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Embryonic Transplantation and Development of the Nervous System.[1]

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With 23 Figures.

It is my intention this evening to give you an account of a certain field of anatomical study, with the purpose of showing how the experimental method may be applied to the solution of embryological problems, and more particularly of those that have to do with the development of the nervous system.

It is generally recognized in science that the experimental method, by which we may deliberately vary the conditions bearing upon a natural event, is a vastly more efficient means of analysis than the method of merely observing phenomena as nature presents them to us. The sciences that have used this means of advancement have attained a much higher degree of perfection than those that have not. While, of course, this has been due in a great measure to the fact that the former, the physical sciences, deal with relatively simple phenomena, still it cannot be doubted that those sciences that have to do with living things might have made greater progress had the possibility of experimentation been more fully realized. In physiology this has been done, and with good effect, but until a comparatively recent date it has not been the case with that branch of biology which deals with the form and structure of organisms, morphology or anatomy. In recent years, however, morphologists have begun to experiment, and now this method of study, introduced about twenty-five years ago by Pflueger, Roux and Born, has found wide application. This is true especially in the field of embryology.

[1]Lecture delivered before the Harvey Society in New York, March 7, 1908.

The complexity of the processes of development, and the extreme delicacy and minuteness of the objects to be studied have, of course, tended to limit the field to which the method could be applied. Especially is this the case with the embryos of the higher vertebrates which are so carefully protected in the maternal body, or by complex fœtal membranes, that they are only to a limited extent amenable to experimentation. We must, therefore, be content with the use of lower forms for this purpose. Yet, fortunately for the possibility of extending our conclusions to many of the phases of human development, we have in the embryos of the frog and the fish forms which are sufficiently closely related to man, and which permit of the most varied kinds of experiments.

In general the methods of experimental embryology have been of two kinds. In the one, the whole organism is subjected to changed conditions, as is the case when the medium in which it develops is altered, for instance, by changing its temperature, illumination or chemical composition. In the second it is the immediate organic environment of the parts of the egg or embryo that is altered, as when the different substances of the egg are separated by means of the centrifuge, or as when certain parts are removed or others added by transplanting living material from one position to another.

One of the most extraordinary discoveries in the latter mode of procedure was made in the year 1894 by the late Professor Born, of Breslau.[2] While experimenting upon the regeneration of lost parts in the frog embryo, this observer was astonished to find that pieces which had been entirely severed from one another might heal together again. Born then showed that it was possible to heal together parts of embryos in any manner imaginable. All that was necessary was to bring two freshly cut surfaces together and to hold the pieces in position for several hours, when they would be found to be firmly and permanently united. In this way individuals of normal form, but with parts taken from two distinct embryos, could be obtained, also any kind of double monster or such with a head in place of a tail or a tail in place of a head (Fig. 1). Some of these specimens were reared past their metamorphosis into the young

[2]For a full account of Born's work see Archiv für Entwickelungsmechanik, Bd. 4, 1896-7. For a general account of the subject of embryonic transplantation see the admirable address of Spemann before the Versammlung Deutscher Naturforscher und Aerzte in Stuttgart, 1906; also Spemann, Zum Problem der Correlation in der tierischen Entwicklung. Verh. d. Deutschen Zool. Gesellschaft, 1907; Braus, Propfung bei Tieren, Verh. d. Naturhist.-medizin. Ver. z. Heidelberg, Bd. VIII.

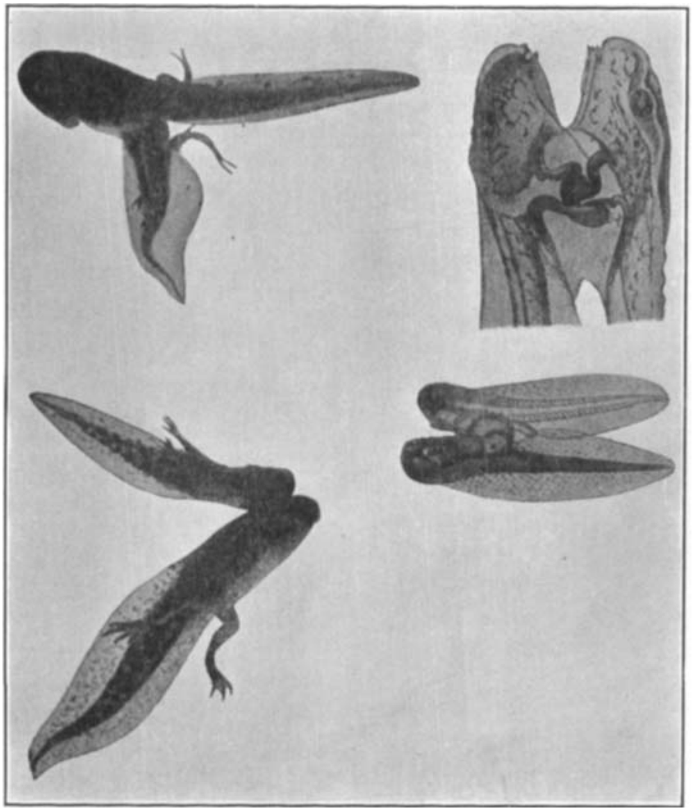


FIG. 1.—Composite tadpoles. (After Born, from Hertwig's Handbuch der Entwickelungsgeschichte.)

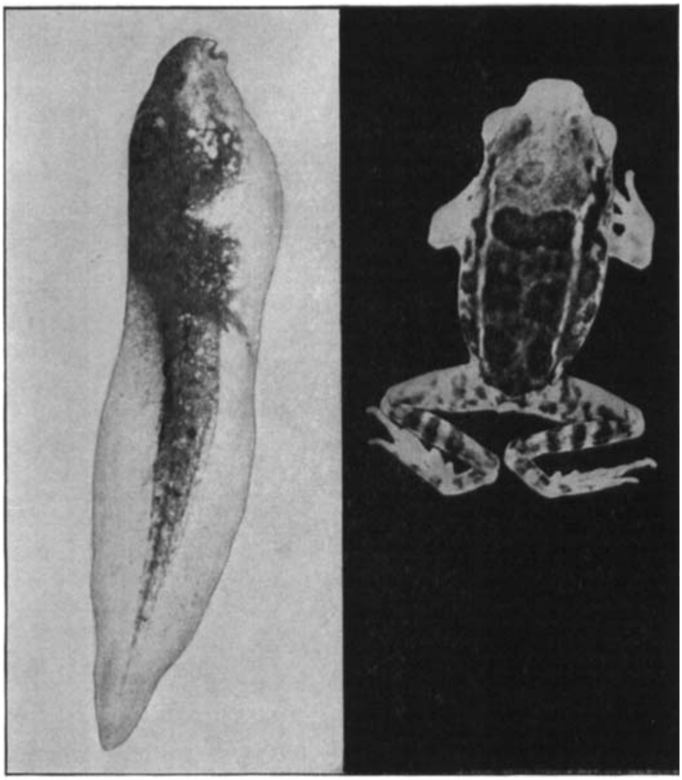


Fig. 2.—Composite individual, as tadpole and as frog; anterior portion, Rana pipiens (virescens); posterior portion, Rana palustris.

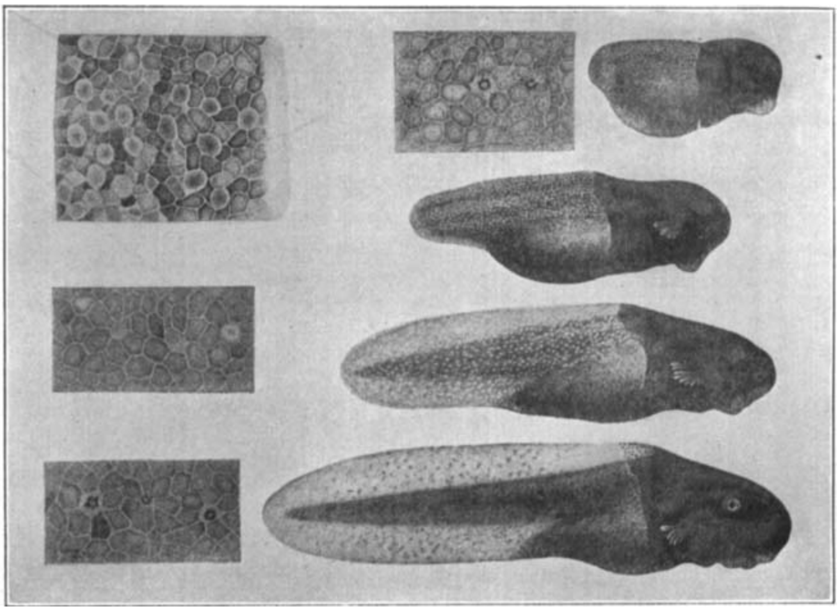


FIG. 3.—Composite embryo; anterior half, Rana sylvatica; posterior half, Rana palustris. The four figures on the right show the same individual at two hours, one day, two days and four days, respectively, after grafting together. The figures on the left show the finer surface markings of the epidermis and the sense organs of the lateral line.

frog. Born showed that even portions of embryos of two distinct species, in fact, organisms as far removed from one another as the common European green frog (Rana esculenta) and the fire toad (Bombinator igneus) could be united.

These experiments have since been repeated, and amongst my own material I have had many specimens of which the anterior half was of one species and the posterior half of another.[3] Many of these lived for a long time and one passed through its metamorphosis, becoming a frog of perfectly normal form, showing in each half the characteristics of the proper species (Fig. 2). Obviously an experiment of this sort would be the ideal one to test the influence of the body upon the reproductive cells within it, though unfortunately the difficulty of rearing frogs in confinement from the egg to maturity has, up to the present time, stood in the way of carrying out the experiment to a successful conclusion. Another use to which the union of parts taken from different species may be put is dependent upon the circumstance that the embryos of the several species are characteristically pigmented. For a considerable period in their development these color differences may be seen in each individual cell, and on this account it is possible to follow in composite embryos the wandering and shifting of the parts during that time. I have made use of this method to study the wandering of the epidermis and the development of the sense organs of the lateral line,[4] which may be traced with great nicety in this way (Fig. 3).

These transplantation experiments are rendered possible by a fortunate combination of qualities in the amphibian embryo. As a factor of first importance we find the extraordinary wound-healing power. The healing is very rapid and always *per primam intentionem*, and this to a degree of perfection never obtained in ordinary surgical procedure. Another important factor is that at the time when the transplantations are usually made there is no circulating medium such as blood or lymph present, but each cell of the embryo is capable of maintaining itself independently of it neighbors, living upon the large quantity of yolk stored within it. On this account it is even possible to make monsters of a great variety of forms, which later, when the yoke is gone, become physiological impossibilities, such as specimens with a tail in place of a head or vice versa. As long as food yolk is present such specimens are capable of maintaining themselves in some fashion, and creatures of

[3]Archiv für Entwickelungsmechanik. Bd. 7, 1898.
[4]Archiv für Mikroskopische Anatomie, Bd. 63, 1903

this character may even be kept alive for a still longer period by a method that I first used to rear tadpoles from which the central nervous system had been extirpated,[5] and which in consequence could not get about in a natural way to obtain food. The method consists in uniting the patient to a normal individual, which serves as a nurse. The vascular and intestinal anastomoses that ensue upon such an operation are sufficient to care for the nutrition of the helpless component, at least for a considerable time (Fig. 4).

In view of the frequency with which the question has been asked how it is possible to perform such delicate and at the same time radical operations, a few words in explanation of the technique will not be out of place. The experiments are usually made upon comparatively young embryos, *i. e.,* from the gastrula stage to those of about 3 mm. in length, with medullary folds just closed. The original method of Born was simple and it was applied to the transplantation of relatively large pieces. The parts to be united were brought into contact along freshly made wound surfaces, were then gently pressed together, and secured in position by placing small pieces of silver wire around them. This is necessary, for, although the embryo at this stage is unable to perform any muscular movements, the action of the cilia which cover its surface is so vigorous as to cause the pieces to separate unless securely held in place. After an hour or two, or even less in favorable cases, the pieces so held will be found to be firmly united and soon afterward all traces of the wound become obliterated.

The refinements of technique introduced by Lewis and by Spemann[6] were made possible by the use of the binocular dissecting microscopes of Zeiss. Under these instruments the frog embryo may be readily magnified to the size of a mouse, and the long working distance of the lenses allows one to operate with every comfort. With very sharp needles, forceps, and small eye scissors, the points of which are sharpened to the fineness of a needle, or, as Spemann recommends, with instruments made by drawing out glass rods and pieces of cover slips to a great degree of fineness, operations of almost incredible delicacy may be performed. The epidermis of the embryo may be lifted, the Gasserian ganglion may be removed to some other part of the body, the ear vesicle may be taken out and replaced upside down, or the right and left ears may be interchanged, as Streeter[7] and Spemann have done. Small organs or

[5]The Journal of Experimental Zoölogy, Vol. 4, 1907.

[6]Verhandlungen der Deutschen Zoologischen Gesellschaft, 1906.

[7]The Journal of Experimental Zoölogy, Vol. 3, 1906, and Vol. 4, 1907.

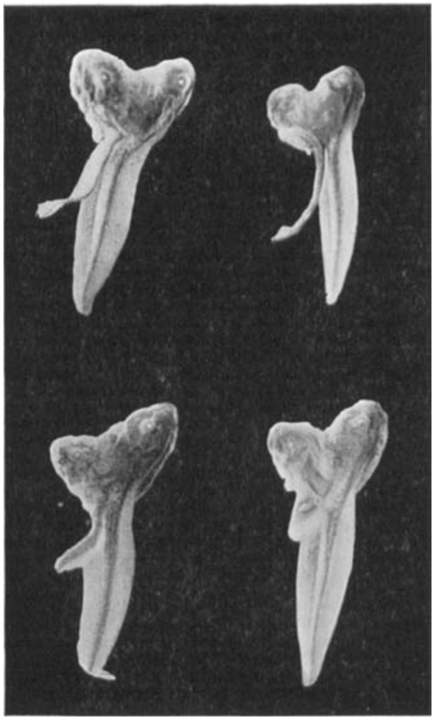


FIG. 4.—Nerveless tadpoles attached to normal individuals which serve as nurses.

pieces of tissue may be transplanted to little pockets made under the skin or between the larger organs, and they will grow readily in their new surroundings. Again, pieces of epidermis may be taken from one part of the body and be made to cover wounds in other regions. It was by means of such experiments that Lewis[8] was able to show that epidermis from any part of the body could give rise to a crystalline lens when brought into contact with the optic vesicle at the proper stage of development.

Such experiments as have just been described are done without anæsthesia because the embryos in these early stages have neither muscles nor nerves differentiated. For operations in later stages, as in the case of Braus's experiments in transplanting limbs, which were made upon tadpoles already able to swim actively, an anæsthetic is necessary. Chloretone is found to be excellent for this purpose, a solution of from two to three parts to ten thousand of water being sufficient to produce a deep narcosis in a few minutes. The recovery after bringing the animals back into pure water takes place with almost the same rapidity. In none of the experiments are there any complications due to sepsis.

Let us now proceed to consider the application of these methods to the study of the development of the nervous system. The problems here involved, which have been the most discussed and which have proved to be the most perplexing, have to do largely with the development of the nerve fiber. We find in the adult nervous system a most intricate maze of fibers, connecting the various parts of the organism, and in each species of animal the arrangement of these fibers is very constant. How can it possibly come about that these interlacing bundles always connect their proper end stations? What are the factors which influence the laying down of the nerve paths during embryonic development? Before these questions can be satisfactorily answered there are other more concrete ones that must be settled. What is the nerve fiber in terms of the cell doctrine? Is it an appendage of a cell or does it consist of a multitude of cells? Again, does the connection between the nerve center and the end organ exist from the beginning, or is it established gradually as development proceeds by extending out from the nerve centers? These questions are old ones, and the various answers now given to them had all been given fifty years ago. Each view has had its vicissitudes, but at the present time it cannot be said that any one of them prevails by weight of authority, for each numbers distinguished investigators among its advocates.

[8] American Journal of Anatomy, Vol. 3, 1904, and the Journal of Experimental Zoölogy, Vol. 2, 1905.

We may first take up the question of the constitution of the nerve fiber. The answer originally given by Schwann,[9] later by Balfour,[10] Dohrn[11] and many others, and again more recently by Apathy,[12] Bethe[13] and O. Schultze,[14] is that the nerve fiber is the product of a chain of cells, which reaches all the way from the center to the peripheral termination, these cells secreting the fibrillæ within their protoplasm much as an embryonic muscle cell secretes the contractile fibrillæ. The opposite answer, first stated with perfect clearness by His,[15] and afterwards ably supported by Ramon y Cajal,[16] von Lenhossék[17] and the neuronists in general, is that the nerve fiber is the process of a single ganglion cell, and is formed by growing out from the cell towards its peripheral connection.

In reality it is difficult to decide between these two alternatives, as may readily be appreciated when we study, for example, the development of a typical spinal nerve in a vertebrate embryo. At an early stage we find the motor root extending out from the medullary cord, and consisting of delicate fibers, with which are intermingled in a most intimate manner numerous spindle-shaped cells. The fibers may be traced into the cord and there they may be seen to proceed from certain cells, which are destined to become the motor nuclei of the ventral horn. The question to be decided is: which of these two kinds of cells, the spindle-shaped cells along the nerve, often called cells of Schwann, or the ganglion cells within the cord, is the essential agent in forming the axis-cylinder of the nerve fibers? The attempt to answer this question, from normal embryos, has been largely a matter of individual interpretation as a glance at the figures will show. Fig. 5, taken from Bethe, expresses one view; Fig. 6, taken from His, and Fig. 7, taken from my own study upon the salmon embryo, express the other view, and the latter is represented in more diagrammatic form in the familiar Golgi picture, taken from Ramon y Cajal (Fig. 8, I). The same is likewise shown in the figure of a section through the chick embryo prepared by the silver reduction method (Fig. 8, II).

[9]Mikroskopische Untersuchungen, 1839.
[10]Development of Elasmobranch Fishes, London, 1878.
[11]Mitteilungen aus der Zoologischen Station zu Neapel, Bd. 10, 1891.
[12]Mitteilungen aus der Zoologischen Station zu Neapel, Bd. 12, 1897.
[13]Allgemeine Anatomie und Physiologie des Nervensystems, Leipzig, 1903.
[14]Archiv für Mikroskopische Anatomie, Bd. 66, 1905.
[15]Archiv für Anatomie und Physiologie, Anatomische Abtheilung, 1886.
[16]Anatomischer Anzeiger, Bd. 5, 1890.
[17]Anatomischer Anzeiger, Bd. 7, 1892.

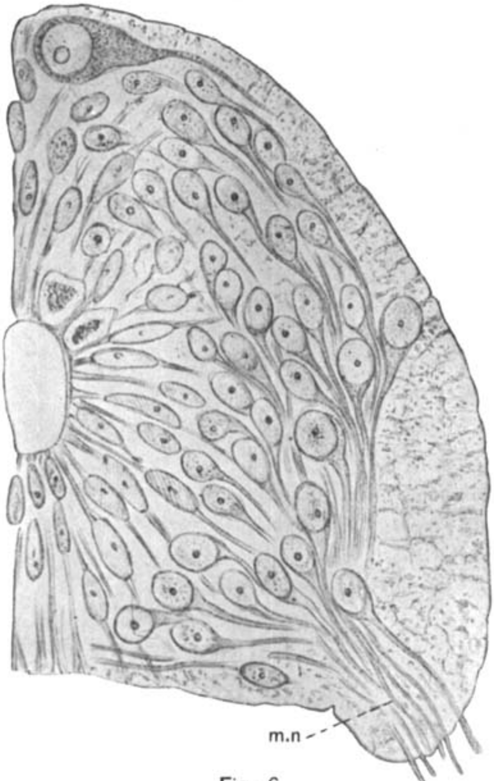


Fig. 6

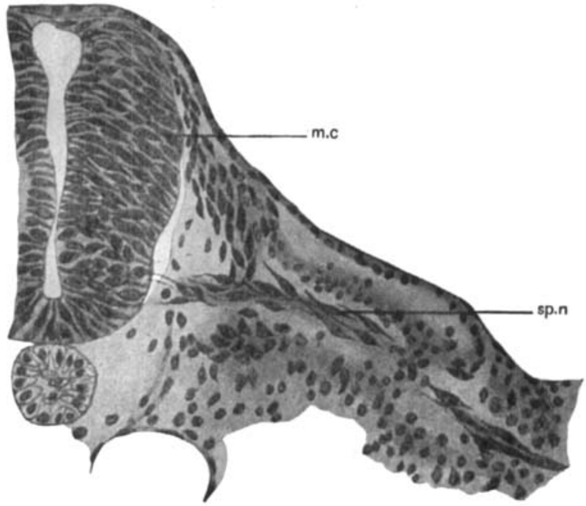
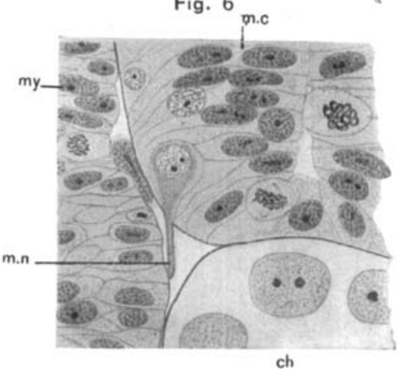


Fig. 5



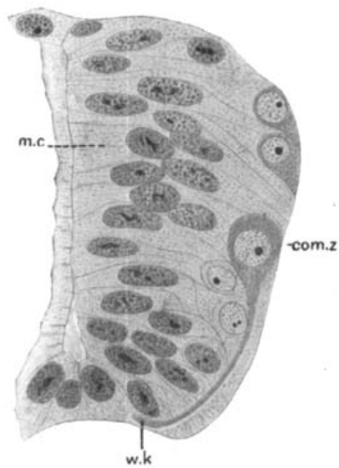


Fig. 7a

Fig. 7b

FIG. 5.—Cross section through a chick embryo of 73 hours to show the beginning of a spinal nerve (*Sp.n*) ; *m.c.*, medullary cord. (After Bethe.)

FIG. 6.—Cross section through the medullary cord of a salmon embryo to show neuroblasts and motor nerve fibers (*m.n.*). (After His.)

FIG. 7.—Cross sections showing part of medullary cord of a salmon embryo. Fig. 7a shows a motor root (*m.n*) consisting of a single fiber proceeding from a single cell within the cord. Fig. 7b shows several neuroblasts and a commissural fiber growing out from one of them, *com. z.*

Although my own work upon the normal development of the salmon and frog[18] had led me to a decided opinion in favor of the cell-outgrowth theory, the attitude of many later investigators showed that we should never be able to obtain evidence from the study of normal development, that would convince everyone alike of the truth of either of the views just stated. A decisive answer to the question, it seemed to me, could

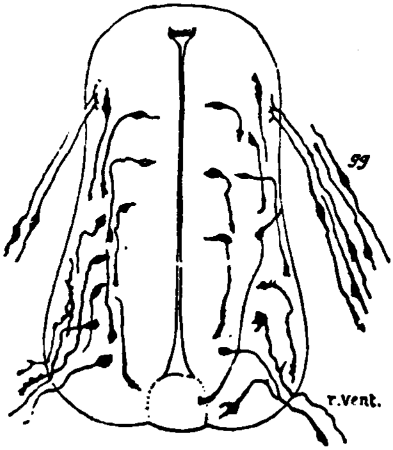


Fig. 8 I

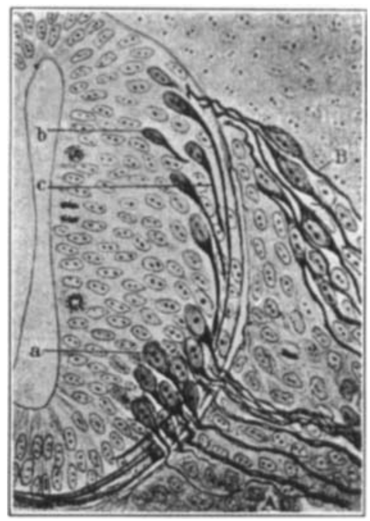


Fig. 8 II

Fig. 8.—I, Semi-diagrammatic cross section through the medullary cord and spinal ganglion of a chick embryo, prepared by the Golgi method. *gg*, spinal ganglion; *r. vent.*, ventral root. II, Section through the spinal cord of a chick embryo of three days. *A*, motor root; *B*, spinal ganglion; *a*, motor neuroblast; *b*, *c*, commissural neuroblasts. (After Ramon y Cajal.)

be obtained only by a more exact method of study. *i. e.,* by the elimination, in turn, of each of the two conflicting elements.

This was accomplished by operations upon the embryo,[19] in which certain parts were removed before the development of the peripheral nerves had begun. The first task, viz., the removal of the source of the spindle-shaped cells of Schwann, which may also be referred to as sheath cells, was complicated by the circumstance that there had been no agree-

[18] Archiv für Mikroskopische Anatomie, Bd. 57, 1901.

[19] Sitzungsberichte der Niederrheinischen Gesellschaft für Natur- und Heilkunde zu Bonn, 1904; American Journal of Anatomy, Vol. 5, 1906.

ment among embryologists as regards their place of origin. As the weight of opinion seemed to lean towards the derivation of these elements from the ganglion crest, and as this structure is easily accessible to the knife, the experiments were begun by removing this structure. Embryos of Rana esculenta were used for this purpose, and, as it was necessary to begin with a stage in which there was as yet no differentiation of the nerve cells or fibers, embryos were taken in which the medullary folds had just closed over to form the tube. Such embryos are about 2.7 mm. long. With the aid of the fine scissors, a thin strip was cut off the dorsal surface of the embryo (Fig. 9), removing the dorsal half of the medullary cord, which includes the neural or ganglion crest. The embryo was thus left with its central nervous system as an open groove along the back, the walls of which contained the elements which were to become the motor cells of the spinal cord. In order to reduce to a

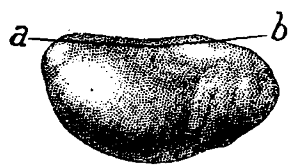


FIG. 9.—Frog embryo 2.7 mm. long. The line a-b indicates the incision for the removal of the ganglion crest.

minimum the possibility of regenerative processes setting in and vitiating the results, two embryos which had been operated upon in this way were in each case brought together by the wound surfaces and were readily healed together. They grew normally, except for the defects due directly to the operation (Fig. 10), and after an interval of six to eight days they were preserved and examined, either in serial sections, or else preparations of the obdominal walls were dissected out and examined *in toto* (Fig. 11). It will be understood that in removing the ganglion crest, the source of the spinal ganglia had been eliminated as well as the presumed source of the Schwann cells. The result was that the embryos were found to lack spinal ganglia entirely, as well as sensory nerves, except those derived from the cranial ganglia, which had been left intact in the operation. What interests us most is the character of the motor spinal nerves. These are found to be present, but to differ entirely from the normal condition (compare Fig. 11, b and c). They are mere naked threads or strands, which extend in normal position from the spinal cord to the periphery. They may be followed to their extreme terminal points in the abdominal muscle near the ventral mid line. In cases in which the operation has been exact, not a single Schwann cell can be found in the whole course of the nerves; and in cases in which a few cells are found, sections show that small groups of spinal ganglion cells are present, indicating that the ganglion crest has not been

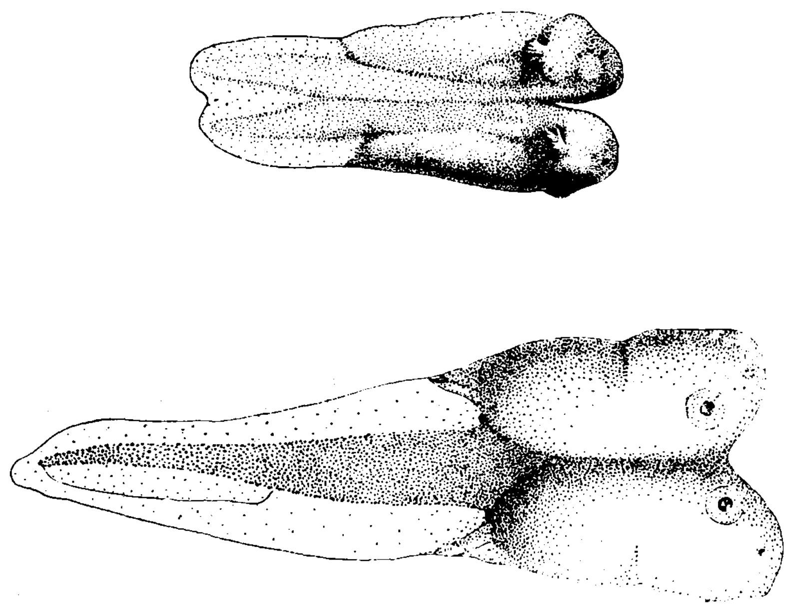


Fig. 10.—Two double embryos from each of which the ganglion crest has been removed. Upper figure, two days after operation; lower figure, six days after.

entirely removed. The nerves are thin and delicate, and have a fibrillar structure, although it has not yet been possible to test whether they give the specific neurofibrillar reaction with the silver or gold methods.

The experiment shows, first, that the source of the sheath cells, both of the motor and sensory nerves, is in the ganglion crest, and secondly, that these cells are unessential to the formation of the nerve fiber. The ganglion cells of the ventral part of the medullary cord are capable by themselves of forming the motor nerves. While it has not been possible to corroborate this by experiment upon sensory nerves, owing to the fact that the sheath cells and ganglion cells of these nerves are derived from the same source, still we have evidence, from normal conditions, which bears out in a striking way the correctness of this conclusion. In the case of the nerve fibers derived from the dorsal giant cells of Rohon-Beard, we have simply naked axis-cylinders (Fig. 12). Likewise in the newt larva the sensory nerves of the tail fin are entirely devoid of cells for a short while during their early development. In other words, nature has here performed an experiment for us, in that she holds back the sheath cells from some nerves until after they have extended out to full length, thus showing that in the formation of these nerves the sheath cells are not an essential element. Another of nature's experiments has been recently recorded by Dohrn,[20] and this corroborates in a very striking way the results above described. In dogfish (Pristiurus) embryos it seems that the *n. trochlearis* receives its sheath cells not directly from the neural tube or ganglion crest, but from a particular branch of the trigeminus nerve, the *n. ophthalmicus minor.* In one case which Dohrn describes the latter nerve was inhibited in its development by some unknown cause, and it was found that the trochlearis consisted of naked fibers throughout its entire extent, not a single sheath cell being present from the decussation to the superior oblique muscle.

Having thus established the fact that the ganglion cells could without the aid of sheath cells give rise to the axis-cylinder of the nerve, it now became of great interest to ascertain if the sheath cells were by themselves capable of giving rise to nerve fibers when the ganglion cells were excluded. This experiment involved greater technical difficulties than the first, but nevertheless it was found possible to carry it out as follows: The dorsal half of the cord was separated from the rest of the embryo, as in the operation previously described, except that it was left attached at one, usually the anterior, end; then the ventral half of the cord was cut out and the thin strip containing the dorsal half healed back in place. Thus the ganglion cells of the motor nerves were removed, the source of the sheath cells and sensory nerve cells (spinal ganglia) being left intact. The embryos developed normally and remained almost motionless, though after a few days slight reactions to stimuli in the form of quivering movements were observed, and later these were found to be due to incipient regeneration of the motor cells within the spinal cord. Examination of the abdominal walls showed, however, that here only sensory nerves were present (Fig. 11, d). The

[20] Mittheilungen aus der Zoologischen Station zu Neapel, Bd. 18, 1907.

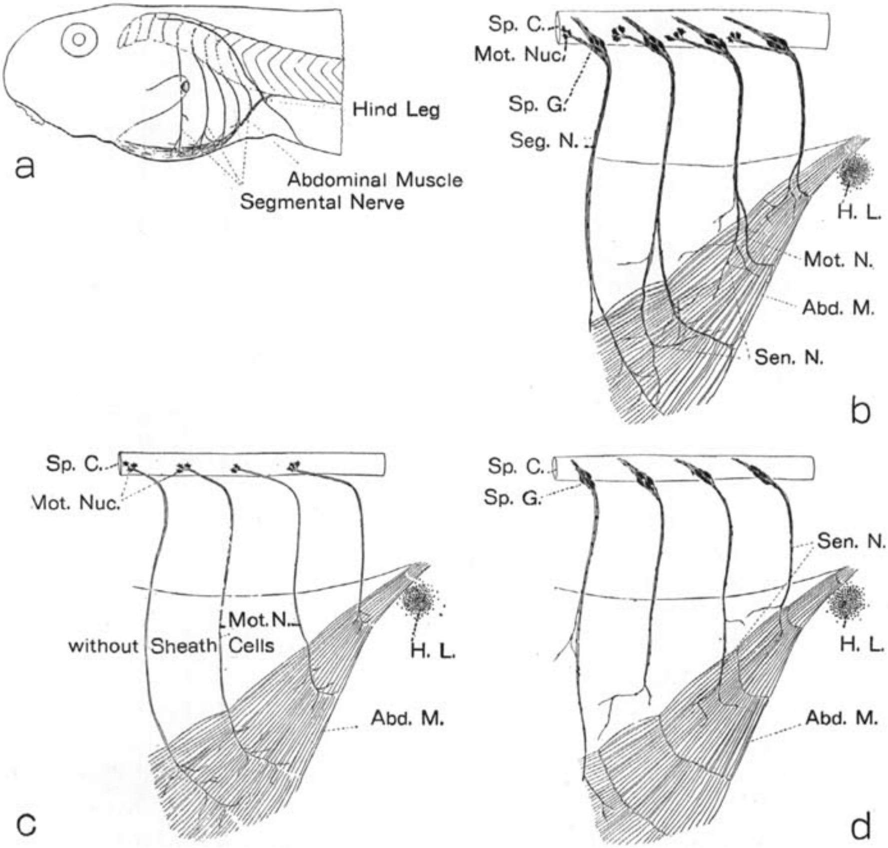


FIG. 11.—Diagrammatic views of the nerves in the abdominal walls of tadpole. a, body of larva showing general arrangement of nerves; b, arrangement in normal larva; c, arrangement in larva from which ganglion crest has been removed, only the motor nerves showing; d, arrangement in larva from which the ventral half of the spinal cord had been removed, showing only sensory nerves. *Abd.M.*, primary abdominal muscles; *H.L.*, hind limb; *Mot.N.*, motor nerve; *Mot.Nuc.*, motor nucleus; *Seg.N.*, segmental nerve; *Sen.N.*, sensory nerve; *Sp.C.*. spinal cord; *Sp.G.*, spinal ganglion.

motor nerves were lacking, although in normal individuals they run for a long distance in common with the sensory nerves, an arrangement which would give the Schwann cells of the latter ample opportunity to form the fibers of the motor rami. The fact that none were formed can only be ascribed to the inability of the Schwann cells to form them.

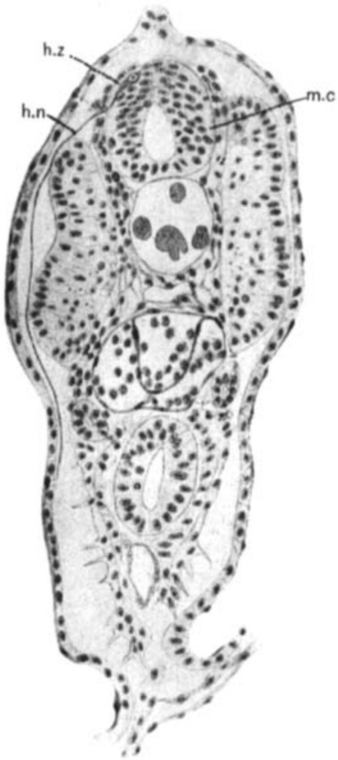


Fig. 12.—Cross section through salmon embryo showing long nerve (*hn*) derived from one of the giant cells (hz) in the spinal cord.

This conclusion has been confirmed by the behavior of nerves that have been deprived of connection with their ganglionic centers after development had begun.[21] In the case of the abdominal nerves of the tadpole, and also of the lateral line nerve, degeneration was found to take place very rapidly after removal of the ganglia, and no signs of

[21] Journal of Experimental Zoölogy, Vol. 4, 1907.

constructive developmental changes were ever observed under such conditions. We may conclude, then, that not only do the sheath cells fail to form nerve fibres, but that they are unable to continue their development or even to maintain the fibres already formed in the absence of connection with the nerve center. The opposite conclusion, which was reached by Brauss[22] and by Banchi[23] upon experimental evidence, may be explained by the fact that these observers did not exclude every possibility of contamination by ingrowth from other nerves, and it is my opinion that this same objection still holds with reference to the evidence for auto-regeneration of peripheral nerve fibers.

Having established the conclusion that the ganglion cells within the nerve centers alone have the power of forming nerve fibers, the spindle-shaped cells merely forming the sheaths of the fibers, we may next inquire into the question as to how the nerve fiber extends from the ganglion cell to its peripheral ending. Is this process a mere differentiation of protoplasmic connections already *in situ,* as Hensen[24] first maintained, or is it an actual outflow of substance from the ganglion cell towards the periphery?

In the past few years the trend of opinion has been unmistakably toward the support of Hensen's theory, according to which protoplasmic bridges are supposed to be left everywhere between dividing cells of the embryo, so that at the time when the nerves begin to differentiate there is already a complex system of protoplasmic connections between various parts of the body; those which function as conduction paths are supposed to differentiate into nerve fibers, while the rest ultimately disappear. There has ever been something insinuating about this theory, putting, as it does, the whole question of the development of nerve paths upon the physiological basis of functional adaptation; but, brilliant and attractive as it seems, very little real evidence has ever been brought forth to support it. In fact, its mainstay has been the imaginary difficulty of conceiving how the alternative view could be true. "How can it be possible," it has often been asked, "that a nerve fiber can grow out for a long distance from its ganglion cell and always reach the right

[22] Anatomischer Anzeiger, Bd. 26, 1905.

[23] Archivio di Anatomia e di Embriologia, Vol. 4, 1905; Anatomischer Anzeiger, Bd. 28, 1906.

[24] Virchow's Archiv, Bd. 31, 1864; Archiv für Mikroskopische Anatomie, Bd. 4, 1868; Zeitschrift für Anatomie und Entwickelungsgeschichte. Bd. 1, 1876; Die Entwickelungsmechanik der Nervenbahnen im Embryo der Säugetiere, Kiel und Leipzig, 1903.

place?" However, within the past three or four years a number of investigators, amongst whom may be mentioned Kerr,[25] O. Schultze,[26] Paton,[27] and, in a modified sense, Held,[28] have sought to place Hensen's theory upon the basis of direct observation. But the point to be decided is really a very difficult one, and one in which the histological method again fails us, refined as the newer neurological procedures may be. It has, on the other hand, been possible to attack the problem experimentally, and with results which seem to me to disprove entirely the theory in question.

It will be readily seen that the two theories differ from one another in attaching to different elements of the embryo chief importance as regards the formation of nerve fibers. According to the outgrowth theory, the ganglion cell situated within the nerve centers is the all-important factor, while on the other view it is the extra-ganglionic protoplasmic structures that play the chief rôle. But, unfortunately for the purpose of devising a clean-cut and crucial experiment, the antithesis between the two views is not complete, for, even according to the first, the organs outside the nervous system are supposed to have some influence in determining the course which a nerve fiber takes as it grows out, while the second view admits that the ganglion cell has a functional or trophic influence upon the processes of differentiation.

The first experiments of my own having a bearing upon this problem were of a comparatively simple nature, but, though helpful, were not crucial.[29] After it had been shown that no peripheral nerves would develop in an embryo from which the nerve centers had been removed at an early stage, thus proving that the ganglion cells were at least one essential element, experiments were made in which the immediate organic environment of the developing nerve was radically altered. This can readily be accomplished by cutting out the spinal cord of an embryo before there is any trace of differentiation in the nervous system. After the wound heals there remains between the notochord, muscle plates and skin a space filled with mesenchyme tissue, which is a portion of the space normally occupied by the spinal cord. Now, if the various fibers which normally arise from the brain and extend as longitudinal funiculi into the cord are formed of protoplasmic processes already situated within

[25]Trans. Roy, Soc Edinburgh, Vol. 41, 1904.
[26]Archiv für Mikroscopische Anatomie, Bd. 66, 1905.
[27]Mittheilungen aus der Zoologischen Station zu Neapel, Bd. 18, 1907.
[28]Verhandlungen der Anatomischen Gesellschaft, 1906.
[29]American Journal of Anatomy, Vol. 5, 1906.

the cord, we should expect to find that no development whatever of these elements would take place. This is, however, not the case, for a few days after the operation very stout bundles of fibers are found extending from the medulla oblongata longitudinally through the mesenchymatic tissue, which has taken the place of the medullary cord. It has been possible to follow such fibers for nine or ten segments, *i. e.*, through the whole length of the trunk region, where they gradually lose themselves in the mesenchyme without showing any definite point of ending. In other words, these fibers have been formed in surroundings entirely different from their natural path and with connections which preclude any possibility of function having played a part in influencing their development. Similarly Lewis[30] has shown that after removal of the embryonic brain the olfactory nerve develops and is found after a few days to extend out from the nasal epithelium and gradually to lose itself in the mesenchyme occupying the position normally taken by the fore-brain. Corresponding results have been obtained in the case of the optic nerve.

The following experiment, which is somewhat more complicated, entirely corroborates the foregoing. The whole trunk region of an early embryo was made sterile as regards peripheral nerves by cutting out the entire spinal cord. Then a bit of the medullary tube, taken from another embryo, was transplanted to a pocket under the skin of the abdominal walls. After the expiration of six or seven days, the abdominal wall was dissected out and mounted *in toto*. The only nerves found in the specimen (aside from the branches of the *r. lateralis vagi*, which come from the head) were those which originated in the transplanted tissue. These were found to radiate in various directions, and not to follow any particular path corresponding to the course of the nerves in normal specimens. It was one of these cases that showed the interesting condition of a nerve crossing the peritoneal cavity. The specimen in question, when dissected out under the binocular microscope, showed an extremely fine thread which extended from the piece of transplanted tissue across the abdominal cavity to the base of the mesentery where it was attached. It was cut off at this end, and, after the specimen was mounted, the thread was found to consist of three axis-cylinders, entirely devoid of sheath cells, which proceeded from a group of three ganglion cells, a miniature spinal ganglion, that had apparently been detached from the main mass of transplanted tissue; root fibers were present connecting

[30] American Journal of Anatomy, Vol. 6, 1907.

this with the main mass. That this nerve had grown where normally no nerve paths ever have been present is clear, although there is some doubt whether it actually did grow through a cavity filled with fluid, for at the time of transplantation it seems that the somatopleure and splanchnopleure were still in contact, the body cavity not yet having been formed between them.

It would seem that these experiments should be interpreted as practically deciding in favor of the outgrowth theory, for the nerve center is shown to be the commanding influence in the development of the fibers. On the other hand the remarkable experiments of Braus[31] have been taken by their author to support Hensen's view. As these experiments are so original in their conception, and lead to otherwise very important results, even though their author's interpretation of them does not seem to me to be justified, a brief account of them will not be out of place here.

Braus transplanted limbs of very young tadpoles to various parts of the body of other individuals and studied the nervous system in the transplanted appendages. It was found that no matter where the limb was implanted, its peripheral nerves would develop normally as regards the distribution of the branches within the appendage itself, though these nerves would have abnormal connections in the host. For instance, a fore limb implanted in place of a hind limb, which had been removed, would develop into a typical fore limb, acquiring a complete system of peripheral nerves, normal in their arrangement, though derived from the lumbo-sacral plexus. A limb transplanted to the head might even acquire normal nerves derived, for instance, from the facial nerve. These nerves are not only normal as regards their arrangement, but they are also functional, for such transplanted limbs not only move in response to stimuli, but they may also exhibit spontaneous movements. These fundamental facts are of extreme importance, though they do not answer in themselves the question at issue, for, either the beginnings of the nerves might themselves have been transplanted with the limb, as Hensen's view would postulate, or else the nerves might have grown in from the nerves of the host, being merely guided in their growth by the structures present in the transplanted part.

In order to decide this point, Braus conceived the very ingenious experiment of transplanting, instead of a normal limb, a limb-bud taken from a larva which had been deprived of its central nervous system at a very early age, and which had, in consequence, developed without any

[31]Anatomischer Anzeiger, Bd. 26, 1905.

peripheral nerves. Braus found in his experiments that even when such a limb is implanted into a normal embryo, no nerves develop within it. In addition to this, he also found that when supernumerary appendages arise from normal transplanted limb-buds, as they do frequently by a process of twinning (Fig. 13), they are likewise devoid of nerves. From these results Braus inferred that within the peripheral parts of a developing organism there is normally some structure which is essential to the formation of the nerve fiber, and which is destroyed when it is cut off for a time from its connection with the central nervous system. In other words, Braus concludes that the peripheral parts of an embryo do not merely serve to guide the nerves in their distribution, but that they actually contribute formed structures to build up the nerve fibers.

Similar experiments which I made in the spring of 1906 upon other species of amphibians[32] show, however, that these results are not of general validity. In the case of the wood frog (Rana sylvatica) and the common toad (Bufo lentiginosus) it was found that the transplanted limbs, whether taken from normal or from nerveless tadpoles, would in the course of their development usually acquire a system of normally arranged nerves, and this was found to hold for the supernumerary as well as for the primary transplanted appendages. The individual cases showed considerable variation as regards completeness of innervation, but this condition could not in any way be connected with differences in the origin of the limbs and could only be referred to slight accidental inequalities in the operations. Were there within the limbs any kind of preformed structures, which give rise to nerve fibres, we should according to Hensen's view, expect them to atrophy through disuse in nerveless individuals long before transplanting, as is actually the case with nerves that are already visibly differentiated. Indications that something was lacking in the nerveless limb would then be shown in the inability of the nerves to develop within it. But this is not the case, for the nerves do develop within such limbs just as in any normal appendage. The results of these experiments, instead of supporting Hensen's theory, add, therefore, further evidence against it.

Distinctly as all of the foregoing facts point to the correctness of the view that the nerve fiber is formed as an outgrowth from the ganglion cell, there is still one defect in the conditions of experimentation which stands in the way of rigorous proof. The nerve fibers have in all of the experiments developed within living tissues and the possibility of the latter con-

[32]The Journal of Experimental Zoölogy, Vol. 4, 1907.

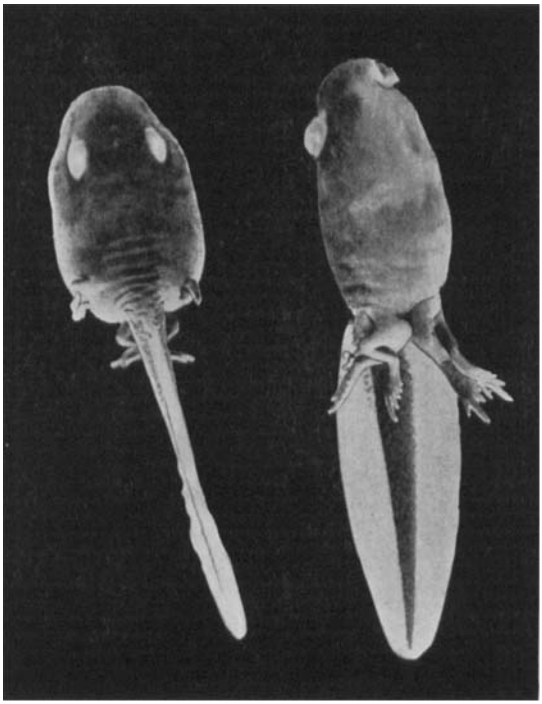


FIG. 13.—Two tadpoles with supernumerary transplanted limbs.

tributing organized material to the nerve elements has not not been entirely excluded. This matter has again recently been taken up by Held[33] and Paton,[34] who have endeavored to show by the aid of exquisite histological methods that the protoplasmic bridges, found between the cells of the embryonic body, do actually take part in the formation of the nerve fibers. Both of these investigators support, in other words, Hensen's view, although Held's conception is a distinct modification of it,

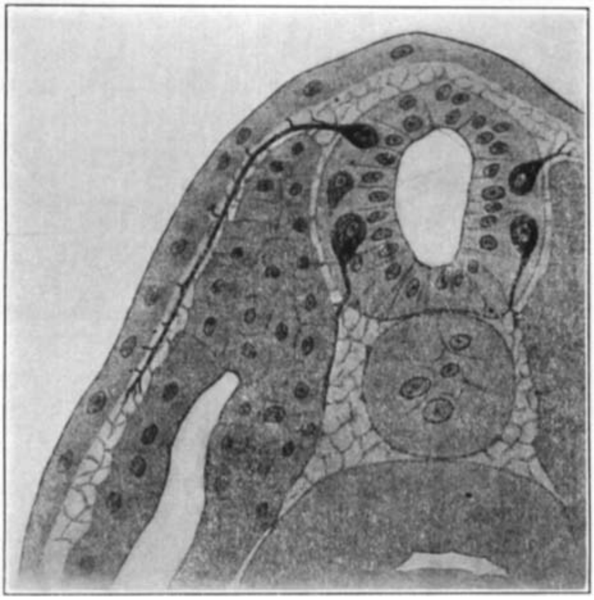


FIG. 14.—Semi-diagrammatic section through the spinal cord and adjacent organs of an axolotl embryo. (After Held.)

in the sense that it approaches measurably the outgrowth theory of His. According to Held the peripheral nerve fiber does not grow out free into spaces between the cells, but it can grow only into the protoplasmic bridges or plasmodesmata which have already been formed by other cells. To translate his own words: "The nerve paths arise through the transformation of plasmodesmata into neurodesmata" (Fig. 14). Striking as Held's preparations are, it does not seem to me that they prove the

[33] Verhandlungen der Anatomischen Gesellschaft, Rostock, 1906; Anatomischer Anzeiger, Bd. 30, 1907.

[34] Mittheilungen aus der Zoologischen Station zu Neapel, Bd. 18, 1907.

essential nature of the protoplasmic bridges. In fact, it is not even proved that these so-called plasmodesmata are not to a considerable extent coagulation products, and even if they are actually present in the living embryo just as seen in preserved specimens, their extremely fine structure would seem almost to preclude the possibility of distinguishing whether the nerve fibers actually grow within them, or whether they entwine themselves amongst them as a vine growing upon a lattice.

That the material upon which Held bases his views is quite capable of another interpretation is evidenced by the fact that Ramon y Cajal,[35] who has studied the same question, upon similar material, making use of the same methods as Held, emphatically supports the outgrowth theory in its original form. The conclusions drawn from such preparations, however definite they may seem, appear, therefore, to be nothing more than a matter of interpretation.

In order to reach a final settlement of this question it thus became necessary to devise a method by which to test the ability of a nerve fiber to grow outside the body of the embryo, where it would be independent of protoplasmic bridges. At first a number of futile attempts were made to cultivate pieces of embryonic nerve tissue in various physiological salt solutions and within the cavities of the normal embryonic body. It then seemed that the outgrowing nerve might be stereotropic, and hence unable to leave a solid mass of cells to grow into a perfectly fluid medium. As the most suitable solid medium in which it would be possible to envelop embryonic tissue and observe its subsequent development, fresh lymph was chosen, first, because the fibrin threads which are formed on clotting might simulate mechanically Held's "plasmodesmata," though they could not be supposed to actually transform themselves into the nerve fiber; and, secondly, because the serum of the lymph would presumably afford a natural culture medium for the embryonic cells. Small portions of various tissues of the embryo were dissected out and removed by a fine pipette to a cover slip upon which was a drop of lymph freshly drawn from one of the lymph sacs of an adult frog. The cover slip was then inverted over a hollow slide and sealed on with paraffine. These manipulations were carried out as far as possible under aseptic precautions. The lymph clots almost immediately and holds the transplanted tissue in place. The specimen can then be readily observed under high powers of the microscope from day to day.[36]

[35] Anatomischer Anzeiger, Bd. 30, 1907; Bd. 32, 1908.

[36] Proceedings of the Society for Experimental Biology and Medicine, 1907

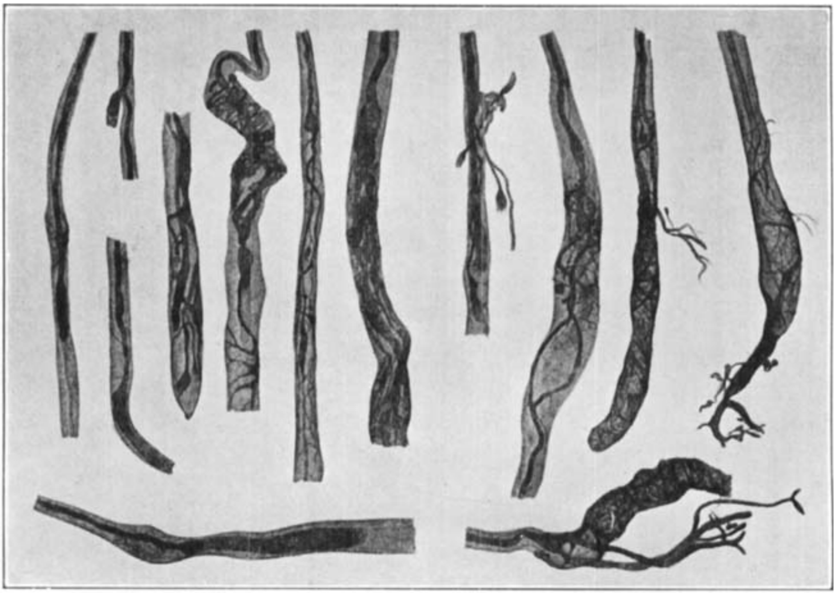


FIG. 16.—Regenerating nerve fibers from the end of the central nerve stump of the sciatic nerve of a dog, taken from six to forty-eight hours after cutting the nerve. (After Perroncito.)

It has been found possible to keep such preparations alive for more than five weeks, and during the first week at least, differentiation takes place in a manner characteristic of each tissue. Cells taken from the muscle plates differentiate into muscle fibers with striated fibrillæ, and when small pieces of spinal cord with portions of the muscle plates attached are taken, twitching movements of the muscle fibers may often be observed on the following days.

In order to understand the behavior of nervous tissue under the conditions just described, it will be well to examine for a moment the appear-

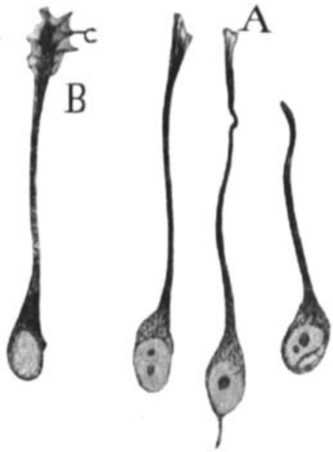


FIG. 15.—A, neuroblasts stained with silver nitrate; B, neuroblast impregnated by the Golgi method; C, growth cone. (After Ramon y Cajal.)

ance of the end of a growing nerve fiber as pictured by various authors from normal preserved specimens. In the figure by Held (Fig. 14) the nerve fiber is seen to run out into a number of fine filaments, which are supposedly the protoplasmic bridges (plasmodesmata) between the cells. According to Ramon y Cajal[37] we find at the end of the growing fiber a swelling (cône d'accroissement), which has a few short processes extending out from it; such endings have been demonstrated both by the Golgi and the silver reduction methods (Fig. 15). In the regenerating fiber, as shown by Ramon y Cajal and by Perroncito[38] there is found a somewhat similar structure at the end of the axis-cylinder (Fig. 16).

[37]Anatomischer Anzeiger, Bd. 5, 1890; Bd. 30, 1907, and Bd. 32, 1908.

[38]Ziegler's Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie, Bd. 42, 1907.

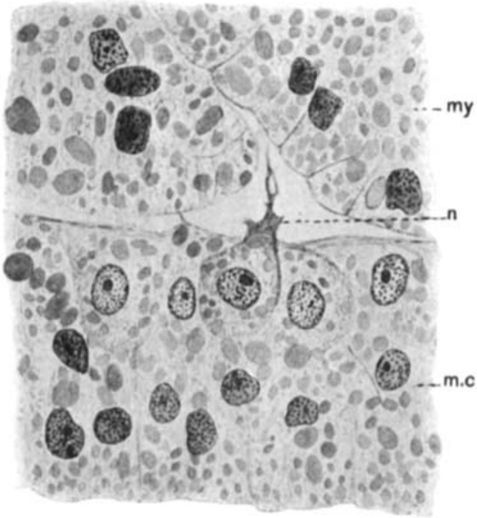


FIG. 17.—Portion of a horizontal longitudinal section through the spinal cord (*m.c.*) and portion of two muscle plates (*my*) of a frog embryo. The cell (*n*) with the branched process is a neuroblast showing the first stage of the formation of the nerve fiber.

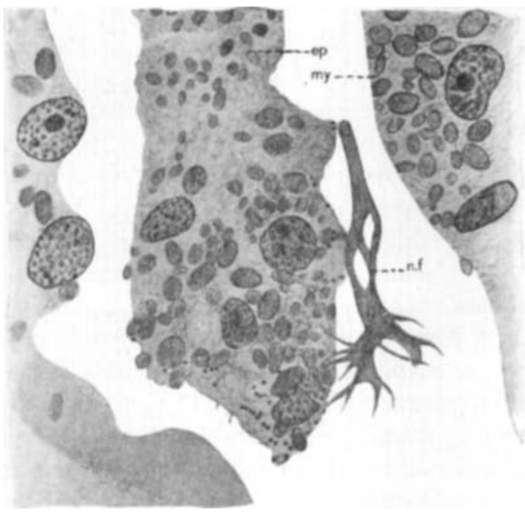


FIG. 18.—End of growing nerve fiber (*n.f*). From a sagittal section through a frog embryo slightly older than the one from which Fig. 17 is taken. The nerve fiber is growing between the epidermis (*ep.*) and the muscle plates (*my*).

A nerve fiber, which is just beginning its development, taken from a section of a normal frog embryo about 3 mm. long is shown in Fig. 17. We see a branched protoplasmic process extending out into the space between the two myotomes and the skin from a cell situated within the medullary cord. If we examine one of these same nerves in a slightly older embryo we find that it has become a fiber of some length,

Fig. 19

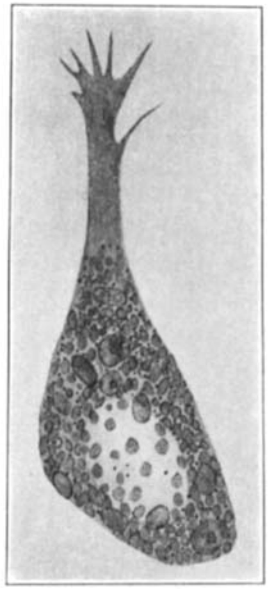


Fig. 20

FIG. 19.—End of growing nerve fiber, as seen in section. Similar to fiber shown in Fig. 18.

FIG. 20.—Isolated cell from a piece of embryonic spinal cord growing in a drop of clotted lymph. The cell body, which is filled with yolk granules, is sending out a hyaline protoplasmic process which undergoes amœboid movements. Drawn from a live specimen.

having at its end a structure (n.f) such as is shown in Figs. 18 and 19. Here there are several fibers bundled together, ending in a mass of hyaline protoplasm resembling a rhizopod with fine branched pseudopodia. These structures may be best seen in the fibers that arise from the dorsal giant cells of Rohon-Beard, and are well brought out by the ordinary embryological methods of fixation and staining.

Let us now observe how the nerve tissue under cultivation in the lymph behaves. It must be borne in mind that when this is taken from the embryo it consists entirely of rounded cells without any signs of differentiation into fibers. Examined after a day or two of cultivation, fibres are found in a considerable number of cases extending out from the mass of tissue into the lymph clot. An early stage of this develop-

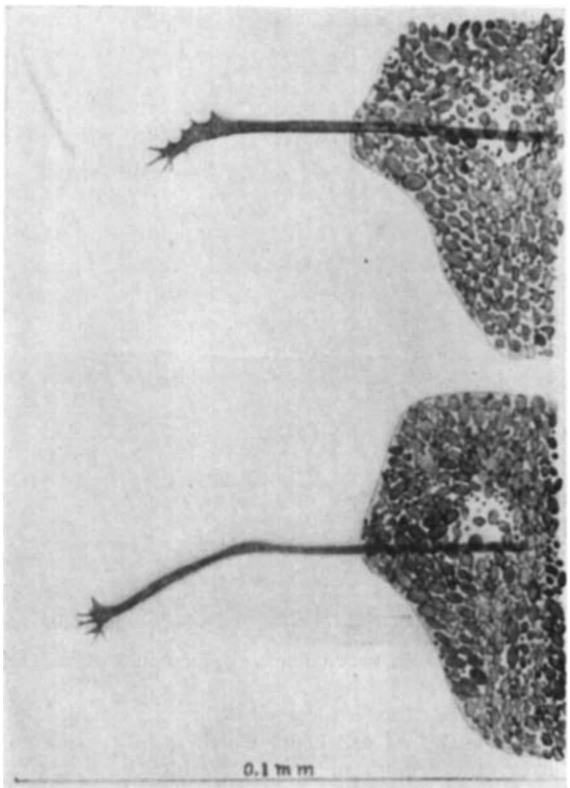


FIG. 21.—Two views, taken twenty-five minutes apart, of the same nerve fiber growing from a group of embryonic spinal cord cells into the lymph.

ment is shown in Fig. 20, which represents a cell that has become detached from the main mass of tissue. This cell is still gorged with food yolk, but at one pole it has sent out a hyaline protoplasmic process, which was observed to undergo distinct changes in form. Fig. 21 shows another case. Here the fiber proceeds from a mass of cells and its own particular cell of origin can not be distinguished. The figure represents two stages of the same fiber sketched at an interval of twenty-five minutes, during which time the fibre has lengthened twenty microns. The case shown in Fig. 22 is a much larger fiber, about 3 microns in

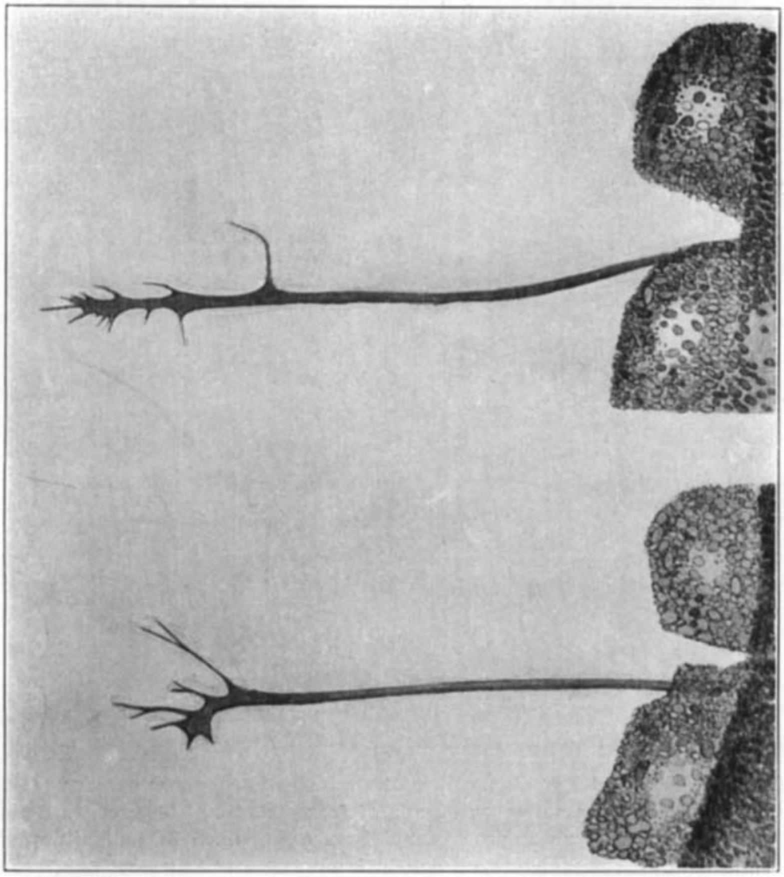


Fig. 22.—Two views of the same nerve fiber, taken fifty minutes apart. Preparation similar to that shown in Figs. 20 and 21.

diameter, with much more protoplasm at the end. The movements of this fiber were extremely active, and the change of form with accompanying lengthening is well shown by comparing the two sketches, which were made fifty minutes apart.

Similar phenomena were observed in the case of pieces of ectoderm taken from the branchial region, which is known to give rise in part to the ganglia of the cranial nerves (Fig. 23). On the other hand, other tissues of the embryo do not give rise to such structures, though kept under exactly similar conditions. This holds for muscle plates, notochord, yolk endoderm, and ordinary ectoderm from the abdominal

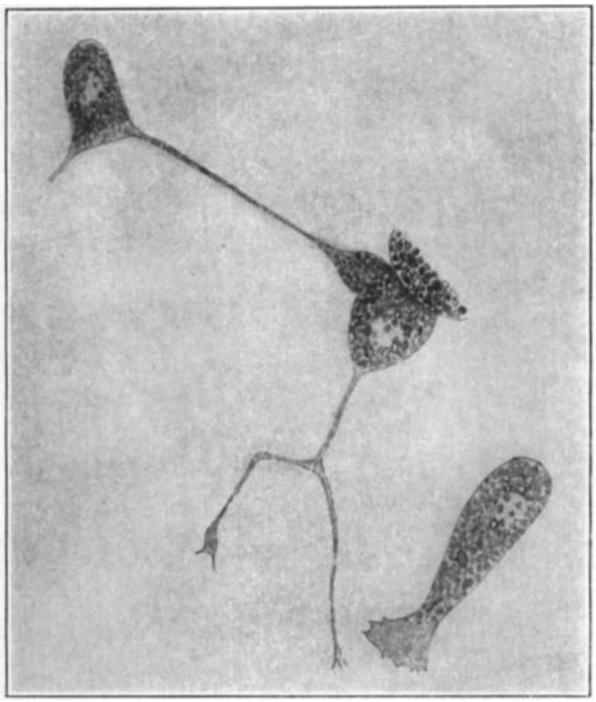


FIG. 23.—Isolated ganglion cell with branched nerve process from tissue taken from the branchial sense organs of frog embryo. The preparation also shows a cell with a short process and twin cells joined by a hyaline protoplasmic fiber. From a live specimen in lymph.

walls. All of these cells exhibit amœboid activity in a greater or less degree, though it does not result in the drawing out of the protoplasm into a filament. There can be no doubt, therefore, that the free-ending filamentous structures are specifically nervous, and when we see the exact morphological correspondence between them and the nerve fibers in sections of embryos of the corresponding age, it becomes certain that the two things are the same.

The foregoing observations show beyond question that the nerve fiber begins as an outflow of hyaline protoplasm from cells situated within the central nervous system. This protoplasm is very actively amœboid, and as a result of this activity it extends farther and farther from its cell of origin. Retaining its pseudopodia at its distal end, the protoplasm is drawn out into a thread, which becomes the axis-cylinder of a nerve fiber. The early development of this structure is thus but a manifestation in a marked degree of one of the primitive properties of protoplasm, amœboid activity. We have in the foregoing a positive proof of the hypothesis first put forward by Ramon y Cajal[39] and von Lenhossék,[40] who based it upon the consideration of the cones of growth found by the Golgi method at the end of the growing fiber.

At present we have but little evidence regarding the influences which bear upon the growing nerve, though now that its mode of growth is known with certainty, we may hope that further experiments will soon throw light upon the problem. From the fact that the nerve fiber is capable of growing out into a lymph clot, and from other facts touched upon in the above discussion, it seems to be established that the mere act of extension is independent of external stimuli, or in other words, that it is due to properties that lie within the cell itself. On the other hand, we cannot escape the conclusion that within the body of the developing embryo there are many influences, exerted by the various organs and tissues, that guide the moving protoplasm at the end of the fiber and ultimately bring about the contact with the proper end organ. The experiments in transplanting limbs show, for instance, that we must seek in the limb itself for the factors which influence the distribution of the ingrowing nerve; for any nerve at all, in whose way a limb may be implanted, may enter the latter and become distributed in a manner normal for that limb. The shifting of parts during development is another factor of importance, as Hensen originally pointed out. For example, the lateral line nerve grows out and establishes its connection with the rudiment of its end organs at a time when its ganglion and the latter are very close together; and the enormous length that the nerve attains in the full-grown tadpole is due solely to the shifting of the sensory rudiment during development. Still, such crude mechanical factors are by no means sufficient to explain the intricacies of the nervous system of a higher animal, and

[39]La Rétine des Vertébrés, La Cellule, T. 9, 1893.

[40]Der feinere Bau des Nervensystems im Lichte neuester Forschungen, Berlin, 1895.

we must seek farther for more subtile influences, possibly such as tropisms, as originally suggested by Ramon y Cajal.[41] Very convincing evidence of chemotropic influences has already been found in the case of regenerating nerves by Forssmann,[42] who showed in a most ingenious manner that degenerating nerve tissue would attract the regenerating fibers. How far such influences, and how far mechanical stimuli determine the course of the nerve fiber in embryonic development, can only be determined by experiment. It is to be hoped that the method of isolation as described above, will here yield results of value.

As regards the theories of nerve development that have been the subject of the foregoing argument, I need scarcely point out that the experiments now place the outgrowth theory of His upon the firmest possible basis,—that of direct observation. The attractive idea of Hensen must be abandoned as untenable. The embryological basis of the neurone concept thus becomes more firmly established than ever.

[41]In the course of the experiments here described, small pieces of tissue taken from the muscle plates or from the epidermis were in a number of cases placed in the drop of lymph along with the nervous tissue. It was hoped to find by this means evidence of attraction or repulsion exerted by these tissues upon the growing nerve fibers. No definite results, however, have as yet been obtained from these experiments.

[42]Ziegler's Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie, Bd. 24, 1898, and Bd. 27, 1900.