements. The outer end of the process sometimes bifurcates forming a V- or T-shaped branch, but as the faintly stained terminal filaments are soon lost in the surrounding mass of protoplasm it is impossible to speak with any certainty regarding their ultimate fate.

All my observations upon the earlier stages of the development of the spinal cord in Selachians seem to point, as already indicated, to the existence of a close union between the cells probably by means of protoplasmic bonds, or, as Held has called them, plasmodesmata. This is the view originally promulgated by Paladino (1894) and apparently confirmed by C. Desta (Riv. Sperim. Freniatria Vol. 30 1904) and by R. Varela de la Iglesia (Contribución al estudio de la médula espinal, Madrid 1904). It is impossible to say, whether the links between individual cells are formed by separate bridges, or whether there is a common matrix composed of material

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ejected from the cells. In Fig. 4, Plate 23 the union between neuroblasts and spongioblasts is clearly shown in a drawing made from the section of a spinal cord of *Scyllium canicula*, length 6 mm. At the edge of the central canal (*c. c.*) is a large deeply stained nucleus, not containing any nucleolus, whose surface near the circumference shows a faint coarse mesh-like structure. Two distinct fibrils pass outward from the outer end of the nucleus to become united into a common strand at *p*. When this point is studied carefully with the aid of the Zeiss Apochrom obj. 2 mm and Compens. Oc. 18 there cannot exist any doubt in the mind of the observer that this nucleus is directly connected with the network of neuro-fibrils belonging to the large cell of Beard represented in the figure and whose nucleus lies just dorsal to the central canal. A second bundle of fibrils (p^3) runs in the direction of the nucleus of the spongioblast but ends abruptly before reaching it. A bundle of fibrils (p^1) the continuation of the fibril *p* marks the inner edge of the fine network connected with the cell of Beard. All the intervening inter-nuclear substance is faintly tinged with eosin but does not show any evidence of fibrillation. The long coarse filaments already referred to, which are seen to pass from the ventral roots on one side through the anterior commissure and then to bend in towards the central canal, where they end, either in a network that surrounds the nuclei in the inner cell layer or spread out over the protoplasm bordering the central canal may possibly be the processes of neuroblasts. On account of the difficulty in following the course of these structures as well as in determining the limits of cell boundaries the question cannot be definitely decided. As development proceeds the connections between neuroblasts and spongioblasts, at least the forms represented in Fig. 4, disappear. Generally when the Selachian embryos have attained a length of 7 mm. or 8 mm. many of the fibrils in and near the ventral roots have split up, forming attenuated filaments at the same time that differentiation is proceeding more rapidly in the inner layer of the cord. The fibrils which at first were not united by lateral connections, now begin to form a definite network, generally most marked over the apical processes of the cells in the regions of the ventral horns. After comparing the figures 1—5 in Held's paper, as well as this writer's description with my own results there seemed at first to be considerable discrepancy between us in regard to the arrangement of the primary fibrils within the cell body. Held's

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preparations undoubtedly convey the impression that from the very beginning the fibrils are arranged in the shape of nets, while in my sections (compare Figs. 3, 5, 8, 9, 12, Plate 23) the first are nearly always parallel without any cross connections. (The cell represented in Fig. 6 is a much later stage than those where the fibrils are parallel.) One explanation for these apparent discrepancies is that the method employed by me seems to be capable of staining the fibrils at a period antecedent to that represented by Held. The longitudinal arrangement of the primitive fibrils is well shown for example in Figure 9 at the points indicated by the letter *f*. In preparations where the differentiation is not well marked and the sections are more than 5 μ in thickness it may sometimes be difficult to recognize this primary longitudinal arrangement, as short sections of fibrils lying in quite another plane may be superimposed on each other so as to give the appearance of a network. The relative value of the method described in Section 3 as compared with that of Ramón y Cajal in differentiating the neurofibrils may be seen when applied to the study of the processes of the ventral horn cells. It is not difficult to show that the coarse bundles are made up of fine fibrils, while the arrangement in the cell processes as represented in Figure 8 of Held is probably the result of an imperfect differentiation, by which individual fibrils are glued together by the excessive silver deposit.

Prior to the period when nets appear in the ventral horns the cells of Beard in the posterior columns have passed rapidly in their development from the stage represented in Fig. 12, Plate 23, to that shown in Figs. 4 und 14 of the same Plate or Fig. 15, Plate 24. In the one instance there are only a few fine fibrils lying parallel to each other within the apical processes, while in the others there is a diffuse network extending over the processes, nucleus and body of the cells and in two of the figures they are shown to form an intimate union between two cells lying on opposite sides of the dorsal commissure.

The importance of the sections represented respectively in Fig. 14, Plate 23 and Fig. 15, Plate 24 is very great, because these two preparations seem to me, as well as to the others who have studied them, to demonstrate conclusively that definite and very distinct connections exist between the neurofibrils in one neuroblast with those in another. It is comparatively easy in sections stained by the method I have employed to find many cells where the general arrangement and distribution of the fibrils seems to indicate

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the probability of the passage of these structures from one cell to another, but it is extremely difficult to find two nuclei with their neuroplasm lying in the same plane, so that the continuity may, without doubt, be established. In Fig. 14 it may be seen very clearly that the continuity between the two cells is established by a network made up of coarse and fine strands, and a similar arrangement is noted in Fig. 15. The network in Fig. 14 is seen to extend laterally, partially covering a third nucleus at the dorsal margin of the central canal. Numbers of small black granules are visible at the various points of junction formed by the meshes of the network, and these represent probably the cross sections of fibrils running at right angles to the plane of section. Careful study, with the aid of high power lenses and brilliant illumination demonstrates, that the fine and coarse strands in the reticulum unite so as to form a single net, and are not two distinct structures. This network or neurospongium is absolutely distinct from the coarse faintly stained structure sometimes seen with in the spongioblasts. At these early periods in the development of the embryo a network of this character is only found at the point where the differentiation of the neuroblasts proceeds with the greatest rapidity, and only in or around the cells of this type. The objection that may possibly be entertained by some, to the effect that at least a part of these fibrils are derived from spongioblasts cannot be accepted as valid as there is no evidence in favor of this view. In the large cells of Beard the centre of differentiation of the protoplasm into fibrils seems to be within the cells, but in the case of the ventral root, as already indicated, it is either in the plasmodesmata outside of the cord or just within the outer triangular zone or the narrow marginal rim of protoplasm between the ventral and dorsal roots. In the case of the spongioblasts the differentiation of these cells is in the opposite direction, beginning in the inner row of cells close to the central canal. In well stained sections there cannot exist the slightest difficulty in distinguishing the neurofibrils from the products formed by the spongioblasts. There is no reason for supposing that filaments from the latter, even if they were produced at this time, may creep into the body of the neuroblasts along the arms which occasionally unite these two different types of elements. At the moment when neurofibrillation begins neither the apical processes, bodies nor nuclei of the spongioblasts contain any fibrils, and all the evidence points to the early atrophy and disappearance of these

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connecting links. There can be no reasonable ground for doubting then that the network (*a*) in Fig. 14, Plate 23 is an integral part of that uniting the two cells (*b*) and (*c*). If the idea is not abandoned, in spite of the absence of any direct evidence showing that this network (*a*) is the product of spongioblasts, it then becomes necessary to attribute to neuroblasts the double capacity of producing both kinds of fibrils. The manner in which neurofibrillation takes place first in the outer layers of the cord and then works in is well shown in Fig. 10, Plate 23. Capping the round nucleus in the outer layer of cells is a process, containing a number of fibrils which are continued inwards almost to the edge of the canal, where the terminal arrangements become too indistinct to be carefully studied. Fibrils coming from the periphery sometimes seem to form a net within this inner rim of protoplasm which bounds the central canal. I have no further knowledge of the relation of the neuro-reticulum at this point to other structures than the absence of all evidence indicating that it is the product of spongioblasts. The cells of the sympathetic nervous system are also united by the neuro-reticulum. In Fig. 27 I, Plate 25 a bundle of fibrils may be seen splitting up (*a*) into several strands which connect the bodies of the two cells and then unite to form a common bundle (*b*) which runs in the same plane until it finally bifurcates (*c*). The curious manner in which fibrils sometimes encircle the nucleus of cells in the sympathetic is represented in Fig. 27 II. An arrangement of the fibrils similar to that shown in 27 I is reproduced in 27 III.

The figures 7 and 11 of Plate 23 and 22 of Plate 25 afford interesting comparisons. In the first, the deeply stained coarse fibrils contrast sharply with those in the other two figures which represent later stages of development. The fact that the first drawing was made from a section of the cord of *Scyllium canicula*, and the other two from *Torpedo ocellata* and *Lacerta muralis* respectively may practically be disregarded, as the splitting up of the fibrils is common to all species examined by me. In Fig. 22 the exceedingly fine character of the reticulum frequently extending down between the nuclei is apparent. If the whole section of the cord from which this area is taken is studied carefully it at once becomes manifest that fibrillation is definitely limited to the outer cell layers in the vicinity of the ventral roots or to the immediate proximity of nuclei lying in the marginal veil. The circumscribed character of the areas of neurofibrillation is very striking, in connection with

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the development of certain of the Cranial nerve nuclei, particularly the large latero-ventral group of cells from which the Acustico-facialis springs. In Fig. 11, Plate 23, there is an arrangement which I have not observed more than once or twice, where the fibrils spread out over the nucleus (*a*). The bundle (*d*) divides into a number of finer branches which spread over the nucleus forming a net with very wide meshes, the filaments again reuniting to form a single bundle (*c*) that is continued in the direction of the central canal. The manner in which the ventral root bundles frequently split up into finer threads, so attenuated that they soon become lost to view in the surrounding structure, is also seen in this same drawing. Any one who doubts the functional importance of the neuro-reticulum, in connection with the conduction of nervous impulses, will find it exceedingly difficult to even suggest an hypothesis which would explain the presence and peculiar distribution of the neuro-reticulum in the ventral roots on the basis that the only purpose it served was to form a scaffolding or supporting substance. As the process of neurofibrillation proceeds in the various areas the protoplasm forming the body and processes of the neuroblasts is still further differentiated from the surrounding matrix. This takes place rapidly in connection with the large cells of Beard. Frequently, as in the case of the ventral root cells, a triangular area with an attenuated long drawn out point, capping the nucleus is the first definite sign marking the formation of the true neuroblast (Stage 1, Figs. 5, 6, Plate 23). Coincidentally with, or just following the appearance of the first fibrils in this apical part a shorter rather blunt basal process is formed which gives the cell a bipolar appearance (Stage 2, Fig. 9). In sections that have been carefully fixed and stained so as to avoid as far as possible all possible shrinkage the short, rounded, comparatively broad basal projection forms a marked contrast to the slenderer and more pointed prolongation extending from the opposite pole. With slight variations, as far as I am able to judge, all neuroblasts in their development pass through this bipolar stage. It is an extremely difficult task to determine at what point the process of a given neuroblast actually ends. All the evidence so far obtained is in favor of the view that the actual cell processes are often shorter than is generally supposed to be the case.

As the first neurofibrils seem to develope at a point lying beyond the limit of the primitive processes of the neuroblasts they

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are at first surrounded simply by the protoplasm out of which they are differentiated, but gradually as they extend become continuous with those lying inside of the cells, which are actually imbedded in the cytoplasm. In what manner it may be asked are the neurofibrils actually differentiated? Do they grow into the neuroblasts as Apáthy believes to be the case, or is their development centrifugal as Held has suggested, so that they grow away from or out of the cells and pass into the bridges, or does a third possibility exist that they are laid down in situ within the protoplasm, and thus their increase in length is the result of a progressive differentiation (etappenweise)? There does not seem to be any direct evidence in favor of the ingrowth theory as first annunciated by Apáthy, so that only the two remaining possibilities have to be considered. Held, on what seems to be purely theoretical reasons, opposes the idea that the fibrils are laid down in situ and grow by the addition at their ends of freshly differentiated material. His objection does not seem to be altogether valid, particularly in the case of the ventral roots where the differentiation in the neuroblasts begins in the apical processes, proceeds towards the opposite pole and is then continued on beyond the apparent limits of the cell in the direction of the central canal. If, with the results of this observation in mind, the attempt is made to explain the development of the fibrils as outgrowths from the neuroblast, the necessity arises of admitting that within the cell is a point from which the process of neurofibrillation spreads in opposite directions: on the one side towards the neuraxon and on the other towards the opposite pole.

By far the easiest solution of the problem seems to be to suppose that the neurofibrils are differentiated in the protoplasm of the cells, and in the plasmodesmata as the result of physiological processes, either purely metabolic in character or initiated by incident stimuli transmitted from the periphery. The outgrowth theory is most difficult of application in regard to the development of the ventral roots, where the whole process of neurofibrillation is so evidently from the external towards the internal layers. In the axons of cells, which may be followed for some distances, fibrils may frequently be detected apparently lengthening as the processes themselves grow. This appearance, however, does not at all preclude the possibility of the differentiation taking place in situ, advancing step by step as the axon increases in length.

As far as one may judge from the study of specimens prepared

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by the method the details of which are given in Section 3, the neurofibrillation in the ventral roots always proceeds from the apex towards the base of cell and never in the opposite direction. The apparent outgrowth of fibrils in connection with the cells of Beard may be explained by the differentiation taking place in situ in the axons as these lengthen. Various theories may be brought forward to explain the manner in which the connections are established between the cells. Held is of the opinion that the fibrils grow out of one cell and at some intermediate point, not yet determined, fuse with those projected from a second cell. In the majority of instances it seems to me probable that a fusion first takes place between the undifferentiated plasmodesmata connecting the cells and then neurofibrillation follows in these bridges. A strong point in favor of this hypothesis seems to be found in the case of the cells in the ventral horns where, as already pointed out the first fibrils appear at the distal end of the axon. If it is supposed that an actual growth takes place the difficulty of explaining how the fibrils at the same time grow out towards a second cell as well as inwards in the direction of the basal process is again presented.

Even in the case where connections are established between cells lying very far apart, it seems to be not at all improbable that undifferentiated bands of protoplasm at first form a span in which the neurofibrils are secondarily differentiated. How far these bridges are the projected arms of neuroblasts, whether intermediate links are made up of protoplasm ejected from cells of a different type, as well as the determination of the point at which fusion takes place are all questions requiring further study. All reference to the bundles of fibrils which constitute true nerves has been purposely postponed, as it is impossible to understand the way in which they are formed without an accurate knowledge of the successive stages in their development.

In Fig. 20, Plate 24 a well developed branch of the N. ventralis spinalis is shown with many elongated nuclei in close contact with this structure. In Selachians many of the cells, which eventually take part in the formation of the sheath, pass out of the cord by way of the ventral roots, so that Harrison's well known experiment if repeated would, at least in this species, give different results. Within this particular nerve the longitudinal direction in which the

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fibrils run and the absence of cross connections are prominent features.

In conclusion it is worthy of note that when neurofibrils give off collateral branches the calibre of these is apparently the same as that of the main trunk.

c) Summary and Conclusions.

It is exceedingly difficult either to summarize or correlate the results of the observations recorded in this paper as a number of them are so detached that it is impossible to even conjecture what their relative value or signification may be. The possibility also exists that the phraseology employed to describe the physiological events or histological findings may unintentionally accentuate the supposed importance of individual facts.

In general, however, it may be said, that the functional activities of the body represented by the beat of the heart and the primitive movements of ab- and adduction of the body begin at a time when these phenomena may as yet neither be designated as myogenic nor neurogenic in origin. In the case of the heart it may be urged that the movements of this organ, in view of the absence of fully differentiated nerve tracts, are to be regarded as the results of muscular activity quite independent of any form of nervous stimulation. Recent observations, particularly those of Marceau, would seem to substantiate this view. One objection however suggests itself which must be answered before "the myogenic theory" (Engelmann) may be finally accepted. At the time when the impulses begin to be transmitted, numerous connections between the cells in the heart have already been formed, and it is not at all improbable that impulses may be conducted and even originate in the undifferentiated tracts of protoplasm which exist. An analogous condition is found in the spinal cord, the best example of which may be noted in connection with the development of the ventral roots. Attention has already been directed to the fact that definite movements of ab- and adduction take place when the only possible paths for the conduction of motor impulses are along the undifferentiated strands of protoplasm connecting the myotome and periphery on the one side, and the myotome and cord on the other.

The neurofibrils which ultimately form the ventral root bundles appear first, either in the myotome, in the outer marginal veil of

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the cord, close to the exit of the ventral roots or in the span between the cord and myotome. About the same time, or in some cases slightly preceeding it, neurofibrils appear in the processes of the large cells of BEARD close to the nucleus.

The appearance of neurofibrils may generally be considered to be an indication that physiological activity has already actually begun, or will soon begin in the tract in which they have been differentiated.

It seems to be not at all improbable that impulses, centrifugal as well as centripetal in origin, may play an important part in the differentiation of the neurofibrils (neurofibrillation).

The opinion held by a number of investigators that the neurofibrils are to be regarded merely as forming part of the supporting substance in the nerves and nerve cells is apparently not substantiated by the facts. The important bearing they have to function, as indicated by APÁTHY, BETHE and others, seems to be confirmed by the following observations. a) Neurofibrils first appear in the tracts which are the earliest to give evidence of functional activity. b) In the nerves, in well stained specimens, it may be seen that the neurofibrils appear as attenuated delicate structures running longitudinally in the axis cylinder without cross connections. c) Their intimate connection with the neuroblasts, at a time when the spongioblast elements consist only of a small body and an attenuated process which generally bifurcates in or near the outer marginal veil without giving off fibrils of any description. In order to prove that the neuro-reticulum in reality serves the purpose of a supporting structure, it must first be shown that the net is the product of spongioblasts, and of this there is not the slightest evidence. d) The neurofibrillation begins in and about the cells which first become functionally active and spreads from this point as a centre. The ventral horn cells in the cord afford an excellent example of this fact. There the neuroreticulum begins in the outer layers of the cord or in the plasmodesmata and creeps inwards towards the spongioblasts and central canal and never grows in the opposite direction. e) The majority of the bundles of fibrils forming the posterior root fibrils first make their appearance in the spinal ganglia. f) Coarse thick fibrils which eventually split longitudinally are seen at a very early period in the outer marginal veil of the cord. The only cells with which these are connected are those lying within this same zone and possessing all the characteristics of neuroblasts. g) The very re-

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markable contrast that is present in the early stages between the rapid and marked degree of neurofibrillation in the medulla, particularly about the nuclei of the Trigeminus, Acustico-facial, Glossopharyngeus and Vagus, in comparison with the almost complete absence at this time of any evidence of this process within the brain. At the same time the spongioblast elements are quite as advanced in their development in the higher as they are in the lower centres.

A review of the principal points brought out by the study of the finer histological relations leads to the following conclusions:

1) At the time when the neurofibrils begin to appear intimate connections exist between many, if not all the cells, within the spinal cord. Whether this union is a genuine syncytium or the result of fusion of material ejected from the cells cannot yet be definitely decided. Even after the differentiation of neuroblasts and spongioblasts has begun, it is still possible to detect connections existing between these two different types of cells.

2) In *Pristiurus*, *Scyllium* and *Torpedo* embryos neurofibrillation begins about the same time at the following places: In the large cells of Beard close to the nuclei, at the point of exit of the ventral roots, within the bridges which connect the cord and the myotome or within the myotome itself.

3) The neuro-reticulum formed within the neuroblasts, as represented by Held, is not the earliest stage in the development of the fibrils as at first they are always parallel and have no cross connections.

4) The primitive fibrils are coarse thick bundles which soon split up longitudinally as originally described by Heidenhain and Apáthy. From the proximal end of one of these primitive bundles fibrils may be seen to go to different medullary cells at some distance from the ventral-root group or are lost to sight in the outer marginal veil of the cord.

5) The connections formed by the neuro-reticulum between neuroblasts are numerous and are already present in embryos of only 5 mm. in length.

6) The methods of staining generally employed fail to differentiate between the neurofibrils and the protoplasm forming the cell process. Although neuroblasts may throw out processes of considerable length, as in the case of the Oculomotorius, in well differentiated sections it may also frequently be seen that the protoplasm

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of the axon apparently ends near the cell and only the fibrils are continued beyond this point.

7) One of the chief histological characteristics of the fully differentiated nerve is that it contains neurofibrils, and every bit of evidence so far accumulated points to the appearance of these structures as marking the period of greatest physiological activity in any given nerve.

In view of these facts it is an assumption to designate the process of any cell as a nerve, unless it can be shown to contain neurofibrils.

Section 3.

Fixation and Staining of Material.

The method of fixing and staining the material finally adopted by me as giving the best results in embryos is a modification of the technique recommended by Bielschowsky. Several of the suggestions made by Wolff (Biol. Centralbl. 25. Bd. 1905) were also acted upon. The chief differences between the method as originally described by Bielschowsky and as I have used it are the following: 1) the substitution of 0,75 or 1% solution of silver nitrate, instead of the 2% solutions generally employed; 2) the combination of formol with hydrochinon as a developer; 3) staining in a neutral gold bath of $^1/_{10}$% instead of 1%, and 4) following the suggestion of R. Gast the subsequent staining of the cytoplasm with eosin or other dyes.

1) The material is fixed in a 4% solution of formol neutralized by the following method. To the ordinary 40% commercial formol sufficient carbonate of magnesia was added to give the fluid a neutral or very faintly alkaline reaction when tested with litmus. Generally after the fluid has been allowed to stand for several hours and the magnesium carbonate has fallen to the bottom of the bottle it will be found necessary to add more of this reagent. After the reaction of the Formol has become permanently and definitely neutral or very slightly alkaline the supernatant fluid is carefully poured off and filtered. One part of this stock solution is then diluted with 10 parts of tap-water as occasion requires. Embryos may be left for any length of time in the 4% solution without interfering with the subsequent staining.

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2) Prior to the silver bath the material must be washed for several hours or better over night in running water, then passed through several changes of distilled water and finally brought into a 0,75% or 1% solution of nitrate of silver. In warm weather, at the end of 4 days, the tissue will be found to have assumed a pale café-au-lait tinge. In cooler weather this reaction generally takes place between the 5th or 7th day, depending on the size of the embryos as well as upon certain other conditions, which are not understood. Of course all light must be carefully excluded from the specimens as long as they remain in the silver solution, and if any metallic deposit occurs upon their surface it is well to rinse them quickly in distilled water and place them in a new solution. For some reason the use of stronger solutions of silver does not give satisfactory results in embryos.

3) The specimens are again rinsed in distilled water and placed for $^1/_2$—2 hours in the dark in the following solution which has been filtered.

0,75—1% Sol. of silver nitrate	20 cc
Caustic soda (40% sol.)	gtt. 4
Liq. ammoniaci caust.	gtt. 10—12

sufficient to dissolve the precipitate.

The material is left in this solution until it has assumed a dark reddish brown color and is decidedly translucent. If the reddish tinge does not appear it is useless to carry the tissue any further. When removed from this solution the specimens are again passed quickly through two or three changes of distilled water and placed at once in a dish containing

4) Distilled water, to every 10 cc of which 5 drops of glacial acetic acid have been added. After a few minutes (5—15) the reddish tinge of the tissue becomes decidedly yellow when it is again quickly rinsed in distilled water and transferred to

5) the developer, composed of hydrochinone (1% solution) 20 cc and neut. formol 2 cc and left in this for 12 hours.

6) For the last time the material is washed in distilled water, preferably in a small vial, as it is then possible to dehydrate rapidly by adding stronger solutions of alcohol. Experience has convinced me that this is a very important step in the process. If, beginning with 35% alcohol, the strength is increased by adding a few drops at a time of a stronger alcohol, the shrinkage in the tissue may be reduced to a minimum. Cedar oil, chloroform, xylol

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or benzol may be used before embedding in paraffine. Cedar oil is useful if it is desired to study the distribution of the nerves prior to embedding and cutting sections, as even large embryos become semi-transparent in this medium.

Selachian embryos of 5—7 mm. in length may be dehydrated and embedded in 3 or 4 hours after they are first placed in alcohol. The weaker solutions of alcohol seem to be far more liable to decolorize the specimens than are the stronger ones.

7) The sections are fixed upon the slide by means of albumen-glycerine. After they are carefully dried it is well to paint them over with $^1/_2$% solution of celloidin.

Following the removal of the paraffin by xylol and the subsequent passage through alcohols the sections are rinsed in distilled water, stained for 1—2 hours in a $^1/_{10}$% gold bath neutralized by lithium carbonate, fixed by the action of a 5% solution of sodium hyposulphite, washed for several hours in running water, dehydrated in alcohol, counter-stained in a 1% solution of eosin in alsolute alcohol, and mounted in the usual way.

Although this procedure is complicated, the results obtained are far more satisfactory and uniform than those given by any other method known to me. With the exercise of care the shrinkage and heavy deposits of silver so common in sections treated by the technique recommended by Ramón y Cajal are avoided.

If the sections are carefully washed after removal from the fixing bath they do not fade and may be kept indefinitely. The neurofibrils should be stained a very deep purple, almost black.

In conclusion I desire to express my thanks to the Smithsonian Institute for the prolonged opportunity given to me, of accepting the exceptionable facilities for carrying on investigations, the assistance, and great courtesy extended to the occupant of this Table by the management of the Zoological Station at Naples.

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Explanation of Figures[1].

B. Protoplasmic strands (bridges, plasmodesmata) uniting Spinal Cord and Myotome.
C. Spinal Ganglion cell.
C. C. Central canal.
E. Commissural tracts.
Ep. Epidermis.
L. H. Lateral horn.
M. Myotome.
N. V. S. Ramus n. ventralis spinalis.
P. Process of giant ganglion cell.
Pl. Plexus.
R. Primitive fibril splitting up into finer filaments within the cord.
Sp. C. Spinal cord.
V. r. Ventral roots.

Plate 23.

Fig. 1. *Pristiurus melanostomus* length 4,5 mm. Protoplasmic bridge (plasmodesma) extending from spinal cord to myotome. (Bausch and Lomb homog. immers. $^1/_{12}$ oc. 2.)

Fig. 2. *Pristiurus melanostomus* length 5 mm. Bridge connecting cord and myotome. Primitive neurofibril embedded in matrix of latter. (Same magnification as in Fig. 1.)

Fig. 3. *Pristiurus melanostomus* length 5 mm. a) Large cell in region of ventral horn with well developed apical process. The black granules are probably the transverse sections of neurofibrils. A single fibril may be seen near apex of process. b) Bundle of fibrils just beginning to split up longitudinally. (Zeiss apochromat. obj. 2 mm. oc. 12.)

Fig. 4. *Scyllium canicula* length 6 mm. Giaut (Beard) Cell connected with process of spongioblast (p) by bundle of fibrils (p^4), with others (p^1) running forward in direction of ventral roots. At p^3 a bundle directed inwards, but ending abruptly before reaching spongioblast. A fourth bundle passing outwards and as seen in succeeding sections has almost reached inner surface of epidermal layer. (Zeiss apochrom. obj. 2 mm. co. 12.)

Fig. 5. *Torpedo ocellata* length 7 mm. *c* ventral root. *d* portion of protoplasm forming apical process which does not contain any fibrils.

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[1] Unfortunately it is not possible to give the exact magnifications of the sections represented by the drawings. Figures 14 and 15 were drawn at the level of the table, on which the microscope stood, and the others in the same plane as the upper surface of a drawing-table of about 14 cm. in height.

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a Fibrils passing beyond cell in direction of central canal. *e* Fibril coming from cell *b* unites with one from cell *d*. (Same magnification as Fig. 1.)

Fig. 6. *Torpedo ocellata* length 6 mm. I. Cell from ventro-lateral portion of spinal cord. *a* Fibril may be traced in unbroken continuity from cell process into bundle running in direction of *b*. *II*. Ventral roots — same section. (Zeiss apochrom. obj. 2 mm. oc. 12.)

Fig. 7. *Scyllium canicula* length 8 mm. At this stage neurofibrils may be seen threading their way among the nuclei, which are now a constituent part of the bridge. At *E* is a small part of commissural tract.

Fig. 8. *Torpedo ocellata* length 6 mm. Within the protoplasm of the bridges (*B*) bundles of fibrils some of which are surrounded by envelopes that are continuous with the process of certain cells (*b*). Frequently the fibrils as they approach the nuclei spread out in a brush-like arrangement, and others (*c*) skirt the edge of these structures as they pass inwards in the direction of the central canal. (Zeiss apochrom. obj. 2 mm. oc. 12.)

Fig. 9. *Lacerta muralis*. Bundles of fibrils (*f*) running in the direction of the central canal. The small black granules represent cross sections of fibrils running at right angles to the plane of section. (Bausch and Lomb homog. imm. $^1/_{12}$ oc. 2.)

Fig. 10. *Lacerta muralis* (Stage 20 of Peter's Atlas). Fibrils encircling nuclei in the outer cell layer of the spinal cord, and running in the direction of the central canal.

Fig. 11. *Lacerta muralis* (more advanced stage than that of fig. 9). Cells in region of ventral roots. a) Basket work arrangement of fibrils about nucleus uniting at *c* and *d* to form single filament. b) Large nucleus from outer cell layer of cord. (Same magnification as fig. 7.)

Fig. 12. *Pristiurus melanostomus* length 5 mm. Giant (Beard) cells in spinal cord. At this stage there seems to be no connection between the individual fibrils. (Bausch and Lomb homog. imm. $^1/_{12}$ oc. 2.)

Fig. 13. *Scyllium canicula* length 6 mm. Giant ganglion cells with the common net in process of formation. (Zeiss apochrom. obj. 2 mm. oc. 12.)

Fig. 14. *Scyllium canicula* length 6 mm. Two giant cells with common net. (Zeiss apochrom. obj. 2 mm. oc. 18.)

Plate 24.

Fig. 15. *Scyllium canicula* length 6 mm. Giant cells with common net. (Zeiss apochrom. obj. 2 mm. oc. 18.)

Fig. 16. *Lacerta muralis* — Stage 20 of Peter's Atlas Spinal ganglion cells. (Same magnification as Fig. 1.)

Fig. 17. *Scyllium canicula* length 11 mm. Spinal ganglion cell (*c*) process of giant cell running just beneath epidermis. (Same magnification as fig. 1.)

Fig. 18. *Pristiurus melanostomus* length 9 mm. Fibrils from N. ventralis spinalis at inner border of myotome creeping in between the nuclei. (Zeiss obj. D. oc. 3.)

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Fig. 19. *Lacerta muralis* — Stage 20 of PETER'S Atlas Neurofibrils thicker than those within the cord, and curiously twisted have reached the inner surface of the myotome. At *R* thick bundles split up into finer fibrils that pass inwards in direction of central canal, while some may be seen running parallel with the periphery to form commissural tracts. (Same magnification as fig. 1.)

Fig. 20. *Torpedo ocellata* length 7 mm. N. ventralis spinal with sheath cells clustering about the bundles of fibrils — in marked contrast to Fig. 18. (Same magnification as fig. 1.)

Plate 25.

Fig. 21. *Pristiurus melanostomus* length 9 mm. Bundle of fibrils extending from cord in the direction of myotome unaccompanied by sheath cells. (Zeiss obj. D. oc. 5.)

Fig. 22. *Torpedo ocellata* length 14 mm. In this section the great contrast between the calibre of fibril-bundles lying without, and those within the cord is very striking. The intramedullary fibrils split up into extremely delicate filaments which seem to form a very fine network best studied in the immediate vicinity of the ventral horn cells. (Zeiss apochrom obj. 2 mm. oc. 8.)

Fig. 23. *Pristiurus melanostomus* length 13 mm. Branches from N. vent. spin. entering myotome. The ends of fibril-bundles are never bulbous as they are represented to be in sections stained by RAMÓN Y CAJAL'S method, when silver is frequently deposited about the distal end of nerve forming an artefact.

Fig. 24. *Torpedo ocellata* — length 14 mm. Plexus of fibril bundles formed by the union of ventral and dorsal roots. (Same magnification as fig. 1.)

Fig. 25. *Torpedo ocellata* Semi-diagramatic representation of course of fibrils in this Plexus *D L.* dorso - lateral branch with filament entering myotome.

Fig. 26. *Lacerta muralis.* Sympathetic Plexus. (Same magnification as fig. 1.)

Fig. 27. *Lacerta muralis.* Cells of Sympathetic Plexus: (*a*) Loose brush-like arrangement of fibrils uniting to form a single bundle (I) which may be followed in the section to (*c*) where it splits up again. — II. Fibrils encircling nucleus. — III. Fibril *a* may be traced in unbroken continuity into the thicker fibril *b*. (Zeiss apochromat. obj. 2 mm oc. 8.)

Fig. 28. *Torpedo ocellata* length 14 mm. Bundle of fibrils from Vagus with filaments entering epidermis. (Same magnification as fig. 1.)

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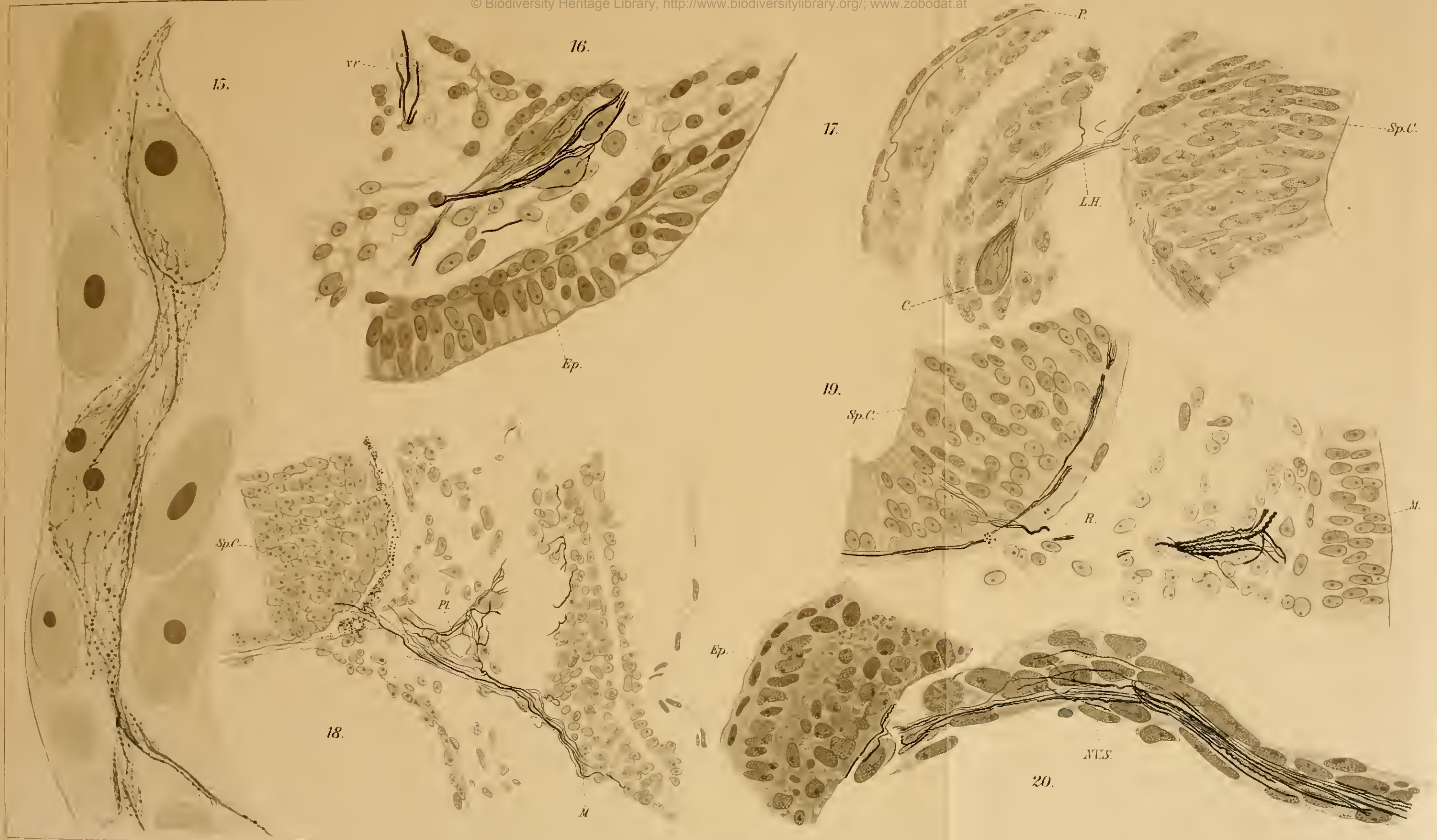

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