THE OUTGROWTH OF THE NERVE FIBER AS A MODE OF PROTOPLASMIC MOVEMENT

ROSS GRANVILLE HARRISON

Bronson Professor of Comparative Anatomy, Yale University

THIRTY-TWO FIGURES

THREE PLATES

CONTENTS

INTRODUCTION

The idea that protoplasmic movement is concerned in the activities of the nervous system has appeared in a variety of forms during the past twenty years. Not only has it been supposed that the processes of nerve cells may be extended and withdrawn, making and breaking connections with other cells during functional activity, but also that the movement of cells and their processes in the course of development has been the chief factor in bringing about the specific nervous connections found in the adult.[1] The latter idea is associated particularly with the name of Ramon y Cajal, who in his memoir on the retina ('92) first put

[1] Schiefferdecker ('06) has discussed at length and in an admirable way the extensive literature bearing upon this subject.

forth the hypothesis of chemotaxis to account for these supposed movements. The discovery, by the same observer ('90), of the *cônes d'accroissement,* found at the end of embryonic nerve fibers very early in their development, had given a clue as to what this growth mechanism might be, for the resemblance of the minute processes borne upon the terminal enlargement of the growing nerve to pseudopodia, naturally suggested that this structure might owe its peculiarities to amoeboid activity. In his larger work on the structure of the nervous system Cajal ('99) elaborates his theory more fully and leaves no doubt as to his meaning regarding the activity of the growth cones. After describing their appearance he says (p. 544–5): "From the functional point of view, the cone of growth may be regarded as a sort of club or battering ram, endowed with exquisite chemical sensitiveness, with rapid amoeboid movements, and with a certain impulsive force, thanks to which it is able to press forward and overcome obstacles met in its way, forcing cellular interstices until it arrives at its destination." From this it is seen that Ramon y Cajal took a considerable step in advance of His ('86–'90), and placed upon a still firmer basis the concept that the nerve fiber is formed as the outgrowth of a single cell.

Although this view has enjoyed wide acceptance, the opposing theory of Hensen ('64–'08), which denies that there is a free outgrowth of protoplasmic substance to form the nerve fibers, has met with increasing support within the past few years, especially in the work of O. Schultze ('04–'08), Braus ('04–05), Held ('06–'09), Paton ('07) and Schaeppi ('09); and it seems that we are really very far from a satisfactory solution of the question, which even the invention of new and marvelously refined histological methods has failed to bring to a final settlement. Nor has Held's[2] compromise theory, which is based upon such methods, and which sees in Hensen's protoplasmic bridges merely a sort of substratum into which the fibrillar substance extends from the neuroblasts or ganglion cells, succeeded in har-

[2] Held's view appears on the surface to be a modification of Hensen's theory and it is usually classed as such, but a full examination of his complete work shows that in reality it approaches much more closely to His's view.

monizing the two views. The wide discussion of the subject which has taken place reached a certain culmination in the controversy between Held and Ramon y Cajal in the years 1906–1909, in which it became clear that the evidence for and against the two theories respectively, rested upon such minute histological details that a decision to which all would subscribe was impossible of attainment. These two observers studied to a great extent similar material, often by the same methods, and, in fact, their prepared material was so much alike that Ramon y Cajal, after seeing Held's specimens, expressed great astonishment at the similarity.[3] Yet the respective interpretations given by them differ diametrically.

Under such conditions a search for evidence of other kinds is indicated. It was with the hope that a study of the problem by entirely different methods might yield such evidence, that the work described in the present paper was undertaken. A crucial experiment was sought that would decide between the two theories. That a decision of this question is of fundamental importance becomes apparent when we consider that the analysis of the factors bearing upon the development of this most intricate system of organs is wholly dependent upon it; for it is obviously impossible to study intelligently the mechanics of development of the nerve paths, unless we know whether we are dealing primarily with phenomena of protoplasmic movement or with mere progressive differentiation without movement.

An extensive series of experiments, as well as observations upon normal embryos, had led me previously to the adoption of the view of His and Ramon y Cajal. These experiments (Harrison '06–'10) showed that the ganglion cells within the nerve centers are the one essential element in the formation of the nerve fiber, inasmuch as pieces of the embryonic nervous system transplanted to any part of the body may give rise to nerve fibers, while no fibers ever develop in the absence of ganglion cells. It was recog-

[3] R. y Cajal, 1908, p. 3, footnote: Tout récemment pendant un voyage en Allemagne, nous avons eu le plaisir d'examiner à Leipzig, les excellentes préparations de M. Held. Ainsi que nous l'attendions elles sont très réussies, mais à notre grande surprise elles montrent à peu près les mêmes images que les nôtres.

nized, however, that in all of the first experiments the nerve fibers had developed in surroundings composed of living organized tissues, and that the possibility of the latter contributing organized material to the nerve elements, stood in the way of rigorous proof of the view that the nerve fiber was entirely the product of the nerve center. The really crucial experiment remained to be performed, and that was to test the power of the nerve centers to form nerve fibers within some foreign medium, which could not by any possibility be suspected of contributing organized protoplasm to them.

Two lines of experimentation were taken up with this end in view. The one was to introduce small pieces of clotted blood into the embryo, in the path of the developing nerves. This gave positive results, in that nerve fibers were found several days after the operation, extending from the medullary cord into the blood clot, and the sole possible disturbing factor in these experiments was the presence of scattered embryonic cells, which began to organize the clot within two days after its transplantation (Harrison '10).

The second line of experimentation, which consisted in the isolation of pieces of living tissue in unorganized media, gave considerable difficulty at first, but in the spring of 1907 a method was finally devised, which satisfactorily accomplished the purpose.

The present paper contains a complete account of these experiments, which have been described previously in a preliminary notice.[4] In addition, a brief description of the early development of the nerve elements in the normal amphibian embryo

[4] The first of these experiments were made in the Anatomical Laboratory of the Johns Hopkins University. After my removal to Yale University they were continued during the seasons of 1908 and 1909 in the Sheffield Biological Laboratory. The repetition of the work gave results which not only confirmed those of the first season, but which also met many possible objections that might have been raised against the original experiments. The preparations obtained during the second season's work were, on the whole, much more convincing than those of the first, and they have been used almost exclusively in making the illustrations for the present paper. The first account of the work was given in a paper before the Society for Experimental Biology and Medicine in May 1907, and later the results were incorporated in a lecture before the Harvey Society of New York, in March 1908.

will be given here, in order to afford a basis for comparison with the protoplasmic filaments formed by the isolated pieces of nervous tissue. Fortunately the part descriptive of normal development need not occupy very much space, for we now have a large mass of facts available in the recent work of Ramon y Cajal ('07–'08) and in the exhaustive monograph of Held ('09).

The method which I have used is, in a word, as follows: Small pieces of embryonic tissue, taken before the histological differentiation of nerve fibers has begun, are placed in hanging drops of lymph, and the sealed preparations kept under observation for a number of days. It is found that the embryonic cells under these conditions manifest striking amoeboid activities, which are especially pronounced in cells taken from the nervous system, and result in such cases in the formation of long threads of hyaline protoplasm. These fibers bear a perfect morphological resemblance to undoubted nerve fibers found in sections of normal embryos of a corresponding stage of development. So striking is the similarity between these structures that no hesitancy is felt in regarding them as identical with one another.

This method, which obviously has many possibilities in the study of the growth and differentiation of tissues, has two very distinct advantages over the methods of investigation usually employed. It not only enables one to study the behavior of cells and tissues in an unorganized medium free from the influences that surround them in the body of the organism, but it also renders it possible to keep them under direct continuous observation, so that all such developmental processes as involve movement and change of form may be seen directly instead of having to be inferred from series of preserved specimens taken at different stages. While these two advantages have not heretofore been combined in a single mode of procedure, the first named has been attained by Loeb ('02) who has embedded pieces of tissue, chiefly epidermis, in blocks of agar or clotted blood and transplanted them to spaces in the body of living animals. It is interesting to note that under these conditions epithelial cells undergo changes which apparently resemble closely the activities of embryonic cells observed in the present investigation, as a comparison of Loeb's figures with my own shows.

EARLY DEVELOPMENT OF NERVE FIBERS IN THE NORMAL EMBRYO

Conditions obtaining in the central nervous system antecedent to the differentiation of fibers

In the walls of the medullary groove and the medullary tube just after it has been completely folded off from the epidermis, one can distinguish in sections a number of irregular layers of cells, mostly oval in shape, with long axis placed radially with respect to the tube as a whole. Sometimes these cells are seen to be bound by a membrane, but usually they are indistinctly defined except where they are deeply pigmented, in which case the pigment granules are thickest around the periphery of the cells. At this period the individual cells do not extend through the whole thickness of the tube from the central canal to the external limiting membrane.

In slightly later stages, *i.e.*, when the tail bud is barely distinguishable, the epithelial cells begin to stretch out radially and then many of the individual cells are seen to extend from the inner to the outer wall of the tube. The boundaries remain indistinct, unless, as before, the cells are marked off from their neighbors by pigmentation. After the elongation of the epithelial cells constituting the walls of the medullary tube has taken place, it is seen that certain cells, less elongated in form, and containing a round nucleus, remain in the outer zone of the wall of the tube. These are the first of the neuroblasts of His, the cells destined to give rise to the nerve fibers.

There are as yet no peripheral nerves, nor are there any nerve fibers visibly differentiated within the walls of the medullary tube. The cranial ganglia are marked off and occupy approximately their definitive position, and in the anterior part of the trunk region the ganglion crest is beginning to break up, its cells extending to the dorsal border of the muscle plates. In the middle of the trunk the crest is intact and it rests entirely upon the medullary cord, while near the tail bud it can scarcely be distinguished at all.

At this point it will be profitable to inquire a little more fully into the supposed syncytial nature of the central nervous system. When sections alone are studied, there may be an apparent justification for regarding the walls of the neural tube as a mass of protoplasm with nuclei embedded in it,[5] for, as has already been pointed out, the cell boundaries within the medullary cord are difficult to make out unless they happen to be indicated by pigmentation. When examined in the fresh condition, an entirely different state of affairs is revealed. It is astonishing how easily the cells, which in sections seem to be baked together in a mass, come apart when the medullary cord is dissected out of the living embryo and teased in water or salt solution. The cells appear as round glistening vesicles under the binocular microscope, and under the oil immersion they are found to be very clearly defined, each being surrounded by a very delicate, though perfectly distinct, cell membrane. The cells are gorged with yolk granules, and the nucleus appears as a clear space near the center of each cell (fig. 15). There is not very much difference in the appearance of the cells in the different media named, though in water and the more dilute salt solution (0.2 per cent) there is some imbibition of water, which may result in the formation of a more or less eccentric clear zone just beneath the cell membrane (fig. 15 c). No sign of protoplasmic bridges can be made out. From these observations the conclusion seems clearly justified that the medullary cord of the frog embryo is made up of perfectly distinct cells. It is in no sense a syncytium, and statements to the contrary based upon the insufficient evidence from stained sections, are to be received with skepticism.

The medullary cord is sharply marked off from all surrounding structures except where the ganglion crest is breaking down. The cord is in direct contact with the muscle plates and the notochord, but in the angle between the two latter structures, and in the the grooves between successive somites there are small spaces, which at this period are entirely devoid of cells. Just what is the structure of the material that fills these spaces in the living

[5] Cf. for instance Weysse and Burgess (1906) on the histogenesis of the retina.

embryo is not, in my opinion, certain, but in sections of preserved specimens, as Held has described in great detail, a delicate network, is visible. The character of this intercellular reticulum varies from specimen to specimen and, as will be seen, varies very greatly according to the mode of preservation. It seems to be beyond doubt that the structures in question are due in part to coagulation, though just to what extent it is not easy to say. In order to test the matter a series of embryos were preserved in osmic acid, which, as Fischer ('01) has shown, fixes protoplasm without bringing about any visible change in structure, and which after prolonged action (24 hours, 1 per cent, in the case of Amoeba proteus) so fixes it that alcohol causes no further change. Sections of these embryos show plainly that the spaces between the organs described above are almost perfectly clear; only occasionally do very delicate filaments appear bridging the spaces. The contrast with specimens which have been preserved in a corrosive sublimate-acetic mixture is very great; and very much more pronounced still is the difference shown by embryos preserved in Hermann's fluid, which is, however, otherwise a very ill adapted preservative for this material.[6] It is not intended on the basis of the foregoing observations to deny the existence of protoplasmic bridges in embryos of this stage, but it does seem proper to call attention to the facts just stated, in order to show the necessity for caution in ascribing significance to the connection between such fine structures and the developing nerve fibers.

Differentiation of nerve fibers

The embryo last described is in the stage which was used for most of the experiments. It is the oldest stage of which it can be said with certainty, without microscopic examination, that there are no nerve fibers present. In the next stage to be con-

[6] On account of the large amount of yolk, which becomes very brittle after prolonged treatment with osmic acid, the amphibian embryo is not a favorable object for the study of this question. It was found necessary to impregnate the embryos with celloidin before embedding in paraffin, and even then the sections were not perfectly satisfactory. It would be of great interest to have an exact compari-

sidered, an embryo of R. sylvatica, 4.1 mm. long, the beginnings of the peripheral nerves, and of some of the principal central bundles are plainly visible. Of all the peripheral nerves the *r. ophthalmicus* of the trigeminal, seems to be furthest advanced. A very early phase of this nerve is shown in fig. 2 (*nf*), drawn from an embryo of R. esculenta, 3 mm. long, which is in about the same stage of development as the sylvatica embryo just mentioned. Protoplasmic processes of the cells within the ganglion are seen to extend for a short distance into the mesenchyme, without having any special relation to the cells of that tissue. The ends of the processes are branched and filamentous. In the sylvatica embryo under consideration, a considerable number of peripheral nerves in addition to the ophthalmic are already laid down. There are at least four ventral spinal roots, corresponding to the second, third, fourth and fifth muscle plates, to which they may be traced; several of the dorsal nerves of Rohon-Beard, extending out between the myotomes and the epidermis; and some fibers in the *r. lateralis vagi.*[7]

The early characteristics of the developing nerve are most clearly shown by the fibers which originate in the dorsal cells of Rohon-Beard. These grow just beneath the epidermis in the space between the muscle plates, where at this period there are no loose mesenchyme cells, and they remain free from sheath cells throughout their growth. The clearest cases of the earliest beginning of these nerves have been found in an embryo of Rana palustris, 3.6 mm. long, which is almost identical in degree of development with the sylvatica embryo just described. The

son of the protoplasmic bridges fixed in osmic acid with those seen after fixation in the usual preservatives made upon such vertebrate embryos as those of the selachian, the teleost, or the bird, in which there is little or no yolk in the tissues at the time when the first nerve fibers differentiate.

[7] These early nervous connections, which are important for the proper interpretation of the relation between structure and function in the neuro-muscular system, have been ignored by a number of investigators. In his histogenetic study of the nervous system O. Schultze ('05) has overlooked these stages of development completely and has thereby been entirely misled in his views regarding the early development of nerve fibers and the formation of the cutaneous plexuses (Cf. Harrison –'04, '06). Held ('09) has recently subjected Schultze's work to a searching criticism, all the main points of which seem to be entirely justified.

cells which give rise to the dorsal nerves form a column in the dorso-lateral part of the wall of the medullary tube just within the external limiting membrane. In this stage certain of the cells are seen to have put forth fine branched processes, which extend for a short distance laterally in the notch between successive muscle plates (fig. 1, *nf*). The processes end in extremely fine filaments, so fine that their exact delimitation is often very difficult to determine. The cell shown in the figure gives off another process quite as extensive as the one shown, but which is seen only in the section next to the one drawn. The structures in question are segmentally arranged, and correspond in the embryo under consideration to the intervals between the muscle plates from the second to the thirteenth segments. A much more advanced condition is shown in an embryo but very slightly older (3.7 mm. long). The dorsal nerves are here composed of several fibers in a bundle, each fiber connecting with a cell. The nerve shown in fig. 3 is composed of four such fibers (*nf*) which arise in pear-shaped cells (*nbl*) and converge toward the point where they leave the medullary cord between the second and third myotomes. The endings are not shown in the section because the fibers bend just beneath the epidermis and run dorso-ventrally. They stain intensely with Congo red, as do the cone-shaped processes of the cells from which they originate, and they show a fairly distinct fibrillation, even when stained merely by this method.

The ends of the fibers are best seen in sagittal sections taken just between the epidermis and the underlying muscle plates. In a series of sections made from an embryo of Rana pipiens, 4 mm. long, they show particularly well. In fig. 4 the end of a bundle of three fibers situated between the ninth and tenth segments is shown. This terminal structure (*npl*) consists of a mass of hyaline protoplasm having a form suggestive of a rhizopod. The mass extends out into a number of very fine filaments. Such structures are found in each segment. Another, more highly magnified, is shown in fig. 5. Further towards the head of the embryo the fibers are longer and more branched (fig. 6), each branch ending in one of the peculiar enlargements just described. The young fibers of the *r. ophthalmicus* end similarly, although the ending

cannot always be made out with such clearness, owing to the existence of the branched mesenchyme cells in their immediate vicinity. In other nerves, as in the case of the *r. lateralis vagi* of Amblystoma, there is a slight enlargement at the end of the growing fiber, though branched filaments are not clearly shown there.

It is a striking fact that in these early stages of development, each nerve fiber, in fact each branch of a nerve fiber, ends in an enlargement of this kind. The enlarged ends, as well as the fibers throughout their whole length, are attached to surrounding organs by fine threads, but, as stated previously, I am unable to find any safe criterion to distinguish between natural protoplasmic filaments and products of coagulation. Aside from these fine filaments, the nerve fibers are found to end free, and anastomoses between different nerves are not present at this stage. This is perfectly clear in the case of the cutaneous nerves formed by the cells of Rohon-Beard. A little later, however, as seen in a R. pipiens embryo, 6 mm. long, the branches of the individual segmental nerves are found to have extended so far as to come into contact with those of the next segment, the result being the formation of a beautiful plexus of nerve fibers beneath the skin overlying the muscle plates. This is composed of fibers devoid of sheath cells, and in specimens hardened and stained by vom Rath's picro-platino-osmo-acetic mixture, the fibrillae are shown very clearly. Plexus formation is thus seen to be secondary, resulting from the accidental coming together of the growing ends of nerve fibers which have origin in different segmental nerves (text fig. 1). In all cases the nerve fibers are found to extend gradually out from the center, and the end of each small twig is characterized by an enlargement made up of hyaline protoplasm, provided with fine filaments, just as the main stem of the fiber itself is at first.

The above observations upon the ends of the developing nerves agree substantially with those of Ramon y Cajal, although they are based upon specimens preserved by entirely different methods. The enlarged ending provided with protoplasmic filaments is in all probability the *cône d'accroissement* first described by him, the filaments being shown in these cases perhaps more completely

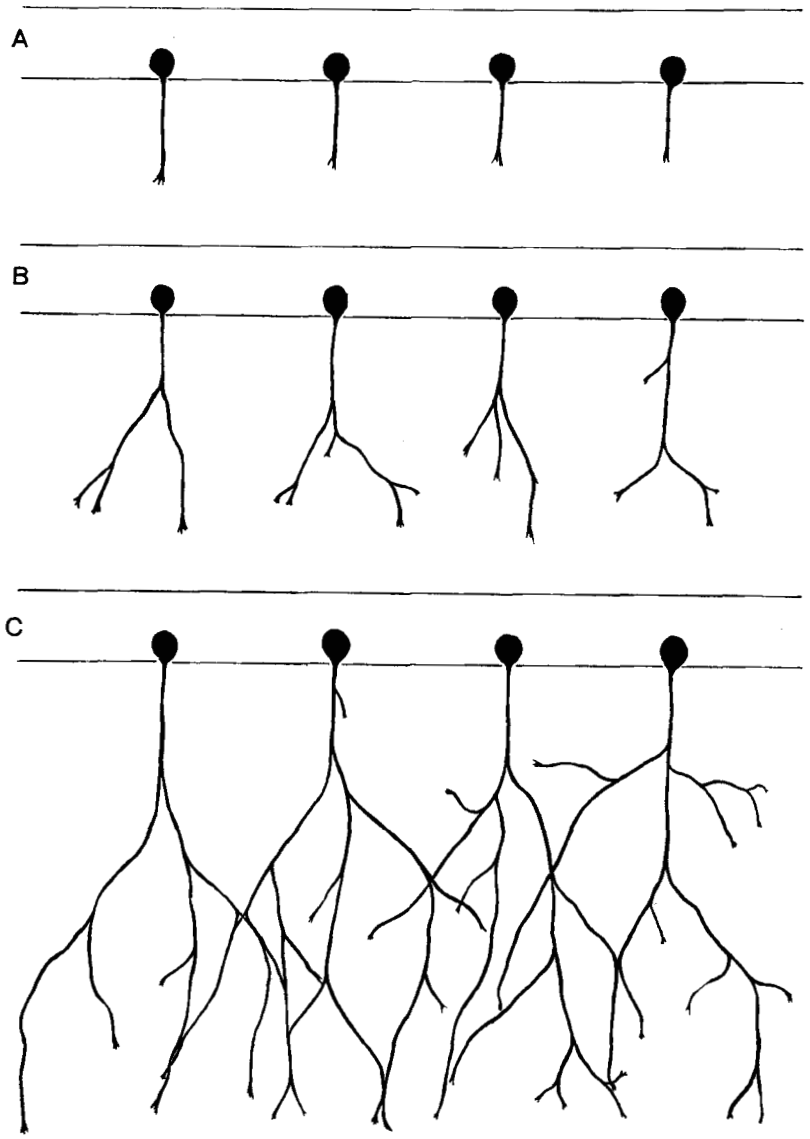


Fig. 1 Diagram illustrating the mode of development of the sensory nerve plexus, derived from the dorsal cells of Rohon-Beard. Each segmental nerve is represented in its simplest terms as a single fiber originating in a single cell of the neural tube. A, early stage in which the nerve fiber has just begun to grow out. B, Later stage in which each segmental nerve has begun to branch. C, Final stage in which neighboring nerves form anastomoses with each other.

by the ordinary embryological methods.[8] Cajal has figured in a number of places his growth cones, as seen both in Golgi and in silver nitrate preparations. Those shown in his book on the Structure of the Nervous System, vol. 1, p. 515, are in most striking agreement with the figures here presented. Again, there is no sharp discrepancy between these figures and those of Held, whose figures, like those of Cajal are sharper than the present ones, since they represent the specific coloration of the neurofibrillae. The only essential difference shown by those of the former observer is in the relation of the young nerve fibers to the protoplasmic net-work between the cells and this to my mind is wholly a question of interpretation. Considering the uncertain nature of the intercellular net-work, as pointed out above, the unusually positive views of Held regarding its rôle in the development of the nerve fibers seem but very insecurely founded.

EXPERIMENTS UPON EMBRYONIC TISSUES ISOLATED IN CLOTTED LYMPH

Description of methods

The first attempt which I made to study the development of isolated bits of embryonic nervous tissue gave entirely negative results. The tissue was dissected out from the embryo and put either into physiological salt or Locke's solution, but no differentiation was observed, before disintegration began. Later a more natural environment for the isolated tissue was sought in the ventricles of the brain and in the pharynx of young embryos. The tissues were transplanted to these cavities and the specimens were killed after from two to seven days and examined in serial sec-

[8] In salmon embryos preserved and stained by the ordinary embryological methods, no protoplasmic filaments are shown attached to the growing ends of the nerve fibers within the central nervous system, and for this reason the latter were figured as smooth in my paper on the histogenesis of nerves (Harrison '01). Ramon y Cajal has pointed out that this condition is likely due to the insufficiency of the methods. While I agree that there is some ground for this criticism, it seems nevertheless probable that there are actual differences between the growing ends found in different places and in different species.

tions. These experiments likewise resulted negatively. In no case were nerve fibers found extending from the transplanted piece free into the cavity, although the pieces themselves often showed differentiation of fibers, and in cases where the graft had grown fast to the walls of the medullary tube, fibers passed from the former to the latter. The only conclusions which could be drawn from these results were either that the nerve fibers were built up by the differentiation of formed protoplasmic structures, according to the view of Hensen and Held, or else that the growing fibers were positively stereotropic and hence remained within the solid tissue instead of passing out into the surrounding fluid.

Acting upon the latter assumption, the next step was to try a fixed medium. Two such were employed, one of which, gelatine, gave no results at all, the transplanted embryonic tissue remaining entirely unchanged after imbedding. The other, clotted frog's lymph, gave the results that are here recorded. It was rather to be expected that this medium would yield positive results, if indeed such were to be obtained at all, for it would be chemically the most natural medium, and the fine net-work of fibrin threads, bathed by the fluid serum, would in a measure simulate mechanically the protoplasmic net-work, which, according to Hensen, Held and others, seems to exist in the tissue spaces in which the peripheral nerves undergo their early development.

In the first experiments made with the lymph, the technique employed was comparatively simple. The tissue to be studied was dissected out of the embryo under the binocular microscope in 0.4 per cent sodium chloride or in Locke's solution without sugar. It was then transferred to a cover-slip by means of a capillary pipette, and a drop of lymph drawn from one of the lymph sacs of an anaesthetized frog was quickly dropped upon it. The cover-slip was then inverted over a depression slide and the preparation kept in a moist chamber. In order to avoid evaporation while the specimens were under examination it was necessary to seal the preparations, which was done most satisfactorily by applying melted paraffine around the cover-slip with the edge of a warm plate.

Although the first definite results were obtained by the above methods, it was found that bacteria quickly invaded the preparations, often destroying them as soon as the second day after implantation. Continued observation over a long period was therefore impossible, and many otherwise good specimens were spoiled before they had yielded anything but negative results. After experimenting a little with antiseptics such as thymol and acetone-chloroform, it became apparent that satisfactory preparations could not be obtained except by working aseptically. The procedure necessary for this involved much tedious detail, though it offered no insuperable difficulties.[9] All glassware, such as slides, covers, pipettes and dishes, was sterilized by dry heat, either in a hot air sterilizer or by passing them through a flame. For cloths and filter paper an Arnold sterilizer or an autoclave was used, and the needles, scissors and forceps were sterilized by boiling. The sterilization of the embryos and the frogs from which the lymph was to be taken offered greater difficulties, and in fact was accomplished only approximately, though the number of organisms seems to have been so reduced as not to interfere with the purpose of the experiments. The embryos were simply cut out of their jelly in water which had been boiled or passed through a Pasteur-Chamberland filter. They were then washed in about six successive changes of this water. The salt solution in which the operations were performed was sterilized in the same way. The frogs were chloroformed and then washed thoroughly in sterile water, laid out upon moist filter paper and kept in a covered dish. In some cases they were first washed in mercuric chloride (0.1 per cent) and in others they were kept for 24 hours before chloroforming in a solution of copper sulphate one part to 500,000, but I am not prepared to say whether these means were sufficiently effective to be of material advantage.

The results of these manipulations are altogether satisfactory as regards asepsis, although the making ready of the apparatus consumes so much time, and the constant attention to the

[9] I am greatly indebted to Prof. Leo F. Rettger for valuable suggestions as to this procedure, and for his generosity in putting at my disposal the apparatus in his laboratory.

details of manipulation during operations is so fatiguing, that only a small number of preparations can be made in one day. Many preparations proved to be absolutely sterile. In some of these the tissues were kept alive for over five weeks, and in a great many for one or two weeks. Some were contaminated, most frequently with Bacillus subtilis, but even in these cases the organisms did not usually appear in sufficient number to injure the living tissue until after it had been kept under observation for four or five days, which was long enough for the present purpose. Several epidemics of mould (Penicillium) were encountered, but this too grew slowly, usually from a single spore or two, and as it does not seem to kill the embryonic cells, it interfered but little with observations.

The tissue to be studied is dissected out from the sterilized embryos in a small flat dish containing dilute salt solution. After this is done the next step is to obtain a drop of lymph from the frog, which has already been prepared. The animal is suspended or placed in an upright position, and after cutting into the lymph sac near its upper end a long fine pipette is introduced and a small drop is drawn from the bottom of the sac. This is then placed upon the cover-slip, and the piece of tissue is quickly transferred to the lymph by means of the same pipette, with care to take with it as little of the salt solution as possible. Then the cover-slip is inverted over a depression slide and the preparation sealed by means of paraffine. It is important to have the depression in the slide deep enough to prevent the drop, which must also be small, from coming into contact with the bottom.

The procuring of the lymph is the most difficult part of the whole procedure, and the variability in its quality and in the amount obtainable, introduces into the work an element of inconstancy, which is a serious disturbing factor, preventing, as it does, a sharp clear-cut process of experimentation with exact controls from being carried out. The composition of the lymph varies not only amongst individual frogs but also in the different lymph sacs of the same individual, according to the position in which the animal has lain, the time since anaesthetization, and other factors of unknown nature. In general it may

be said that the first lymph drawn is the best; it clots readily and is less hemorrhagic, though this is by no means always the case. Usually a single frog can be used for five or six drops. The first two drops were taken in most cases from the femoral sacs. After opening these sacs, the lymph in the crural sacs becomes so watery that it will not form a sufficiently firm clot for the purpose, but the abdominal, lateral, and dorsal sacs of the trunk, as well as those of the forelimb will usually each yield a small drop which clots firmly. The quantity obtainable from a single sac is often too small to be of use. In fact, whenever any very large amount is to be had, it is very watery in quality, as is especially the case in the sacs which happen to lie lowermost. This oedematous condition is no doubt due to weakening of the heart, action but oddly enough it is more pronounced in frogs which have been pithed than in those chloroformed. Perhaps if the animals were anaesthetized by cold, the lymph obtained would be more uniform, and the low temperature would retard the clotting somewhat, which would be a distinct advantage. Even after taking the foregoing circumstances into consideration it is impossible always to get lymph of the proper composition. It may be very thin and fail to clot; or it may be so rich in fibrinogen that it clots immediately, even before it can be got out of the pipette, or in any case before the tissue can be transferred to it upon the cover. During this time, which is variable, some evaporation takes place and thus another factor of uncertainty is introduced. Still another variable is the amount of lymph relative to the amount of salt solution taken up with the embryonic tissue. It is not surprising, therefore, that there should be variations in the results of the experiments, which cannot be ascribed to any particular cause. On the other hand it apparently makes no difference from what species of frog lymph is taken, Rana sylvatica, pipiens, palustris and clamitans, all having yielded satisfactory material. Nor does it seem to be of consequence that the lymph should be of the same species as the embryonic tissue.

Embryos of R. sylvatica, R. pipiens, R. palustris, and, in a few experiments, of Bufo lentiginosus, were used, all in very nearly the same stage of development, corresponding to that used in most

of the previous experiments upon the development of the nervous system. The medullary folds are just completely closed and the tail bud is barely visible. The reason for choosing this stage is because it is the latest in which there is no histological differentiation in the nervous system or muscle plates. All of the cells are compact and no fibers whatever are present. The tissues are thus got into the lymph before their histogenetic development has begun.

The transference of the tissue to the lymph drop cannot be accomplished without a considerable amount of tearing. Often single cells or small groups are torn loose from the main mass and individual cells are fragmented, setting free yolk and pigment granules, but the fibrin holds the main masses together, unless the lymph is too thin, in which case the embryonic cells round off and separate from one another. This same kind of disintegration has been observed also in some cases in which the clot was firm. Even in the absence of bacteria the cells in these specimens may remain entirely unchanged, manifesting none of the peculiar protoplasmic activities seen in successful preparations. It has not been possible to assign any particular cause for this condition, and it must be attributed to slight deviations from the normal in the composition of the medium. All such experiments, and these have formed but a small percentage of the whole, have been rejected as inconclusive, and have been so indicated in the tabulation of results.

While the methods of preparation were practically the same in all cases, the experiments themselves were varied considerably as regards the tissues isolated. The chief object of the work being to test the power of embryonic nerve cells to form fibers by outgrowth, the largest number of experiments were made with nervous tissue. In some cases the medullary cord was dissected out entire, though it usually broke when transferred, and in others it was purposely fragmented by teasing. In quite a number of cases portions of the muscle plates were left attached to the medullary cord. In other experiments pieces of ectoderm from the branchial region, together with the underlying ganglia were taken. The behavior of this tissue, as regards the formation of fibers, was altogether similar to that of the medullary tube, and has

thus served to confirm the conclusions which have been drawn from the study of the former. On the other hand, the experiments have been controlled by observing the behavior of other embryonic tissues, such as muscle plates, ectoderm from the abdominal region, notochord, and yolk endoderm, under the same conditions. The results have shown, that while all tissues have certain features in common, each has nevertheless its specific activities, and these peculiarities coincide, as far as they go, with the activities shown by the respective tissues in the normal embryo. In other experiments separate pieces of ectoderm or muscle plates were placed in the lymph close to the nervous tissue, with a view to testing the power of the former tissues to influence the growth of nerve fibers. For instance in some experiments the medullary cord of the trunk was divided into its dorsal and ventral portions, and each was implanted separately with pieces of epidermis or of myotome, in the hope that it might be possible to show in this way that each of these tissues exerted some characteristic influence upon particular kinds of nerve fibers, the epidermis upon the sensory and the muscle tissue upon the motor. The results of the latter experiments were entirely negative; but since they were few in number and since the conditions of experimentation were not ideal, hope that this method may ultimately yield important discoveries need not necessarily be abandoned.

The total number of preparations made was 211. Permanent records have been kept for 150 of these, the remaining ones having given no promise from the beginning. Of the 150 cases, 35 have been rejected because they were found to be in bad condition before they could be expected to yield positive results. Table 1 shows how the experiments were distributed amongst the various embryonic tissues.

The specimens were studied almost exclusively under the water immersion lens, D* of Zeiss. In fact this lens is almost indispensable for the work. It has such a long working distance that the depths of the preparation can be readily examined without fear of breaking the cover. The magnification obtainable by the combination of this objective with eye-piece No. 4 is about 400 diameters, which is sufficient for all practical purposes. It was

TABLE 1

Summarizing the results of the experiments

TISSUE ISOLATED	NUMBER OF CASES RECORDED	NUMBER OF CASES REJECTED AS BELOW STANDARD	NUMBER OF VALID CASES	NUMBER OF CASES IN WHICH PROTOPLASMIC NERVE FILAMENTS WERE FORMED	PROPORTION OF CASES SHOWING NERVE FILAMENTS	NUMBER OF CASES IN WHICH MUSCLE FIBRILLA WERE OBSERVED	NUMBER OF CASES IN WHICH MUSCLE TWITCHING WAS SEEN	NUMBER OF CASES IN WHICH CILIARY MOVEMENT WAS SEEN.
Medullary cord[1]	90	19	71	35	.49	4	12	2
Branchial ectoderm	15	4	11	6	.55	..	..	7
Abdominal ectoderm	18	5	13	..	..	..	..	5
Axial mesoderm alone	30	8	22	1[3]	..	3–5[4]	..	..
Notochord	3	0	3	..	..	..	..	..
Endoderm (yolk)	2	0	2	..	..	..	..	..
Total	158[2]	36	122	42[3]	..	7–9	12	14

[1] In many of these cases no attempt was made to exclude all of the axial mesoderm. This accounts for the presence of muscle fibers in some.

[2] The excess of this number over the total number of recorded experiments is due to the fact that in some preparations several kinds of tissue were included.

[3] This isolated case is one of a series in which the attempt was made to separate the myotomes from the medullary cord along their natural boundary. This is very difficult to do with absolute accuracy and it is supposed that in this case some cells from the medullary cord were left attached to the mesodermic tissue. In subsequent experiments cutting in close proximity to the nervous system was avoided and only the lateral portion of the mesoderm was taken.

[4] The small number of cases recorded as showing striations is due to the fact that the preparations were examined only in toto. Had sections been cut it is believed that the number of positive observations would have been considerably larger.

only in certain cases that the oil immersion could be used, and then it was found to have no great advantage over the water immersion. A large number of sketches were made, nearly all with the camera lucida. In making these especial care was used to show the length of the fibers, and the form of the end organ correctly. Owing to the extreme fineness of the terminal filaments and the constant changes which they undergo, it is not, however, possible always to draw them with absolute accuracy in every detail. Still

is believed that any deviations which may have crept in have not misrepresented the essential character of the structures. The original sketches were made only in outline. The finished drawings, which are reproduced in the plates, were traced from these, details of texture being filled in in accordance with studies made for the purpose. Individual cells, when appearing by themselves, have been in most cases drawn in with the camera, but in indicating the larger masses of cells nothing more has been attempted than to give their general character. For instance, the exact arrangement of yolk and pigment granules was not copied because it was felt that this was not essential, and it would have required much time to the exclusion of the study of essential features.

Study of the material has been confined almost entirely to the fresh preparations. In fact it must be admitted that one serious defect in the work has been the impossibility of obtaining satisfactory preserved specimens. The ideal procedure would be first to study the growth of a particular fiber, recording the events by frequent sketches, and then to preserve that same specimen, demonstrating by suitable histological methods the structural identity between the fibers studied and the nerves found within the embryo.[10] Owing to the extreme delicacy of the structures and to the almost fluid consistency of the lymph drops, it has, however, been impossible to do this, since the mere immersion of the preparation in any fluid brings about a disarrangement of the tissue, and in many cases the clot with the implanted tissue becomes loosened from the cover, or the tissue falls out of the clot. The method which has given the greatest promise is fixation in osmic acid vapor with subsequent hardening in Tellyesniczky's bichromate acetic mixture, and staining in alcoholic haematoxylin by the method of Oskar Schultze '04. In some of these preparations

[10] Since this was written Dr. M. T. Burrows of the Rockefeller Institute, while working with me has devised a satisfactory method for obtaining permanent preparations. He has shown that embryonic nervous tissue of the chick, when isolated in the proper medium, gives rise to the same long filamentous processes as does that of the frog; and further, that by staining the preparations in Held's molybdenum haematoxylin the neurofibrillae in these filaments are brought out very clearly. An account of this work will be published at an early date.

isolated cells of various kinds have been well preserved (fig. 12) but satisfactory preparations of the nerve fibers have not been obtained. Some of the preparations have been cut into serial sections. Nerve fibers were found within them, but in all cases they were broken off at the surface of the tissue.

This defect in method has in a measure been offset by the experiments described elsewhere ('10) in which the nerve fibers from the medulla oblongata were shown to have grown into a blood clot implanted in their path.

General description of material

The developmental processes which have been observed in specimens prepared as described in the last section involve only the histological differentiation of the tissues. The gross morphological changes have no resemblance to those which take place within the embryonic body. This is as might be expected even on purely mechanical grounds, for the stresses and strains which are brought to bear upon the developing organs when enveloped in the fibrin must be entirely different from those within the intact embryo.

From the time when the tissue is implanted in the lymph it shows a tendency to spread out (fig. 16), and often broad laminae made up of a single layer of cells (*l*) are found at the periphery of the mass, while individual cells may move off entirely by themselves. This is the case with both nervous and axial mesodermic tissue, as well as with pieces of ectoderm, though the latter more often roll themselves into complete spheres. One notable peculiarity that has frequently been observed is the formation of large round or oval openings in the flattened tissue (*fen*), which may be surrounded by very narrow bands or rings of tissue with cells sometimes in single file (*cd*). This phenomenon may possibly be due to the mechanical action of the fibrin upon the implanted tissue, but the spreading out of the cells into thin sheets seems to result largely from the activities of the cells themselves. These activities, which are common to several tissues, in fact to all except the very inert yolk-laden endoderm and, perhaps, the notochord, may be referred to a form of protoplasmic movement having its

seat in the hyaline ectoplasm found at the angles and sometimes at the borders of the cells. The movement cannot be observed clearly in the larger masses of cells on account of their opacity, but it may be seen very clearly in those cells which leave the main masses and wander off by themselves. These cells are irregular in shape, varying from unipolar to multipolar form and having a varying amount of ectoplasm at their angles (figs. 23 and 27). The movement is amoeboid in character and results either in a change in shape of the cells or in their movement as a whole (text fig. 2). Such cells are found usually in greatest numbers in preparations of the medullary cord, and it is here that they are most active, though cells from the mesoderm are often quite similar. However, it is only the protoplasm of cells from the medullary cord and from the cranial ganglia (branchial ectoderm), that gives rise by its movement to long fibers. Cells of the epidermis show their power of movement in somewhat different form. As has frequently been observed, the general tendency of isolated bits of epidermis is to round off into small vesicles, which, when left in water, may move about for days by means of their cilia. Within the lymph the same thing frequently takes place, although there is apparently greater resistance to the process of rolling up, and the cells may often remain together in the form of extensive sheets. Along the free border of these sheets of cells there often appears a fringe of hyaline protoplasm, which undergoes continuous amoeboid changes (figs. 13 and 14*pl.fr.*) In one case of this kind it was observed that the sheet of cells gradually spread out toward the side on which this fringe was placed. Since the work of Peters ('85–'89) it has been generally admitted that wound healing in the epidermis is primarily due to the movement, in part amoeboid, of the epithelial cells, so that it seems quite possible that in this fringe of hyaline protoplasm above described, we have one part of the mechanism by which the movement of cells in wound healing is brought about. The most inert of all the tissues is the endoderm, which will remain for days in the lymph, practically unchanged, gorged with yolk and devoid of hyaline ectoplasm. The notochord is also very inactive, although large pieces of this structure may show after a time the early stages of normal differ-

entiation, unaccompanied, however, by growth, *i.e.*, increase in length.

The changes which take place through the protoplasmic activity of the embryonic cells can usually be distinguished from those which are due to the action of the clot or the sudden spreading out of the drop of plasma. Likewise the fibrin can readily be distinguished from the hyaline protoplasm of the cells, although even in the fresh specimen it varies considerably in appearance. Sometimes the fibrin filaments, in spite of their extreme fineness, are plainly visible, and in other cases there are comparatively few to be seen. They may be found singly or in bundles, and often run for a long distance in a straight line, or sweep around in circles, the individual filaments running from one strand to another. The threads are seen to radiate from the transplanted tissue, and often they may be traced from the hyaline ectoplasm of the embryonic cells, upon which they apparently exert considerable tension. This may result in drawing out the ectoplasm to a narrow fringe (figs. 9, 10, and 28), which differs, however, from the fringe of active protoplasm described above, in that it does not continually undergo changes in form. Evidence of still greater tension is found in cells which are drawn out into spindle shape, and which often seem to be pulled along bodily, as may be seen in figs. 9, 10 and 11 which show three successive views of the same cell (ct_2.) Sometimes long chains of cells in single file or slightly overlapping one another may be formed. Direct evidence of mechanical tension may be had in observations like the following: A long thin fiber-like structure was observed in a preparation containing branchial ectoderm extending, tightly stretched, from a pear-shaped cell to a mass of cells some distance away, when suddenly this strand of protoplasm broke, contracting into a short thick process which remained attached to the cell. Again very fine protoplasmic threads are frequently found spanning the round openings in the masses of tissues, which have been described above (fig. 16 *fil.*). These threads are always taut and are apparently due to the stretching of originally shorter protoplasmic connections between the cells, as the holes in the tissue enlarge.

Similar filaments are often found extending from one cell to another (figs. 17 and 27).

The histological differentiation of the tissue in successful preparations is specific and normal, although it does not proceed so rapidly nor become so complete as when the tissues are left in their normal environment. No doubt one factor which contributes to this retardation of development is the insufficient supply of oxygen within the moist chamber. This is indicated by the slow rate of absorption of the yolk, as compared with its rate of absorption in the embryo. It is only in those specimens that have been kept alive for a week or longer that there is any great diminution of the yolk contained in the cells. The most noticeable histological differentiations that have been observed in the various isolated tissues are the following: the formation of typically striated fibrillar substance in cells taken from the axial mesoderm; the development of the cuticular border in ectoderm cells and the growth of cilia which may continue in action for days; the formation of typical chromatophores, most probably from cells derived from the medullary cord; and the formation from the central nervous system and from the cranial ganglia, of the long protoplasmic filaments, which are identical with the nerve fibers of the embryo and which are the especial subject of the present investigation.

Muscle fibers have been found not only in cases where a portion of the axial mesoderm was left in contact with the medullary cord, but also where it was isolated entirely from all other tissue, showing that the cells of the muscle plates at this stage, *i.e.*, before visible differentiation has begun, have the power of self-differentiation in the highest possible degree. This is in conformity with the results obtained from the study of muscle tissues in embryos deprived of the central nervous system. It is of course only in certain favorable cases that the presence of muscles fibrillae can be observed in the fresh specimen, since the tissue, unless it spreads out, is too opaque to permit of satisfactory observation in toto. While it is only the first stages in differentiation that take place, the yolk never being completely absorbed, the most

characteristic structure of the muscle fiber, the striated fibril, is formed with the alternating dark and light bands as well as Krause's membrane plainly visible. In many specimens in which the myotomes have been taken out with the medullary cord, muscle twitchings have been observed, beginning to occur the next day after isolation, and continuing sometimes up to the sixth day. No contractions have ever been witnessed in muscle completely isolated from nervous tissue.

Characteristic pigment cells have been observed a considerable number of times to arise apparently from pieces of the medullary tube from which large numbers of single cells had separated. But only fragmentary information is at present available regarding the development of these cells. In one case, which was under observation for several days, it was found that the pigment first arose as a round mass of granules lying just to one side of the nucleus. This gradually increased in size and then the pigment granules became scattered through the cytoplasm. In the meantime the yolk was almost entirely absorbed. After the cells are fully differentiated, observations from day to day showed that the individual cells changed slightly in form (figs. 24–26, *a*). While the evidence is by no means conclusive, especially since no great care was taken to exclude the presence of mesoderm cells, the fact that pigment cells were frequently formed from pieces of medullary cord, suggests the possibility that these cells may normally take origin in part from this source, most likely from the ganglion crest. This suggestion is borne out by the fact that pieces of medullary cord or cranial ganglia when transplanted to various regions of the embryonic body often break down and give rise to large numbers of pigment cells.

The above observations are recorded here primarily for the purpose of showing that the mode of procedure employed in the experiments permits the characteristic differentiation of various tissues to take place. It is of importance to establish this fact in order that the interpretation which has been given the observations upon the behavior of nervous tissue under these conditions may not be called into question as being based upon something entirely abnormal. It must of course be admitted that some of

the phenomena which have been observed may not have their counterpart in the embryonic body, but fortunately the careful comparison with what takes place in the latter enables us to discriminate with a fair degree of accuracy between the abnormal and the normal.

Description of the behavior of nervous tissues

The early changes which isolated pieces of medullary cord undergo are not very different from those seen, for instance, in pieces of mesoderm. There is merely greater protoplasmic activity. This frequently results in the separation of numerous cells which may move off individually from the main mass of tissue (figs. 10, 11, 16 and 27), or it may result in the formation of sheets of cells, one layer thick, which form a more or less complete fringe around the main mass (fig. 16). The formation of rings, as described above, is also frequent. These changes begin on the day on which the tissue is implanted, and may continue for several weeks, although the first week—usually the first four days—witnesses practically all the essential changes that take place. Observed from time to time, these cells may be seen to change their shape and their position relative to one another. Frequently they show anastomoses, though in a great many cases apparent continuity is often found on continued observation to be merely due to very close contact which may later be relinquished.

The striking peculiarities of this tissue have never been observed earlier than on the next day after isolating. Then it is that the long filaments, identical in form with the nerve fiber of the normal embryo, begin to appear. Two and three days after implantation they show their greatest activity. After the fifth day they are usually no longer to be found.

It will be well to begin with a description of one of the most striking typical cases and to consider the more aberrant forms afterward. In the case chosen for this purpose (experiment 137) the tissue was isolated from an embryo of R. palustris and the lymph was taken from an adult R. pipiens. The day after the preparation was made, there appeared on one side of the main

mass of tissue a long stout process of hyaline protoplasm, which, when first observed, was about 90 μ in length, and extended out from a tapering cell (fig. 7). Examined an hour and a half later, this process was found to be only a little (about 17μ) longer, but the form of the end had changed considerably (fig. 8). It could also be seen that there were two separate fibers instead of a single one, one partly overlapping the other. Eight hours and a half later the specimen was again examined and found to have undergone remarkable changes (fig. 9). No less than four fibers could then be distinguished diverging from one another in their direction of growth, and each with its characteristic branched end (*npl*) continually undergoing change in form. The longest fiber (nf_3) was about 220μ in length. At this time the preparation closely resembled the condition described above in the normal embryo (figs. 3 and 4). Another interesting and important feature shown by this preparation was the action of the fibrin (*thr*) upon certain of the cells (ct_2), and the independence of the protoplasmic filaments from the fibrin threads. Twelve hours later, on the following morning, the change noticed was again very striking (fig. 10). Two of the fibers (nf_2 and nf_3) were branched and all had lengthened materially, the longest being about 480 μ in length. The ends of the fibers continued to show the same activity as before. Throughout their entire length the fibers consist of hyaline protoplasm, with no yolk nor pigment granules whatever. Slight varicosities are present in places, and often the fibers show a faint fibrillation and sometimes are slightly mottled. The thickness of the fibers, 2–3μ, in this case is rather unusual. Changes in the cells are also important. The number of loose cells has increased, and they move along slowly from place to place, while changing their shape. Their movement is, however, entirely independent of the fibers. The tension of the fibrin filaments upon some of the cells is clearly shown. The cell (ct_2) noticed previously is very much drawn out as compared with its condition the day before. Eleven hours later still further elongation and branching of the nerve fibers is to be seen (fig. 11), the longest now being about 600μ. The loose cells are more numerous and some of the fibers are partly obscured by them. The exact

extent and manner of ending was therefore not observed in all fibers—nf_1, and nf_2, for instance, being only incompletely recorded. The cell to which the fibrin filaments were attached is now drawn completely out of the main cell mass. Twelve hours later the longest fiber is found to extend 557 μ beyond the point of ending the evening before, having thus grown at the rate of 46μ an hour, the total length now being about 1.15 mm. Many branches are present and many new fibers are visible in the same region, but the proximal part of those described has become covered over by the loose cells which have wandered out from the main mass. These circumstances rendered it impossible to make further accurate observations of the changes which took place, and the specimen was therefore preserved. Unfortunately, the region which had been observed most carefully was completely disarranged in the course of fixation and the preparation in permanent form was almost useless for further purposes.

This same preparation showed a number of other fibers of interest. Among these was one which arose from a single isolated cell, and which was visible throughout its entire length (fig. 21). When first observed this fiber had a total length of 453μ. At a distance of 303μ from the cell it bifurcated, the longer branch being 150μ, and the shorter, which afterward grew to be the longer, 107μ. At this time, the ends were not very active and that of each branch was almost globular, with but one blunt pseudopodium. The cell itself was unipolar. Examined at the expiration of four hours and three quarters (fig. 22), the change in the fiber was found to be very great. The cell itself was unchanged but the fiber then had a total length of 631μ, and one of the original branches had again bifurcated. All three of the terminal enlargements were exceedingly active, and all were provided with a number of fine filaments. The increase in length from the cell to the tip of the longest branch was 221μ, which is at the rate of .77+μ per minute, or 46.5μ, per hour. Comparison of the two stages shows that the greater part of this was due to terminal growth, but the distance between the cell body and the first bifurcation increased 21μ, and the curvature of this part of the fiber was partially straightened out.

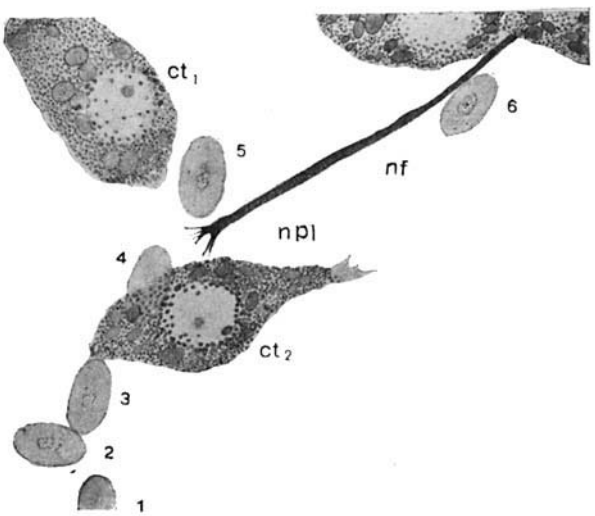


Fig. 2 Three views of a growing nerve fiber, observed alive in a clotted lymph preparation. 1, 2, 3, 4, 5, red blood corpuscles in fixed position; ct_1, and ct_2, single cells which were seen to wander across the field; *nf*, nerve fiber; *npl*, growing end of motile protoplasm. × 420. A, As seen at 2.50 p.m., two days after isolation of the embryonic tissue. B, As seen at 4.40 p.m., the same day. Note change in form and position of the loose cells. C, As seen at 9.15 p.m., the same day. Movement of cells has covered over the proximal part of the fiber.

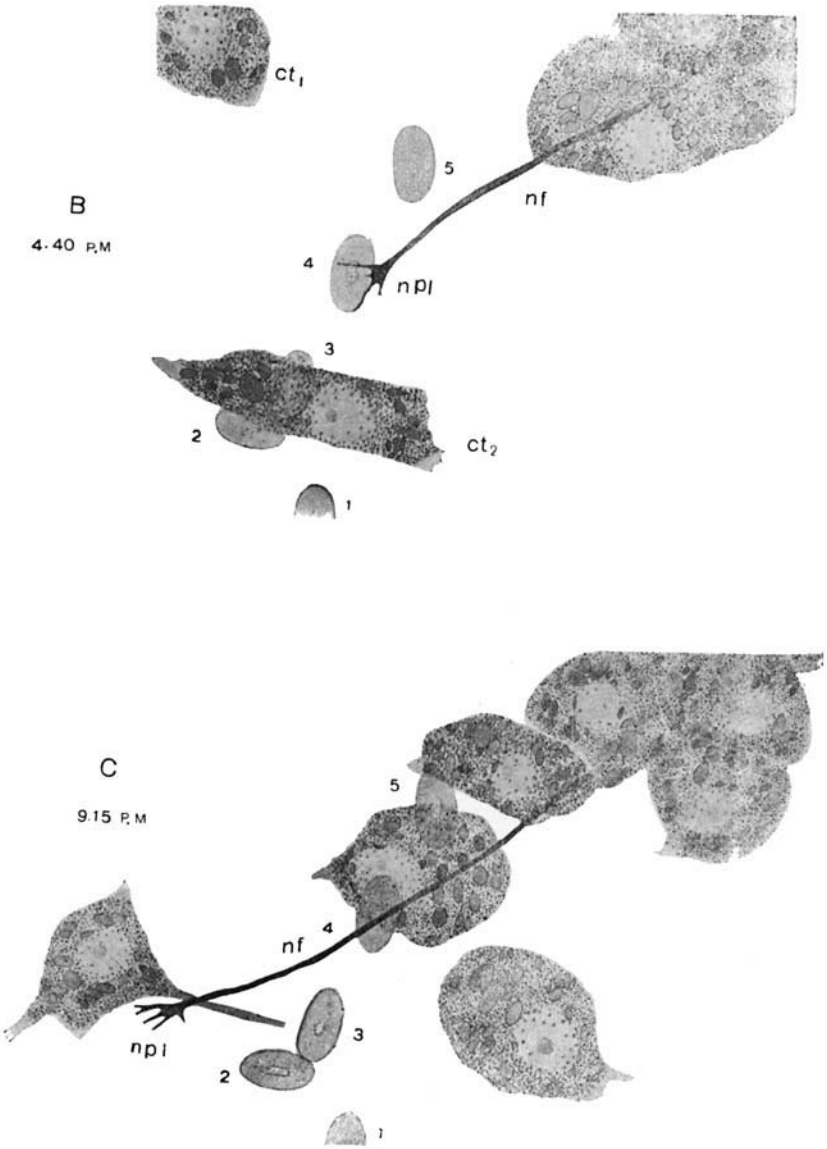


Fig. 2 (Continued)

Still another feature of importance was shown in the preparation under consideration. In the previous observations upon the growing filaments, it had been impossible to find absolutely fixed points from which to measure their increase in length, so that there remained the possibility that the extension of the fibers was not due to the active movement of the enlarged end but rather to the passive shifting of the cells from which the fibers were seen to arise. While such an objection would not hold in cases like the one just described, where the fiber is visible throughout its entire length from the cell of origin to the amoeboid end, such instances are not very common, and it is desirable to have other means of determining which structure is moving. The specimen under consideration has demonstrated that in good preparations where the clot is firm, the red blood corpuscles, some of which are nearly always to be found mixed in the lymph, can be used for this purpose. One particular case (text fig. 2) will serve to illustrate the point clearly. A short stout fiber (*nf*) was observed emerging from a mass of cells, its cell of origin not being visible. Near the end of the fiber (*npl*) was a group of five red blood corpuscles (1–5) arranged characteristically, and at the point of emergence of the fiber was another single corpuscle (6). In the vicinity of the end of the fiber were two large separate cells (ct_1, and ct_2) derived from the implanted tissue. The relative position of the structures just mentioned is shown in fig. A. The end of the fiber was then only fairly active. An hour and fifty minutes later (text fig. 2 B) it had progressed only 25μ, as measured by its relation to corpuscles 4 and 5. The loose cells (ct_1 and ct_2), however, had moved considerably, but in a direction approximately at right angles to the direction taken by the fiber, showing that they could not have been concerned in the movement of the latter. At the same time these cells had changed their shape materially, as is shown especially in cell ct_2, this change in shape being undoubtedly due to the activity of the hyaline ectoplasm. Six hours and twenty-five minutes after the first observation was made (text fig. 2 C) the distance through which the end had moved was 100μ, *i.e.*, at the rate of 15.6μ per hour. This rate is slow as compared with that observed in other cases, though it is considerably faster

in the first interval than in the second. Considerable numbers of loose cells had by this time moved into the field, so that the proximal part of the fiber, which was visible earlier,was then covered up, but the five corpuscles were still plainly in view and their relative position remained still unaltered. During all this time the end of the fiber had been changing its form continually. It is inconceivable that the fiber could have been pushed past the corpuscles by any force acting from behind. Again, it is impossible that the five corpuscles could have shifted materially and at the same time have retained their same relative position. Nor can we account for the movement by tension upon the fiber from beyond, for such tension would act also upon the corpuscles and upon the cells in like manner; and the continual change in the form of the end and the lack of any appearance of tension likewise speak against this mode of accounting for the movement. We therefore cannot escape the conclusion that the extension of the fiber is due to the activity of the enlargement at its end.

The character of the movement that takes place at the end of the fiber is difficult to describe. The filaments in which the fiber ends are extremely minute and colorless, showing against their colorless surroundings only by difference in refraction. The eye perceives, therefore, only with difficulty an actual movement, though when an active end is observed for five minutes it will be seen to have changed very markedly, so that in making drawings one encounters the difficulty of having the object change before the outline can be traced. In order to show the change of form that does take place, a series of sketches are reproduced in text fig. 3, which show the same fiber at intervals of from five to nine minutes. The specimen was a portion of the ectoderm with the underlying cranial ganglia taken from the branchial region of a sylvatica embryo. When first seen the fiber was about 450μ long. The next day, April 6, 1909, four days after the preparation was made, the fiber had increased in length to 800μ when the first sketch was made at 10.50 a.m. The changes shown in the sketches took place between 10.50 and 11.37 a.m. The end of the fiber was just beyond a red corpuscle, the position of which was fixed. This is shown in outline in each figure. It will be

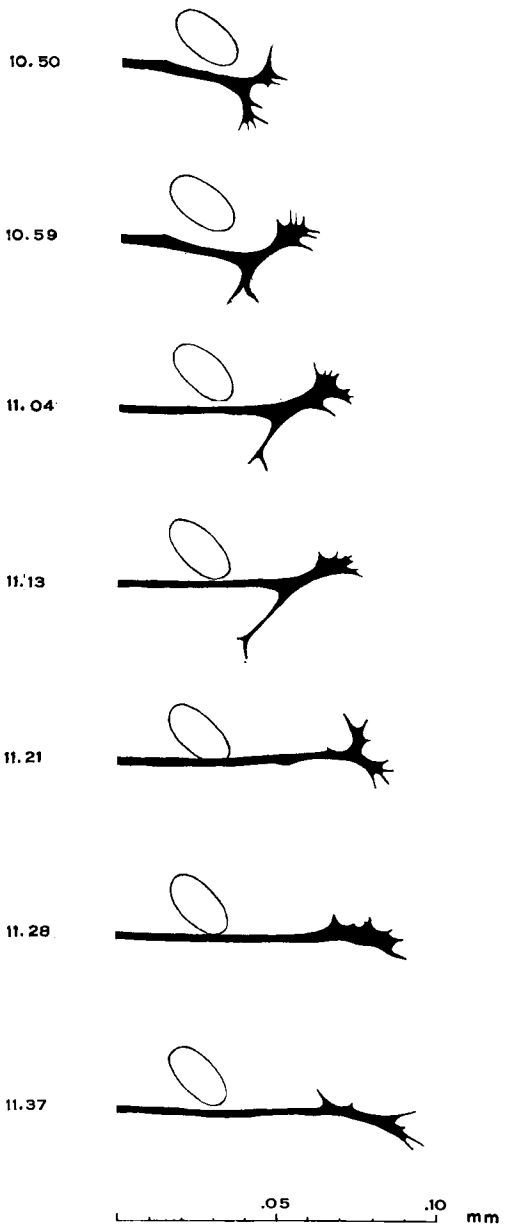


Fig. 3 Seven successive views of the end of a growing nerve fiber showing its change of shape and progression. The sketches were made with the aid of a camera lucida at the time indicated on the left. The red blood corpuscle, shown in outline, marks a fixed point. The observations were made upon a living preparation of ectoderm from the branchial region, isolated in lymph, four days after isolation. The total length of fiber at that time was 800μ.

seen that during the time in which it was under observation, new processes or pseudopodia were formed and some present at first were withdrawn. The movement of the end was 44μ during the 47 minutes in which it was observed. This is at the rate of .94μ per minute or 56μ per hour, which is the most rapid extension that I have ever observed. This is all the more remarkable because of the great length of the fiber, for one would naturally expect a gradual lessening of the activity as the limit of growth is approached. There can be no doubt that the red blood corpuscle was a stationary point. Its position with reference to five other corpuscles remained fixed throughout the period of observation, and even on setting the preparation on edge, *i.e.*, turning it 90°, no change in relative position was observed, although the plasma in the meshes of the clot flowed perceptibly. The direction of growth of the fiber served, however, to draw the fiber close to the corpuscle. One feature shown in the present case and observed also in a number of others, was the formation of processes of considerable thickness and length which were afterward withdrawn (text fig. 3), indicating that the movement of the end is not directed constantly toward a particular goal."[11]

As already mentioned, the fibers that are found in preparations of the nervous system usually proceed from masses of cells so opaque that their exact mode of origin cannot be determined. Fibers whose origin could be seen in single cells have been observed a number of times and several are shown in figs. 18 and 20. Fig. 18 differs from the others in so far as the cell itself has wandered out from the mass, remaining connected with the latter by a long filament (*b*) about 300μ long. The hyaline process (*a*) at the distal end of the cell is relatively short.

This leads to the consideration of the formation of protoplasmic fibers by the drawing apart of cells. Fig. 19 shows a bipolar cell the processes of which were formed in this way. They are tightly stretched and at both ends terminate in masses of cells, so that it is not possible to make out their exact mode of termina-

[11] This is akin to what Held has termed "Prinzip der Auswahl" for which he has adduced evidence (*Op. cit.*, p. 270).

tion. The cell resembles strongly a spinal or cranial ganglion cell, and as a matter of fact this particular one was derived from a piece of branchial ectoderm, but the case is not specific, for similar cells have been found in other tissue, even in pieces of muscle plate, and drawn out fibers of this kind have been observed in large numbers of cases, though more frequently in nervous tissue than in any other. They demonstrate the great extensibility of the embryonic protoplasm and show how a nerve fiber may be passively drawn out to enormous length, as no doubt occurs in the embryonic body, for instance, in the case of the *r. lateralis vagi*.

Frequently large numbers of cells become loosened from the main mass and scatter themselves in the periphery. Such cells may remain separate or they may often be connected with one another by hyaline protoplasmic filaments (fig. 27). In many cases, however, the connections are more apparent than real, as cells that seem to have been joined will frequently glide apart and demonstrate the supposed continuity to be merely a close contact.

In a few cases the long nerve fibers have apparently formed distinct nets. One of the most remarkable observed is shown in fig. 29. There are a large number of free endings in the group, as well as many apparent anastomoses. This specimen was observed late one evening, and while it was under observation one of the connections (x) was actually resolved. On the following morning it was found that, with two possible exceptions, all of the anastomoses had been severed, each fiber being independent of the others. Many of these apparent protoplasmic fusions were obviously to be accounted for by our optical limitations. The protoplasmic filaments are very delicate, colorless, and without visible limiting membrane. When two such structures touch one another, an appearance of fusion is readily given and one must be extremely cautious in interpreting observations. On the other hand, there are undoubted instances of strong adhesion between the cells by means of filaments, as shown by the tension upon them if the cells move apart, but it must be borne in mind that very slight differences in the physical properties of the protoplasm of which the cells are composed would suffice to permit

or prevent fusion between contiguous elements, and the same elements might therefore at one time be separate, and at another continuous.

In some cases large numbers of the protoplasmic fibers have been found matted together, in an inextricable tangle (fig. 17). These have undoubtedly been derived from groups of cells, and the specimen reminds one of the condition that is found after the medullary cord of the embryo is removed from the trunk region, when the fibers from the brain grow out in a large bundle and lose themselves ultimately in the mesenchyme.

DISCUSSION OF RESULTS

The significance of the experiments in the interpretation of normal development

In attempting to estimate the significance of the foregoing experiments as elucidating the processes of normal development, we are at once confronted with the question whether the conditions in the experiments are sufficiently like those in the embryonic body to warrant any comparison at all. This can be answered most satisfactorily by carefully comparing the activities of isolated tissues with the activities of the tissues in the normal embryo. Such an empirical determination must have more weight than any amount of *a priori* argumentation upon the subject. The phenomena which can be compared and interpreted most readily are those of movement and of tissue differentiation.

The movement of the embryonic cells in the lymph clot is very distinct, and is due beyond doubt to the activities of the hyaline ectoplasm (figs. 23 and 27), which is accumulated especially at the angles of the cells. It there forms extremely fine filamentous pseudopodia, through the activity of which the cells may change their shape or move from place to place. The exact character of the movement is not the same in all kinds of cells and it varies greatly in intensity. Axial mesoderm and medullary cord yield cells that frequently wander for considerable distances by themselves; epidermis, when it does not roll up into bands or spheres,

may form a hyaline fringe (figs. 13 and 14), and spread out considerably; pieces of the central nervous system and the primordia of the cranial ganglia give rise to the fiber-like structures described in the last section; the endoderm and notochord remain almost inert.

It is, of course, needless to point out here the wide occurrence of protoplasmic movement in the normal development of organisms, and it will suffice to mention a few special cases which bear more directly upon the present problem. Within the body of the vertebrate embryo at the stage of development under consideration there is ample evidence that this kind of movement takes place. It is then that the mesenchyme is beginning to form by the breaking down of the epithelial mesoderm and the shifting of its cells to regions far removed from their source; and similarly the cells of the ganglion crest leave their place of origin and wander for a considerable distance before grouping themselves together as the spinal ganglia. In these cases we are dealing largely with the movement of single cells. A notable example of the active movement of masses of cells is afforded by the lateral line rudiment, which, in the course of several days, extends all the way from the head to the tip of the tail, as experiments show, by its own motile force (Harrison '03). At a later period of development after the first nerve trunks are laid down, there is an actual movement of Schwann cells along the nerve fibers, as I have been able to observe in the tail fin of the living tadpole ('04). In the same object Clark ('09) has watched the growth of lymphatics by sprouting at their ends. This observer has not only seen the actual amoeboid movement of the endothelial cells, but has also been able to show that the movement may be stimulated and directed by definite bodies, such as, extravasated red blood corpuscles. Within the central nervous system there is undoubted shifting of groups of ganglion cells, such movement having been taken into account by Cajal ('92, '99) in his original hypothesis of chemotaxis, and more recently by Kappers ('08) in his papers on neurobiotaxis, though it must be admitted that the evidence for active movement in the two last cases is merely inferential. In the closure of wounds we have another example

of the amoeboid activity of cells, as was first pointed out by Peters ('85, '89) in the cornea of the frog, and the observations of Barfurth ('91) and Born ('96–'97) on the epidermis of amphibian larvae and embryos confirm this view. More recently Eycleshymer ('07) has observed directly the movement of epidermal cells over a denuded wound surface in Necturus embryos.

The phenomena of movement, which may be observed in the embryonic cells isolated in lymph, must, in view of the above considerations, be considered as manifestations of activity similar in kind to those shown by cells within the normal embryo. The differences which may exist are unimportant for our present purpose. For instance, the peculiarities of form (fig. 6) assumed by the larger masses of cells when transplanted, are not to be taken as an index of a marked abnormality of conditions which might introduce entirely new features into the movements of individual cells, for these strange formations may be accounted for by the peculiar mechanical conditions obtaining in the clot. Analogous deviations from the normal in the gross form of parts are found, accompanied, however, by normal differentiation of tissues, when pieces of the medullary cord are transplanted to strange regions within the body of the embryo.

The phenomena of differentiation permit the drawing of a much closer parallel between the behavior of the tissues in their normal environment and when isolated in lymph than do the motor activities. Each type of cell follows the same course of differentiation which it would have taken had it not been removed from the embryo, as is seen, for instance, in the formation of striated fibrillae in cells from the axial mesoderm, a cuticular border in cells from the epidermis, and typical chromatophores from the walls of the medullary tube. Ciliary activity, which may persist for days in the case of tissue from the medullary cord or the ectoderm, and muscle contractions, which occur in the muscle plates when transplanted along with parts of the central nervous system, bear witness to the possibility of normal functioning under the conditions of the experiments.

It is seen from the above that the behavior of embryonic cells when transplanted to lymph is specific as regards the character

and degree of their motility, the quality of their differentiation, and their mode of physiological activity when differentiated. Even had we but scant knowledge of the normal development of nerve fibers, we should therefore be justified in concluding that the phenomena witnessed in preparations from the central nervous system are a representation of what occurs when the nerve fibers develop in the body of the embryo. The study of normal development strengthens this conclusion immeasurably by revealing, in the young stages of peripheral nerves, structures which are strikingly similar to the protoplasmic fibers found in the lymph. (Cf. figs 4–6 with figs. 8, 9 and 21). Such fibers with their active amoeboid ends are formed by cells taken both from the walls of the medullary tube and from the rudiments of the cranial ganglia, and must be regarded as specifically nervous. It is thus scarcely conceivable that the identity between the fibers found in the lymph and true embryonic nerves can be questioned, for that conclusion, as just pointed out, is based not only upon the general fact that the differentiations taking place in the isolated tissues are normal and specific, as far as they go, but also upon the particular morphological resemblance between the structures in question and undoubted nerves. It must be admitted that the case might be made even stronger were it possible to preserve the isolated nerves satisfactorily and stain them by the specific stains for neurofibrillae, though this is in no sense essential to the argument here advanced (see footnote, p. 807).

The bearing of the experiments upon the theories of nerve development

As the experiments clearly show, one of the fundamental characteristics of the neuroblastic protolplasm is its high degree of motility, which, being manifested by only a limited portion of the cell, results in the drawing out of the protoplasm into a long filament representing the axone of a nerve fiber. The extreme tip of the fiber, which is the *cône d'accroissement* of Ramon y Cajal, remains remarkably mobile, while the body of the fiber evidently soon acquires a firmer consistency and considerable tensile strength.

The latter is probably the result of neurofibrillation, since, as the work of Held and of Ramon y Cajal shows, the neurofibrillae extend almost to the tip of the fiber even in very young nerves.

The movement of the neuroblastic protoplasm, which is brought about not by passive extension but by its own activity, will take place in a medium foreign to the embryonic body, and there can here be no possibility either of accretion by transformation of living protoplasm already *in situ*, or of outgrowth of fibrillar substance within such protoplasmic connections, since there is nothing of the kind present, the solid parts of the culture medium being nothing but fibrin. Quite aside from this consideration, the character of the movement, as observed directly, precludes all possibility of extension according to the conception of either Hensen or Held.

The criticisms of this conclusion that have appeared up to the present time, have been directed against my preliminary notices, in which the data were very briefly recorded and illustrations were few. Those who have expressed themselves adversely to the claim of conclusiveness are Hensen ('08), Schaeppi ('09), Kerr ('10) and Held ('09). Hensen, however, raises no specific objection and Schaeppi[12] is merely not convinced that the actual growing end of the nerve fibers was observed. The criticism of Kerr ('10) is more specific, though of the same kind as that of

[12] Schaeppi's criticism is directed mainly against my earlier experiments (Harrison '06), which had not then been published in full. I regret that through inadvertence no notice was taken of Schaeppi's appreciative though adverse critique in my full paper (Harrison '10), in which the logical bearing of the various experiments is set forth at some length. No claim of absolute rigorousness of proof for the non-participation of protoplasmic bridges in the formation of nerves, was there made for any particular experiment, except in the case of the one in which the nerves grew within the implanted blood clot; and even in this case it was admitted as a remote possibility that the embryonic cells which rapidly organize the clot might form protoplasmic bridges. Taken together, however, these experiments afford a mass of evidence against the protoplasmic bridge theory, which to my mind far outweighs that which has been brought forward in its favor. The experiments certainly rob the theory of any claim upon functional activity as a factor in the early development of nerves, even though the experiments with acetone-chloroform are not admitted as evidence. Turning now to the criticism of the present experiments, I feel confident that if Schaeppi had had before him the figures which I am able to present here, he would hardly have asked: "Wer in aller

Schaeppi. He has urged that in the lymph experiments the excised fragments may have included protoplasmic bridges such as he has figured from Lepidosiren embryos, and that these might differentiate later into nerve fibers. In reply to this it may be pointed out that the embryos used in the experiments were of a relatively younger stage than those Kerr has in mind and contained no protoplasmic connections of the kind figured, as serial sections readily show.[13] Furthermore the pieces of the tissue were cut out with sharp scissors, sometimes by a single clean cut between the neural tube and the notochord, or in other cases, after the neural tube had been separated by the scissors from the muscle plates. All protoplasmic filaments must have been severed by this mode of cutting, and while, of course, short ends may possibly have been left attached to the cells, and have remained invisible in the preparations, they would by no means be able to account for the great length—over a millimeter in some cases—attained by the fibers. Besides, what is much more conclusive, *the end of the fiber is an actively motile mass of protoplasm.*

Held's ('09) criticism takes a different turn. He admits[14] that the fibers seen in the lymph are really the beginnings of nerves (Ansätze einer Nervenbildung), though on the next page he maintains—on what grounds it is not clear—that if the nerves of an embryo did develop exclusively in the manner described by me,

Welt will mich denn davon überzeugen, dass das, was unter dem Mikroskope als das Ende einer Faser erscheint, nun wirklich in Tat und Wahrheit das Ende ist?"

In doubting that such ends, in which motion and extension can be observed with absolute certainty, are actual ends, it seems to me that the supporters of the protoplasmic bridge theory are pushing skepticism about one thing beyond the utmost limit, and at the same time are placing an equally unbounded faith in the invisible. One cannot but think of the epithet "noli me tangere" by which Hensen designated the outgrowth theory, and wonder if it might not be applied with much greater appropriateness to the plasmodesm hypothesis. Could the botanist, if pressed for an absolutely rigorous proof that the roots of a plant grow out from the radicle and are not preformed in the soil, give an answer based on evidence of any different kind from that given here for the outgrowth theory of nerves?

[13] To my mind the protoplasmic bridges which Kerr figures are simply the processes that have grown out from the cells in the ventral part of the cord.

[14] *Op. cit.*, p. 260.

they would after a time degenerate as do those which develop sporadically in the ventricular fluid. It is quite true that the nerves which grow out into the lymph-clot do ultimately shrink and disintegrate, but it is purely gratuitous to assert that this is *in consequence* of their original mode of growth, when it is in all likelihood due to the effect of continued unfavorable surroundings upon their subsequent development. Held also intimates that the nerves grown in lymph are incapable of functioning, though no sufficient ground for this statement is offered. But when he says "Die Beobachtungen Harrisons zeigen . . . mit welcher *Energie die neurofibrilläre Zellsubstanz aus der fibrillogenen Zone des Hisschen Neuroblasten hervorwächst,*" and when he says further on "Dass die fraglichen Experimente . . . die *elementare Bedeutung der Hisschen Neuroblasten* für die von ihnen herausgehende und vorschreitende Bildung der spezifischen Substanz des Nervengewebes illustrieren," then I can only express my cordial agreement, since in these sentences Held practically admits all that I have ever claimed for the experiments, viz: that they show the nerve fibers to be the product of the neuroblasts, and to be capable of being formed without the aid of protoplasmic bridges.

It would seem from the above that Held and myself were in pretty fair agreement regarding the question at issue, and I shall endeavor to show below just how our views are related, but it will be necessary first to consider the important difference that appears in the next following paragraph of Held's work, in which he maintains that the histogenetic study of the embryo shows more than the experiments, since it reveals the presence of a connective substance between the individual cells and organs of the body, which is used in the formation of the definitive nerve paths.

Held's sections are of exquisite beauty and show beyond doubt the structures he has described, but they fail on the other hand to prove that the same have any *essential* connection with the formation of the nerve paths. While it is true that the developing nerves seem to be very intimately joined with the protoplasmic bridges which Held describes, the very ubiquity of the latter in the embryonic body precludes the possibility of proving, by the

study of normal material, however clearly stained, that they are essential to nerve building. So long as we keep an animal in pure air without ever varying its surrounding medium, we have no means of knowing whether the nitrogen constituent is essential to its life or not. It is only by eliminating or at least by varying this part of medium that it can be shown not to be necessary. In the embryonic body, according to the descriptions of Held, no nerve can grow along a normal path without coming into intimate contact with the protoplasmic bridges or the protoplasm of the cells within the central nervous system. Until these are eliminated or modified, therefore, we can have no knowledge whether they are essential to the growth of the nerves or not. This is the crux of the whole question and it is this that the present experiments have settled, adversely to the view taken by Held, by substituting for the supposedly essential protoplasmic bridges. unorganized fibrin threads, which afford merely mechanical support to the growing nerves. In view of this I find it altogether impossible to accept Held's conception as correct, though just to what extent the protoplasmic net work which he describes may influence mechanically the growing fibers remains problematical, there being no ground for denying it a place as a subsidiary factor along with the other structures of the embryonic body. Notwithstanding this difference of opinion I think that it will become clear from the following, that, in the main, the relation between Held's work and my own is not antagonistic but complementary.

The elementary factors of nerve development

When we consider the elementary phenomena of nerve development in the light of the present experiments and of recent histogenetic studies, the first thing that stands out is that two separate processes are involved: the one is the protoplasmic movement, which results in the drawing out of a part of the neuroblast into a thread of protoplasm, the primitive nerve fiber; the other is the differentiation of this protoplasm by the formation within it of neurofibrillar substance. These may be referred to as the motor and the differentiation phenomena respectively. The existence of the former has either not been recognized or has been openly

denied by the adherents of Hensen's theory. Early protoplasmic connections have been observed by them, and by assuming these to be primary, as Kerr has done in the case of the motor roots of Lepidosiren, or at least by not realizing that connections of that kind are established by the outflowing of the protoplasm of the neuroblasts, they have been advanced as evidence in support of the protoplasmic bridge theory. Held and Paton, in their recent work, have emphasized the differentiation phenomena, and have totally failed to recognize the importance of the protoplasmic movement which precedes. This is of course a natural consequence of relying entirely upon specific staining methods. Means of demonstrating neurofibrillae at the earliest possible moment were sought and found, and though undoubtedly one result has been a great advance in our knowledge of the processes of neurofibrillation, it is equally without doubt that another result has been the production of a one-sided view of the development of the nervous system. This is exemplified in the opening sentence (following the historical sketch) of Held's monograph where one reads[15] "Bei der Frage nach der Entwicklung des Nervengewebes handelt es sich um den Nachweis der ersten histologischen Characteristika des späterhin so eigentümlich ausgeprägten und im Tierkörper weit verbreiteten Nervengewebes" (meaning by this the neurofibrillar substance). Held, it is true, holds that the fibrillar substance is formed by the neuroblasts of His, and he describes the pushing out of the fibrillae from the center into the peripheral protoplasmic net work; he also enters upon a discussion of the influences which bring about the nervous connections found in the adult organism,[16] so that there is a close

[15] *Op. cit.*, p. 10.

[16] Held recognizes a number of principles in the outgrowth of the fibrillar substance which are closely similar to those stated by His and followed here, as, for instance, "das Prinzip der Achsenstellung" and "das Prinzip der Wegstrecke" (*op. cit.*, p. 278); and in his reference to chemotaxis (p. 148), and to the "Prinzip der Auswahl" (p. 270), Held's views lean strongly toward those of Ramon y Cajal. It must be borne in mind, however, that in all these cases Held refers solely to the movement of the *fibrillar substance* within the preformed reticular protoplasm, instead of recognizing that it is a mass of undifferentiated neuroblastic protoplasm that moves, and that this afterwards forms the neurofibrillae within itself by differentiation.

analogy between his view and the one advocated here. It is, however, the peculiar relation of the outgrowing nerve substance to the intercellular net work to which Held attaches the most fundamental importance, and it is in this respect, as comparison of his work with that of Ramon y Cajal shows, that he goes further than the methods he has employed would, in my opinion, warrant. What Held's figures really show, objectively expressed, is that the neurofibrillae are laid down within protoplasm and that they are always connected with the neuroblast, extending further peripherally in later than in earlier stages of development. This is in no wise incompatible with the results of my experiments, for the only difference concerns the source of the protoplasm in which the fibrillae develop, Held holding that it is formed of cells scattered all through the embryonic body, while I maintain on the basis of the experiments, that it flows out from the central cells, and thereby establishes the paths in which the neurofibrillae are formed. But it is this laying down of the primary nerve paths by means of a form of protoplasmic movement, rather than the process of neurofibrillation, that constitutes the specifically intricate problem in the development of the nervous system. The differentiation phenomena are naturally of great interest too, but though chemically specific, they are essentially of the same class as the differentiation phenomena witnessed, for example, in muscle or in the connective tissue cell; and they are not in any way comparable, as regards complexity of special relations, to the phenomena of the establishment of the primitive nervous connections by the outflow of the neural protoplasm. In order to discover the factors which influence the formation of the nerve paths, we must, therefore, in the first instance take into consideration this property of protoplasmic movement. This is of the utmost importance, and any theory of nerve development which fails to do so is sure to be misleading.

Analysis of the factors which produce the specific arrangement of the nerve paths

In proceeding with the analysis of our problem, the first point to consider is the localization of the energy which produces the outgrowth. The experiments answer this clearly: the primary act of extension by which the protoplasm of the neuroblast is drawn out into a fiber, the primordium of the axone, is due to forces immanent in the neuroblast itself at the time when outgrowth begins and probably for a considerable time before. This conclusion is based upon the direct observation that the movement takes place in the protoplasm itself without the application of any external physical force, and it is corroborated by the fact that outgrowth occurs even when the normal surroundings are radically modified, as in the present experiments or as Lewis ('07) and myself ('06, '10) have previously shown. That the original direction taken by the outgrowing fiber is already determined for each cell before the outgrowth actually begins, so that when it does begin it is dependent upon forces acting from within, follows first from the fact that the nerve fibers within the embryo tend to grow out in a given direction even when quite different surroundings are substituted for the normal, and secondly from the fact that the nerve fibers which grow into the clotted lymph, are there surrounded on all sides by an isotropic medium, which cannot conceivably be held to produce movement in a definite direction.

The formation of the protoplasmic nerve tracts falls, according to the foregoing, within Roux's definition of self-differentiation, by which is meant, not that the process is entirely independent of external conditions, but simply, as Roux ('85) in first defining the concept pointed out, that the changes in the system, or at least the specific nature of the change, are determined by the energy of the system itself.[17] In the particular case in question this means that within a medium compatible with the life and growth of the neuroblasts the formation of nerve fibers may take

[17] *Op cit.*, p. 423. In the "Gesammelte Abhandlungen," p. 15.

place without the application of any further external force either as stimulus or as motive power.[18]

The experiments indicate that some solid support is one of the essential conditions of growth, the fibrin threads apparently affording this support in the experiments with clotted blood and lymph. At least it has not been possible to induce growth in purely fluid media, and I am therefore inclined to hold to the hypothesis stated in the beginning (see p. 800), that some form of stereotropism plays a rôle in the outgrowth of the fibers, as Loeb

[18] Miss Shorey ('09) objects to calling this self-differentiation on the ground that the lymph used in the experiments contains the products of metabolism of various organs of the body, including organs such as muscles whose physiological activities are similar to those of the embryonic parts which the nerves in question would normally innervate; and these products of metabolism are the substances which, according to Miss Shorey, stimulate the growth of embryonic nerves. While it is of course true that lymph is a complex medium and may contain a variety of such products, it is nevertheless pretty hazardous, to say the least, to assume that they are the same in the lymph of an adult frog as in the interstices of a young embryo, and, in the absence of experimental evidence, it is entirely without foundation to assume that it is these particular products that stimulate the neuroblasts to grow out. In my opinion, the lymph is merely a medium in which these structures are capable of growing as a mouse grows in air or a fish in water. There is no specific relation between these media and the kind of organism that develops. Roux had such cases in mind in casting his definition of self-differentiation. Within the lymph the growing nerve fiber is bathed on all sides by the same medium and it is impossible therefore for the latter to exert any directive action. In interpreting her own striking and very important experiments Miss Shorey has ignored a number of facts, brought out by Lewis ('06 and '07) and myself (Harrison '06), which are opposed to her theory, as, for instance, the experiments in which part of the medullary tube was removed, with the result that the longitudinal fibers from the remaining part grew straight out into the mesenchmye. Since these fibers would normally have grown within the substance of the cord, and since, to carry out Miss Shorey's hypothesis to its logical conclusion, it must be supposed that that tissue emits some product of metabolism capable of stimulating the growth of the intrinsic nerves, we should consequently find in the experiments the self contradictory condition of nerve fibers growing directly away from that particular tissue, the remaining part of the neural tube, which alone should be stimulating growth toward it.

Miss Shorey's experiments show that the extirpation of peripheral material has a marked effect in partially inhibiting the development both of the nerves supplying it and of the central neuroblasts themselves. It is significant that in these experiments the effect of removal was found to be much more marked after a considerable time had elapsed, and also that in no case was the development of peripheral nerves and their neuroblasts entirely suppressed. This indicates that the

('98 and '07) has thought to be the case in the regenerative growth of epithelium. This, however, would not necessarily preclude the possibility of the bridging of very small spaces by the outgrowing fiber.[19]

There are cases which have been described where nerve fibers are found extending for considerable distances in the ventricular fluid, as for instance Reissner's fiber, and all those cases which Held refers to as developmental curiosities, but these do not contradict the view here advanced, for it is quite possible that the fibers originally grew along the surface of the lining of the neural tube. I am also of the opinion that the case described by me in which a nerve was found crossing the peritoneal cavity is not altogether against this view, because the attachment of the nerve was probably effected before the separation of the splanchnopleure and somatopleure took place.

Given a form of protolasm with power to extend itself in a definite direction so as to form a fiber, the next step is to determine the influences which may modify the direction of its growth and produce the specific arrangement of nerve tracts found in the mature organism. His ('88) was the first to show that developing nerves in the normal embryo begin their growth in a straight line, and that this direction may afterward be modified by various agencies such as the shifting of parts, or by meeting obstacles in the path of growth. The normal amphibian embryo affords abundant confirmation of these observations which His made upon the human embryo. The peripheral fibers from the dorsal nerves of Rohon-Beard, for instance, run at first laterally in a straight line through the notches between the muscle plates; they soon reach the epidermis and are there deflected through an arc of nearly 90°, to a dorso-ventral direction, with other minor deflections of a variable nature. In many cases it is apparent that the nerves follow definite paths, which are preformed in the sense

first nerves to grow did so independently of the peripheral conditions, as my own experiments show. The inhibition has affected principally the fibers which grow later, when distances are much greater and conditions much more complex; in other words, it has affected those fibers which grow in the period when differentiation is more dependent upon function, rather than those of the earlier period of self-differentiation.

[19] Cf. Ramon y Cajal, 1908.

that they are marked out by the configuration of other organs. Grooves or spaces between the more solid embryonic organs seem to be paths of predilection. Thus the dorsal nerves, just described, after leaving the medullary cord, run in the small spaces left between adjacent myotomes and the epidermis. Likewise the spinal nerves—at first only the motor constituents—run ventrally in the groove between successive myotomes on the inner side, to reach the extreme ventral part of the musculature. In these cases it does not seem necessary to assume that any special directive factors of a chemotactic nature play a part. However, we are far from being justified in generalizing too freely from the facts just stated, for while the nerves which have been mentioned apparently follow paths of low mechanical resistance, others again, grow where the resistance is probably considerable, as when the first fibers in the central nervous system bore their way through the solid ependyma cells.[20]

A striking feature of the development of the peripheral nervous system is the fact that the principal nerve paths are laid down very early. In the frog the main branches of the cranial nerves, the sensory spinal nerves from the dorsal cells, and the motor spinal nerves are all formed within two or three days of the closure of the medullary folds, a considerable time before the complete absorption of the yolk. The sensory nerves from the spinal ganglia follow the above named nerves after an interval of a day or two. The point to be emphasized in this connection is that none of the peripheral nerves have very great distances to grow before connecting with their proper end organs.[21] The . *ophthalmicus*

[20] Harrison, '01.

[21] In criticising a previous statement to this effect Hensen ('08) expressed himself as follows: "Ich glaube mich richtig auszudrücken, wenn ich sage: es ist kümmerlich sich damit helfen zu wollen dass *nur kurze* Wegstrecken (Wie kurz doch wohl?) zu durchwachsen sind. Der Zusammenhang muss *zwangsmässig* gesichert sein." When we consider that, as the present experiments show, nerve fibers have power of independent growth of over a millimeter, that there are obviously conditions in the embryo which may direct this growth for distances of that magnitude, and that scarcely any peripheral nerves have normally much greater distances to grow, then it does not seem to me to be either futile or preposterous to assume that such conditions are sufficient to conduct the developing fiber to its proper end organ.

grows out from the trigeminal ganglion across the optic stalk toward the skin of the front part of the head, spreading out in the region above and in front of the eye and above the nose. The *r. lateralis vagi* connects almost immediately with the rudiment of the lateral line organs and is drawn out as the latter extends towards the tail. The outgrowth of the nerve fibers from the dorsal cells has just been described, and as pointed out, the definitive arrangement can be accounted for by power of growth in a straight line modified by deflection as a result of minor obstacles in the path. The ventral branches of the spinal nerves reach the grooves between successive myotomes and pass ventrally in them to the ventral border of the muscles. As the latter are carried in mass to near the ventral median line the nerves are elongated. These nerves, like the others referred to, have no great distance to grow, and one of the guiding factors, the myosepta, is obvious. The formation of the limb plexuses, comes about by slight deflections from the main path of growth. The nerves to the limbs reach the base of the limb buds in the same manner as the ventral nerves reach the abdominal musculature, *i.e.*, along the grooves on the inner surface of the muscle plates. The nerves are accordingly present in the limbs practically from the time when the latter begin their development, and as the limbs grow the nerves lengthen with them.[22] Thus the principal paths are all at first relatively short and subsequently become lengthened by the shifting of parts which takes place during the development and growth of the organism.

It is obvious that the primitive peripheral nerves, which are laid down in early embryonic life, consist of but very few fibers—in the frog often of not more than two or three at first. These first fibers may be called the path-finders; the remaining ones fol-

[22] Regarding the period of development at which the nerves reach the limb buds, my own observations ('07 a) differ from those of Braus ('05). In referring to this discrepancy I failed to consider that it might be due to specific differences between Rana and Bufo on the one hand and Bombinator on the other, instead of to errors of observation, as implied in my criticism. Braus has since called my attention to these specific differences, and I am glad to have this opportunity to express my regret at having overlooked this reasonable explanation of the difference in our observations.

low them little by little. Those that develop later, after the growth and shifting of the various parts of the organism has taken place, have much longer distances to grow, but the paths are already laid down by the pioneers and the later ones have only to follow where the others have led.

Plexus formation by outgrowth is admirably illustrated in the nerves arising from the dorsal cells. These fibers begin their growth in a direction perpendicular to the axis of the body, and run intersegmentally approximately parallel to one another, being directed at first from the neural tube towards the epidermis (figs. 1 and 3). Reaching the latter they begin to branch (fig. 6), though not perfectly regularly, owing to slight variations either in the nerve protoplasm itself or in the pathway. In branching, a small deviation from the purely transverse direction of outgrowth takes place, and since the nerves run along the inner surface of the epidermis, and in fact are squeezed in between this layer and the muscle plates, the branches from adjacent segmental nerves must soon come together, and cross one another or form anastomoses. This last stage, which is not figured here from an actual specimen, may be readily observed in parasagittal sections taken just under the skin of Rana embryos about 6–7 mm. in length. It has been possible to observe the like of these processes also in the live specimens in lymph; for instance, the formation of branches, the crossing of two fibers growing in different directions, and the fusion or intimate contact (anastomosis) between separate fibers that happen to come together. The diagram on p. 798 (text fig. 1) illustrates the process; the early stages are shown in figs. A and B, and the completion of the plexus in fig. C. This outline of development will account quite satisfactorily for the general features of a cutaneous plexus, *i.e.*, definite areas for each segmental nerve, a considerable overlapping of one segment of distribution upon adjacent ones, and minor irregularities in the mode of branching and anastomosis. A further feature, the oblique course of the nerve trunks in the lower part of the body and the tail, while perhaps in part due to the original direction of outgrowth, is largely brought about by the general shifting of the epidermis over underlying organs, which

takes place during development after the first connections between the nerve fibers and the epidermal cells have been established.

There is nothing in the present work which throws any light upon the process by which the final connection between the nerve fiber and its end organ is established. That it must be a sort of specific reaction between each kind of nerve fiber and the particular structure to be innervated seems clear from the fact that sensory and motor fibers, though running close together in the same bundle, nevertheless form proper peripheral connections, the one with the epidermis and the other with the muscle. That the connection is not long deferred is shown in a large number of instances where the nerves reach their end structures, and function is established very early in development. The foregoing facts suggest that there may be a certain analogy here with the union of egg and sperm cell. The nerve fiber during its growth comes into contact with a cell of the proper kind. Assuming the latter to be in a condition of ripeness, a more intimate contact or perhaps even actual fusion may take place between nerve twig and end cell. A connection of this kind once established would terminate the susceptibility of the cell to further innervation, and nerve fibers growing subsequently in the same path would pass along to other end cells which were mature but not yet innervated. The nerve fiber itself, however, apparently retains its power of growth and ramification, for it usually becomes connected finally with a large number of end cells, as is plainly the case with muscle and ordinary cutaneous endings. It is in the establishment of the definitive connection with end organ rather than in the determination of the direction taken by the main nerve trunks, that influences such as chemotaxis may be expected to operate.

The present experiments suggest, of course, a possible method for further study of this problem. If it could be shown that there is an attraction between growing nerve fibers taken from a certain part of the nervous system and a particular kind of peripheral cell, and between another type of central neuroblast and a different peripheral cell, then we should have direct evidence for the existence of those more subtile factors which seem to

be necessary to account for the definitive establishment of particular nervous connections. The few experiments which I have directed to this end have given negative results, which is not surprising when the crudities of the method are borne in mind, but since it is possible to introduce many refinements into these methods, an ultimate solution of the problem in this way does not seem to be beyond hope of attainment.

The specific arrangement of the fibers within the central nervous system affords a morphogenetic problem of much greater difficulty. Still there is nothing in the conditions in the walls of the neural tube which is inconsistent with the development of the nerve fibers in accordance with the view here represented. The growing fibers are clearly endowed with considerable energy and have the power to make their way through the solid or semi-solid protoplasm of the cells of the neural tube. But we are at present in the dark with regard to the conditions which guide them to specific points.

In pointing out the above factors which seem to be involved in the development of the nervous system, I am aware of the great imperfections in our knowledge of the subject, and of the little progress that has been made beyond the ideas of His and Ramon y Cajal. Nevertheless, although our present conception of the secondary factors which influence the nerve paths may have to be modified in the light of future knowledge, the primary factor, protoplasmic movement, must be regarded as definitely established and it will have to form the basis of any adequate theory of nerve development. The chief claim to progress that the present work has is that it has taken this factor out of the realm of inference and placed it upon the secure foundation of direct observation. With this it has been shown that the first manifestations of activity observable in the differentiating nerve cell are of the same fundamental nature as those found not only in other embryonic cells but also in the protoplasm of the widest variety of organisms. The movement which results in the drawing out of a compact cell into a long filament, the primitive nerve fiber, it is but a specific form of that general type of movement common to all primitive protoplasm. In studying the secondary

factors which influence the laying down of the specific nerve paths of any organism, we are concerned, therefore, primarily with the laws which govern the direction and intensity of protoplasmic movement, and it is the analysis of these phenomena to which students of the ontogenetic and regenerative development of the nervous system must now direct their attention. The present discussion will not have been in vain if it makes clear that the development of the nervous system in the light of the protoplasmic movement concept is no less capable of rational analysis than is development in general.

SUMMARY

Reference is made throughout the following exclusively to the anouran embryo.

Before histological differentiation of the medullary tube begins, its walls do not constitute a syncytium, but are composed of separate cells each with a distinct cell membrane, as freshly teased preparations show.

The peripheral nerve fibers in their earliest stages, as seen in sections of normal embryos, extend from the neural tube or cranial ganglia as finely branched processes of single cells, which in slightly later stages become extended to long fibers; the end of each fiber is a rhizopod-like structure with very fine processes or pseudopodia.

Pieces of undifferentiated embryonic tissue, when isolated under aseptic precautions in clotted lymph, will live for weeks and undergo at least the initial stages of normal histological differentiation: cells from the axial mesoderm give rise to striated muscle fibers; epidermal cells form a cuticular border; typical chromatophores and a mesenchyme-like tissue are formed from pieces containing portions of the neural tube and axial mesoderm; the walls of the neural tube and the primordia of the cranial ganglia give rise to long hyaline filaments closely resembling embryonic nerve fibers.

Tissues grown in lymph function characteristically, as is seen in the movement of cilia and in the contraction of muscle fibers when left in organic continuity with fragments of the neural tube.

One characteristic that the embryonic cells have in common, is the power of movement. They change their form or move from place to place in the clot by virtue of the amoeboid activity of their hyaline ectoplasm. The amount of activity and its result vary according to the tissue, cells from the nervous system and the mesoderm being most active, while those of the endoderm and notochord are most inert.

In the case of cells from the medullary tube and the primordia of the cranial ganglia the activity is so localized and the ductility of the ectoplasm is such, that the movement results in the formation of long fibers, the primitive axones. The free end of each fiber is enlarged and provided with fine processes or pseudopodia. This part continues its progression and the fiber is gradually drawn out.

The rate of progression (lengthening of the fiber) varies considerably, the extremes observed being 15.6μ per hour (100μ in 6 hours 25 minutes) and 56μ per hour (44μ in 47 minutes).

The longest fiber observed, and this was followed throughout its whole period of growth (53 hours), was 1.15 mm. long.

The nerve fibers take origin usually from masses of cells which are so opaque that their mode of connection with the cells cannot be made out, but in a considerable number of cases the fibers were seen to originate in single isolated cells, the longest one of this kind having had a total length of 631μ and having had several long branches.

In many cases anastomoses have been found between fibers. These have been observed to form through secondary fusion, but two threads on coming together do not necessarily fuse, and anastomoses already formed may be resolved later.

The experiments show that neuroblasts are competent to form primitive nerve fibers within a foreign unorganized medium simply by the amoeboid outgrowth of their protoplasm. By eliminating from the periphery all formed structures which have heretofore been supposed to transform themselves into nerve fibers and leaving only the neuroblasts in the field, it is demonstrated that the latter are the sole elements essential to the formation of nerves. The concepts of both Hensen and Held are rendered untenable.

Taken together with recent histogenetic studies, the experiments show that two elementary phenomena are involved in nerve development: (a) the formation of the primitive nerve fiber through extension of the neuroblastic protoplasm into a filament —protoplasmic movement; (b) the formation of the neurofibrillae within this filament—tissue differentiation.

It is through the former that the specific nerve paths of the body are first laid down. The further analysis of the influences which determine these paths can be made only through the study of the laws which govern protoplasmic movement.

The energy of outgrowth is immanent in the nerve cell, and the initial direction of outgrowth is already determined within the cell before the outgrowth actually begins. The formation of the fiber is therefore an act of self differentiation within Roux's definition.

One of the necessary conditions of outgrowth is in all probability a medium which affords some solid support to the fibers.

The configuration of the various organs of the embryo affords certain paths of predilection, such as small channels or grooves, in which nerve fibers are found to grow. These factors, together with the predetermination of the initial directions of outgrowth within the cell and the motive force of the neuroblastic protoplasm itself, will account for the main features in the topography of the peripheral nervous system.

The first nerves which form are composed of few fibers and have relatively short distances to grow before establishing connection with their end organs. The long paths found in the adult are largely the result of subsequent stretching or interstitial expansion, which takes place as the various parts grow or shift apart. The fibers which develop later follow, in the main, the paths laid down by the pioneers.

The mechanism by which the proper connection between nerve fiber and end organ is brought about is not revealed by the experiments, though a certain analogy with the penetration of the egg by the sperm is suggested.

REFERENCES

APÁTHY, S. VON. 1897 Das leitende Element des Nervensystems und seine topographischen Beziehungen zu den Zellen. Mitt. a. d. Zool. Station z. Neapel, Bd. 12.

BARFURTH, D. 1891 Zur Regeneration der Gewebe. Arch. f. mikr. Anat., Bd. 37.

BORN, G. 1896-97 Über Verwachsungsversuche mit Amphibien-Larven. Arch. f. Entw.-Mech., Bd. 4.

BRAUS, HERMANN. 1904 Einige Ergebnisse der Transplantation von Organanlagen bei Bombinatorlarven. Verh. d. Anat. Gesell. 18. Versamm. in Jena.

1905 Experimentelle Beiträge zur Frage nach der Entwickelung peripherer Nerven. Anat. Anz., Bd. 26.

CAJAL, S. RAMON Y. 1890 À quelle époque apparaissent les expansions des cellules nerveuses de la moëlle épinière des poulets. Anat. Anz., Bd. 5.

1892 La Rétine des Vertébrés. La Cellule, Tome 9.

1899 Textura del sistema nervioso del hombre y de los vertebrados. Tomo I. Madrid.

1907 Die histogenetischen Beweise der Neuronentheorie von His und FOREL. Anat. Anz., Bd. 30.

1908 Nouvelles observations sur l'évolution des neuroblastes, avec quelques remarques sur l'hypothèse neurogénétique de Hensen-Held. Anat. Anz., Bd. 32.

CLARK, ELIOT R. 1909 Observations on living growing lymphatics in the tail of the frog larva. Anat. Rec., vol. 3.

EYCLESHYMER, ALBERT C. 1907 The closing of wounds in the larval Necturus Am. Jour. Anat., vol. 7.

FISCHER, ALFRED. 1901 Ueber Protoplasmastruktur, Antwort an O. Bütschli-Arch. f. Entw.-Mech., Bd. 13.

HARRISON, ROSS GRANVILLE. 1901 Ueber die Histogenese des peripheren Nervensystems bei Salmo salar. Arch. f. mikr. Anat., Bd. 57.

1903 Experimentelle Untersuchungen über die Entwicklung der Sinnesorgane der Seitenlinie bei den Amphibien. Arch. f. mikr. Anat., Bd. 63.

1904 Experimental study of the relations of the nervous system to the developing musculature in the embryo of the frog. Amer. Jour. Anat., vol. 3.

1904 a Neue Versuche und Beobachtungen über die Entwicklung der peripherern Nerven der Wirteltiere. Sitz.-Ber. der Niederrh. Ges. f. Nat. u. Heilkunde zu Bonn.

1906 Further experiments on the development of peripheral nerves. Amer. Jour. Anat., vol. 5.

1907 Experiments in transplanting limbs and their bearing upon the problems of the development of nerves. Jour. Exp. Zoöl., vol. 4.

1907 a Observations on the living developing nerve fiber. Proc. Soc. Exp. Biol. and Med., vol. 3. Also in Anat. Rec., vol. 1.

1908 Embryonic transplantation and the development of the nervous system. Anat. Rec., vol. 3.

1910 The development of peripheral nerve fibers in altered surroundings. Arch. f. Entw.-Mech., Bd. 30.

HELD, HANS. 1906 Zur Histogenese der Nervenleitung. Verh. d. Anatom-Gesellsch. 20 Versamm. in Rostock.

1907 Kritische Bemerkungen zu der Verteidigung der Neuroblas. ten- und Neuronenlehre durch R. Cajal. Anat. Anz., Bd. 30.

1909 Die Entwicklung des Nervegewebes bei den Wirteltieren. Leipzig. Joh. Ambr. Barth.

HENSEN. V. 1864 Ueber die Entwickelung des Gewebes und der Nerven im Schwanze der Froschlarve, Virchow's Archiv., Bd. 31.

1868 Ueber die Nerven im Schwanz der Froschlarven. Arch. f. mikr. Anat., Bd. 4.

1876 Beobachtungen über die Befruchtung und Entwicklung des Kaninchens und Meerschweinchens. Zeitschr. f. Anat. u. Entwick., Bd. 1.

1903 Die Entwickelungsmechanik der Nervenbahnen im Embryo der Säugetiere. Kiel u. Leipzig.

1908 Über das Auswachsen der Nerven im Embryo. Münch. Med. Woch., 55 Jahrg.

HIS, W. 1886 Zur Geschichte des menschlichen Rückenmarks und der Nervenwurzeln. Abh. d. math.-phys. Kl. d. Kgl. Sächs. Ges. d. Wiss. Bd. 13.

1887 Die Entwickelung der ersten Nervenbahnen beim menschlichen Embryo. Arch. f. Anat. u. Physiol. Anat Abtheil.

1888 Zur Geschichte des Gehirns sowie der centralen und der peripherischen Nervenbahnen beim menschlichen Embryo. Abh. d. math.-phys. Kl. d. Kgl. Sächs. Ges. d. Wiss., Bd. 14.

1889 Die Neuroblasten und deren Entstehung im embryonalen Mark. Arch. f. Anat. u. Physiol. anat. Abtheil.

1890. Histogenese und Zusammenhang der Nervenelemente. Arch. f. Anat. u. Physiol. anat. Abtheil.

KERR, J. GRAHAM. 1904 On some points in the early development of motor nerve trunks and myotomes in Lepidosiren paradoxa. Trans. Roy. Soc. Edinburgh., vol. 41.

1910 The development of the peripheral nerves of vertebrates. Presidential Address. Proc. Roy. Phys. Soc. Edinburgh, vol. 18.

KAPPERS, C. U. ARIËNS. 1908 Weitere Mitteilungen über Neurobiotaxis. Folia Neuro-Biologica., Bd. 1.

LEWIS, WARREN HARMON. 1906 Experimental evidence in support of the outgrowth theory of the axis cylinder. Proc. Am. Ass. Anat., Ann Arbor, December 1905. Am. Jour. Anat., vol. 5.

1907 Experimental evidence in support of the theory of outgrowth of the axis cylinder. Am. Journ. Anat., vol. 6.

1898 Ueber Regeneration des Epithels. Arch. f. Entw.-Mech. Bd. 6.

LOEB, LEO. 1902 Ueber das Wachstum des Epithels. Arch. f. Entw.-Mech., Bd. 13.

1907 Ueber einige Probleme der experimentellen Tumorforschung. Zeitschr. f. Krebsforschung. Bd. 5.

PATON, STEWART. 1907 The reactions of the vertebrate embryo to stimulation and the associated changes in the nervous system. Mitt. ad. Zool. Station z. Neapel, Bd. 18.

PETERS, A. 1885 Ueber die Regeneration des Epithels der Cornea. Inaug. Diss., Bonn.

1889 Ueber die Regeneration des Endothels der Cornea. Arch. f. mikr. Anat., Bd. 33.

ROUX, WILHELM. 1885 Beiträge zur Entwickelungsmechanik des Embryo. I. Zur Orientierung über einige Probleme der embryonalen Entwickelung. Zeitschr. f. Biologie. Bd. 21. Also in "Gesammelte Abhandlungen" (No. 13), Bd. 2.

SCAHEPPI, TH. 1909 Kritische Bemerkungen zur Frage nach der Entstehung der Nerven. Anat., Anz. Bd. 35.

SCHIEFFERDECKER, P. 1906 Neurone und Neuronenbahnen. Leipzig, Joh. Ambr. Barth.

SCHULTZE, OSKAR 1904 Über Stückfärbung mit Chromhaematoxylin. Zeitschr. f. Wiss. Mikroskopie., Bd. 21.

1905 Beiträge zur Histogenese des Nervensystems. I. Über die multizelluläre Entstehung der peripheren sensiblen Nervenfasern und das Vorhandensein eines allgemeinen Endnetzes sensibler Neuroblasten bei Amphibienlarven. Arch. f, mikr. Anat., Bd. 66.

1908 Zur Histogenese des Nervensystems. Sitz. Ber. d. Kgl.. Pr. Akad. d. Wiss., Bd. 6.

SHOREY, M. LOUISE. 1909 The effect of the destruction of peripheral areas on the differentiation of the neuroblasts. Jour. Exp. Zoöl., vol. 7.

WEYSSE, ARTHUR W., AND WALDO S. BURGESS 1906 Histogenesis of the retina. The American Naturalist., vol. 40.

EXPLANATION OF PLATES

ABBREVIATIONS WHICH APPLY TO ALL FIGURES

chr isolated pigment cell;
ct embryonic cell exhibiting independent movement in the lymph;
cut cuticular border of epidermal cells;
ep epidermis;
ep.c epidermal cells in lymph preparation;
ery red blood corpuscle;
fen opening found in mass of transplanted tissue;
fil hyaline protoplasmic filament stretching between two cells or cell masses;
gn ganglion;
l thin layer of cells formed by spreading of transplanted tissue;
mc medullary cord;
mes mesenchyme;
ms main mass of transplanted tissue;
my myotome, the suffix denoting the serial number of the segment;
nbl neuroblast;
nf nerve fiber;
npl protoplasmic end of growing nerve;
pl.fr hyaline protoplasmic fringe, often seen in transplanted epidermis;
thr fibrin threads.

PLATE 1

EXPLANATION OF FIGURES

All figures drawn with camera lucida from sections of normal embryos.

1 Part of a frontal section of an embryo of R. palustris, 3.6 mm. long. (Series 5b, Row 3, Section 7.) The section is taken through the dorsal half of the medullary cord at the level of the dorsal cells of Rohon-Beard. One of these cells is shown sending out a branched process (*nf*) into the notch between the ninth and tenth myotomes (my_9 and my_{10}). × 465.

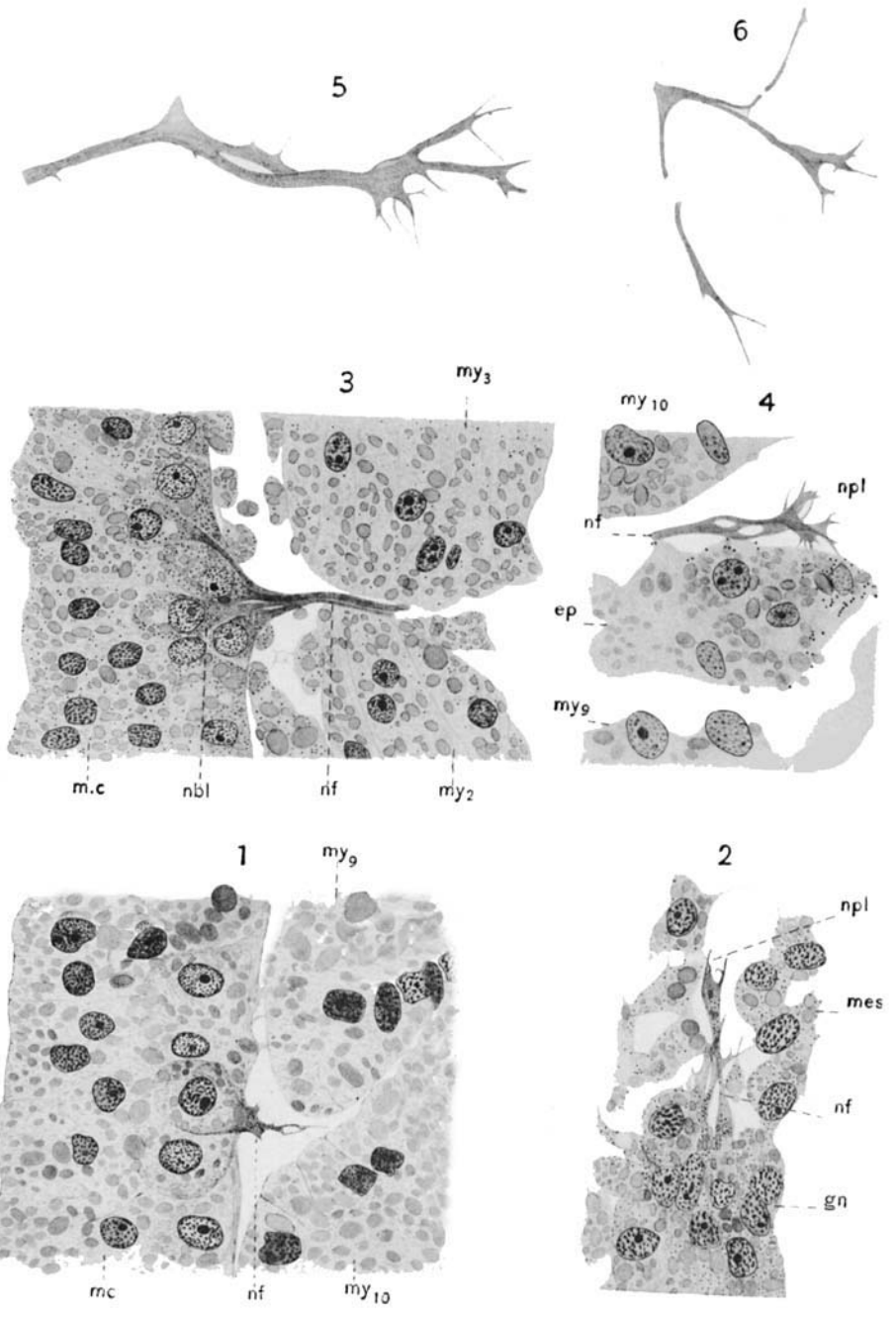
2 Part of a frontal section through the head region of an embryo of R. esculenta, 3.0 mm. long. (Control Experiment Y, 18 hours, Row 2, Section 1.) The figure shows a portion of the trigeminus ganglion (*gn*) with the fibers of the r. ophthalmicus (*nf*) just beginning to sprout. × 465.

3 Part of a frontal section through an embryo of R. palustris, 4.2 mm. long. (Series 6b, Row 4, Section 2.) The section is taken at the same level as that shown in fig. 1. Four dorsal neuroblasts (*nbl*) send out long nerve fibers (*nf*) between two myotomes (my_2 and my_3). × 465.

4 Part of a sagittal section through an embryo of R. pipiens (virescens), 4 mm. long. (Series 7c, Row 5, Section 14.) The end of one of the dorsal nerves consisting of several fibers is shown. The nerve ending occupies the space between two myotomes (my_9 and my_{10}) and a thick ridge of the epidermis (*ep*). × 465.

5 End of a similar nerve fiber, from another section of same series as fig. 4. Taken between my_{13} and my_{14}, Row 3, Section 10. × 930.

6 Branching nerve between myotomes 4 and 5 from a section of the same embryo as figs. 4 and 5. The drawing, as indicated by breaks in the fibers, is combined from two successive sections. (Row 5, Sections 12 and 13.) × 465.



R. G. Harrison, del.

PLATE 2

EXPLANATION OF FIGURES

All figures except figs. 12 and 15 were drawn from camera lucida sketches of living specimens of embryonic tissue isolated in clotted lymph.

7–11 Five views of the same group of nerve fibers made at different times (experiment Is, 137), medullary cord tissue from R. palustris, 3.3 mm. long, lymph from R. pipiens (the interval between the first and last figure represents 34 hours). × 350.

7 Apparently single fiber (*nf*) growing out from a pointed cell (ct_1) which projects from a mass of cells (*ms*) one day after isolation of tissue. April 28, 1908, 12.25 p.m.

8 Same fiber, 2 p.m. Fiber is now clearly double.

9 Same group of fibers. 10.25 p.m. Four distinct fibers (nf_1–nf_4) are now visible. The fibrin filaments (*thr*) shown in this figure were present in the earlier stages but were omitted from the original sketches.

10 Same group. April 29, 11 a.m. nf_3 possibly a branch of nf_2.

11 Same group. 10.30 p.m. Continuation of nf_1 and upper branch of nf_2, unfortunately left out of sketch. Note migration of cell (ct_2). Identity of other isolated cells in figs. 10 and 11 is uncertain.

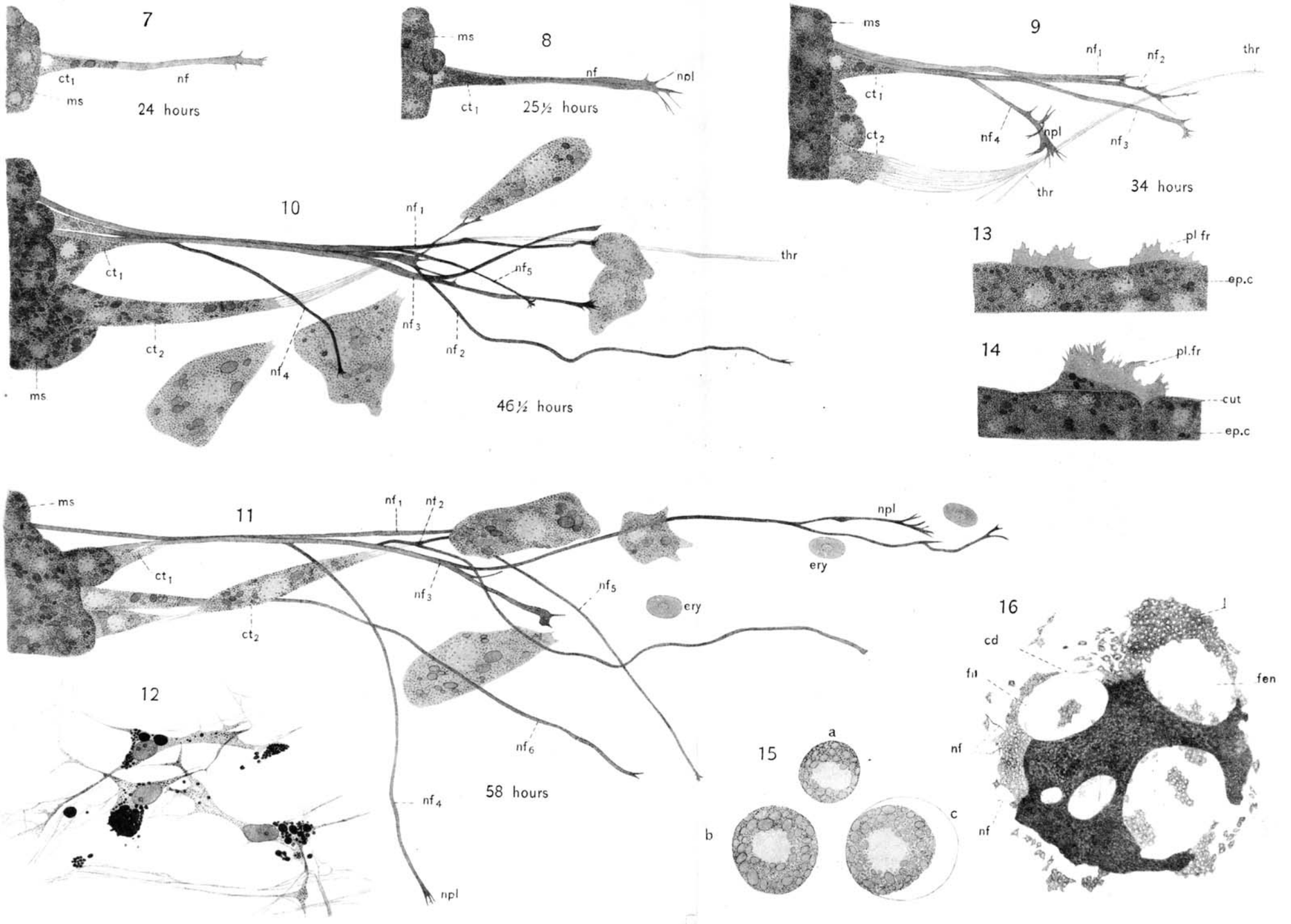
12 Three cells from a specimen preserved five days after isolation. Osmic vapor followed by Tellyesniczky's fluid, stained in O. Schultze's haematoxylin. The cells have much branched processes which end indefinitely in the coagulum which pervades whole specimen. Many isolated cells of this kind are in the specimen, which is quite typical. Experiment Is, 157, medullary cord from R. palustris embryo; R. clamitans lymph. × 350.

13 Row of ectoderm cells from the abdominal region, showing fringe of amoeboid hyaline protoplasm (*pl.fr.*). Experiment Is, 75, two days after isolation. Tissue from palustris embryo in palustris lymph. × 350.

14 Similar specimen. In this case the ectoderm cells, which are taken from the branchial region, show the cuticular seam (*cut*). The hyaline fringe (*pl.fr.*) belongs to cells lying below the main row. Experiment Is, 87, one day after isolation. Tissue from palustris embryo in palustris lymph. × 350.

15 Three cells from the medullary cord of frog embryos about 3.3 mm. long, in which the medullary folds had closed and the tail bud was just beginning to appear, prepared from the living specimens; *a* and *b* taken from an embryo of R. sylvatica, dissected and examined in 0.2 per cent NaCl; *c* taken from an embryo of R. palustris, examined in tap water. The latter cell (*c*) has imbibed water and the cell membrane is very distinct at one side. Nucleus shows as a clear space in each cell. × 350.

16 Whole piece of tissue (medullary cord, with small portions of muscle plates attached) isolated in lymph, two days after preparation. The dark area represents a thick opaque mass of tissue. Thin sheets of cells (*l*) and isolated cells are shown on all sides. *nf*, nerve fibers projecting out into lymph from under the masses of cells. *fil*, threads of hyaline protoplasm bridging spaces between masses of cells. *cd*, band of cells in single file. Experiment Is, 124. Tissue from pipiens embryo, lymph undetermined. × 32.



R. G. Harrison, del.

PLATE 3

EXPLANATION OF FIGURES

All figures were drawn from camera lucida sketches of the living specimens isolated in clotted lymph.

17 Plexus of nerve fibers growing out from a mass of transplanted medullary cord. Experiment Is, 124. Two days after operation. Pipiens tissue, lymph undetermined. × 350.

18 Bipolar cell with protoplasmic processes. *a*, Free end of process; *b*, process connecting with mass of cells not shown in figure. Length 300 μ. Fiber probably formed through movement of cell. Experiment Is, 124, four days after isolation of tissue. Pipiens tissue in undetermined lymph. × 350.

19 Bipolar cell and protoplasmic fiber. In this case the fibers were both stretched between two groups of cells and may have been formed by drawing apart. No free ends were visible. Experiment Is, 87, two days after isolation. Tissue from branchial region of embryo. Embryo and lymph R. palustris. × 350.

20 Long nerve fiber arising from unipolar cell (*ct*) at edge of group of cells (*ms*). Experiment Is, 124. Tissue from medullary cord, three days after isolation. Pipiens tissue, undetermined lymph. × 350.

21 Isolated unipolar nerve cell with long bifurcated nerve filament. Tissue from R. palustris in lymph from R. pipiens. Experiment Is, 137. Seen at 4 p.m., two days after isolation. × 350.

22 Same cell as in fig. 21, as seen at 8.45 p.m. ($4\frac{3}{4}$ hours later).

23 Two cells from a preparation of medullary cord. Experiment Is, 153, three days after isolation. Tissue from R. palustris, lymph from R. clamitans. These forms are typical of the isolated cells found in the majority of the preparations. × 350.

24 Two pigment cells from a preparation of medullary cord, including some mesodermic tissue, thirteen days after isolation. Experiment Is, 133. Tissue and lymph R. pipiens. × 180.

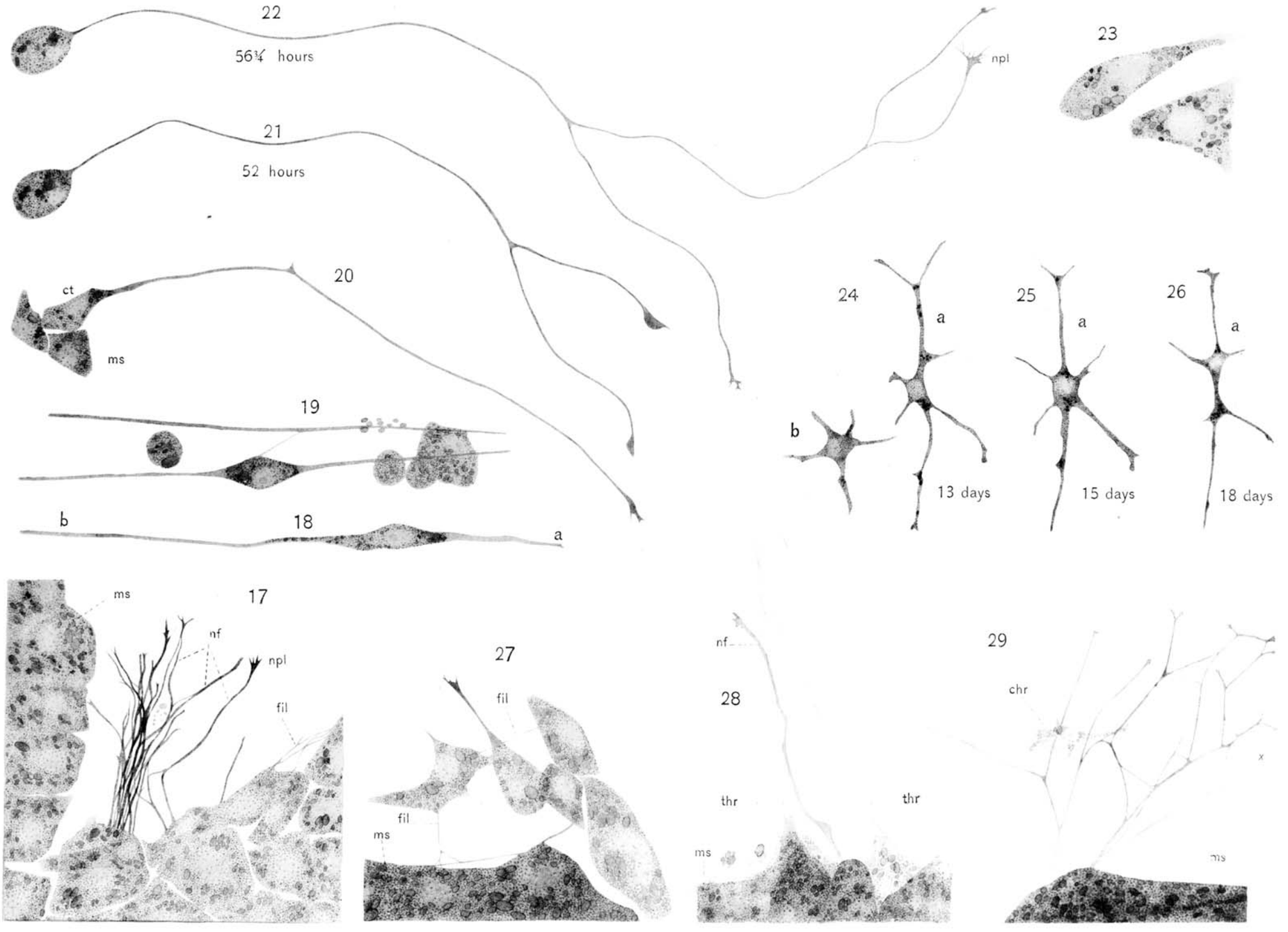
25 Same cell as *a* of fig. 24, fifteen days after isolation. × 180.

26 Same cell as *a* of fig. 24, eighteen days after isolation. × 180.

27 Group of cells from medullary cord showing protoplasmic processes and connecting threads. Experiment Is, 124. Three days after isolation. Tissue R. pipiens, lymph undetermined. × 350.

28 Nerve fiber (*nf*) extending out from mass of cells (*ms*) to show contrast with fibrin thread (*thr*). The fibrin shown is attached to the ectoplasm of several cells upon which it apparently exerts considerable tension. Experiment Is, 69, three days after isolation. The outgrowth of the nerve fiber was observed the previous day. It now shows signs of incipient degeneration. Tissue and lymph from R. palustris. × 350.

29 Plexus of nerve fibers arising from a mass of cells taken from medullary cord. The anastomoses were not permanent. The one at *x* was seen to separate, and the day following, nearly all had been resolved. *Chr*, pigment cell. Experiment Is, 200, three days after isolation. Tissue and lymph R. pipiens. × 200.



R. G. Harrison, del.