

THE GROWTH AND HISTOGENESIS OF THE CEREBRO-SPINAL NERVES IN MAMMALS.

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WITH 15 TEXT FIGURES.

In the following article are given the results of a study of the histogenesis and the general mode of growth of the cerebro-spinal nerves in mammals. Owing to the ease with which the material could be obtained pig-embryos have been those chiefly used, but in addition embryos of man, guinea-pigs and mice have been studied. In all of these animals the development of the nerves seems to be essentially similar.¹

I. OUTGROWTH OF THE CEREBRO-SPINAL NERVES.

In the mammals the development of the peripheral nerves, with the exception of the optic and olfactory, begins by an outgrowth of naked processes from cells lying in the motor root-zone of the central nervous system and in the sensory ganglia. The naked processes belonging to a given cerebro-spinal nerve are usually grouped in bundles which extend out separately into the mesenchyme surrounding the central nervous system but which soon are collected into a common nerve-trunk. This method of development has been clearly described by His in the human embryo, and can readily be verified in any of the mammals more commonly studied. If a pig embryo 8 mm. long, for instance, be fixed in Zenker's fluid and hardened in alcohol, the thoracic nerves may readily be dissected out. At this period bundles of fibres from the motor-root zone and from the spinal ganglion of each thoracic segment have become grouped into a spinal nerve which extends towards but does not enter the body-wall. One of these nerves, with its sensory-root and ganglion, motor-root, and main-trunk intact, may be isolated, together with a slight amount of the surrounding mesenchyme, stained in Dela-

¹The literature on the general subject of the development of the peripheral nerves in vertebrates has been recently reviewed by Harrison, Dohrn, Fürbringer and Nussbaum, each from a different standpoint.

field's hæmatoxylin, followed by Congo-red, dehydrated, cleared, and then mounted in balsam. Clearer pictures may thus be obtained in early embryos than by the osmic-acid, picro-carminé method used by Vignal.

In a specimen thus prepared (Fig. 1), the bundles of nerve-fibres may be seen surrounded by branched, anastomosing mesenchyme-cells. Here and there an elongated cell may be seen closely applied to a bundle of fibres, but special sheath-cells of this kind are infrequent. In places single fibres or small bundles consisting of two or three fibres entirely free from sheath-cells may be followed for nearly half a millimeter. After teasing the tissue of the spinal ganglion or that from the ventral

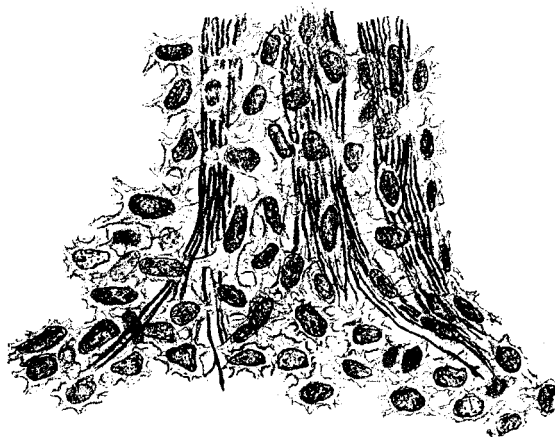


FIG. 1. Portion of the tip of an intercostal nerve isolated from a pig-embryo 8 mm. long. 720 diam.

root-zone of the spinal cord, cells may be seen which give rise directly to the nerve-fibres, but no cells of this kind are to be found among the cells accompanying the bundles of nerve-fibres constituting the nerve-trunk.

As the nerve grows forward new nerve-fibres grow in rapidly from behind, and the nerve-fibres as they grow forward give rise to groups of fine fibrils.

The cells found scattered among the nerve-fibres and nerve-fibrils multiply actively. As the new cells are formed certain of them give rise to a skeletal framework for the support of the nerve-fibrils. The periphery of the nerve, also, at an early period becomes covered with a fairly complete membrane formed of anastomosing cells. The cells within the nerve give rise to branched processes which anastomose with

one another and with the peripheral layer of cells. In the meshes of this framework the bundles of nerve-fibrils run.

In Fig. 2 is shown the extremity of the ventral division of a spinal nerve of a pig 10 mm. long. This nerve was isolated and stained by the method described above. The advancing tip of the nerve is com-

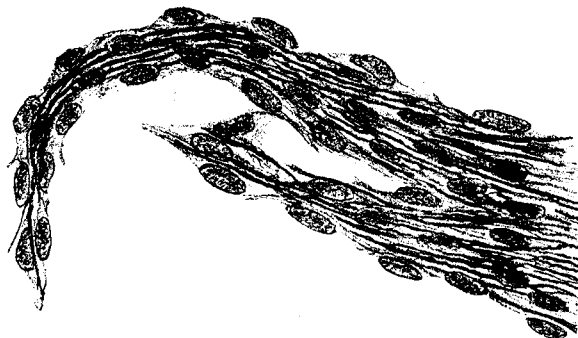


FIG. 2. Nerve-tip isolated from an intercostal nerve of a pig-embryo 10 mm. long. 720 diam.

posed of a few fibrils ensheathed by two cells. Behind this the nerve-fibrils and the sheath-cells rapidly increase in number. A short distance behind the tip the nerve has been partially split during the process of isolation. The curving of the tip is due to mounting.

The various constituent elements of the nerve may be isolated by teasing. The cells applied to the more compact bundles of fibrils present the appearance shown in Fig. 3, *a*. Fig. 3, *b*, shows a cell in the process of division; Fig. 3, *c*, two cells with a nerve-fibre passing between them; and Fig. 3, *d*, two fibres and two cells from the extreme tip of a growing nerve. With care it is possible to isolate nerve-fibres or bundles of fibrils free from any cellular covering for considerable distances. No nerve-fibres can be seen arising from

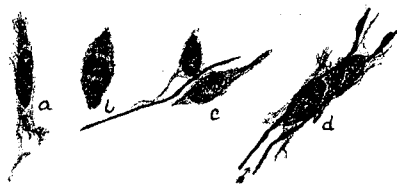


FIG. 3. Elements isolated from the ventral trunk of a spinal nerve of a pig-embryo 11 mm. long. 720 diam.

cells within the nerves. The cells are, however, sometimes so closely applied to small groups of fibrils as to give rise to the appearance of protoplasmic continuity between sheath-cells and nerve-fibrils. This is especially apt to be the case in longitudinal sections.

Fig. 4 shows a cross-section of the median dorsal branch of a thoracic nerve of an embryo 18 mm. long. This nerve is similar in struc-

ture to the main ventral branch of an embryo of 10 mm. About the periphery of the nerve cells may be seen which give rise by flattened anastomosing lateral processes to an inclosing membrane. Within the body of the nerve many cells may be seen giving rise to processes which anastomose with one another and with processes arising from the marginal cells. On staining in hæmatoxylin and then in the Van Gieson mixture the processes of the marginal cells and of the internal cells take a purplish tint, while the cross-sections of the nerve-fibrils appear orange in color. A slight bluish tint, often taken by the areas intervening between the nerve-fibrils and the sheath-cells, indicates that some substance is present there. This corresponds to the homogeneous material which Vignal has described as constituting the stroma of the bundles of nerve-fibres.

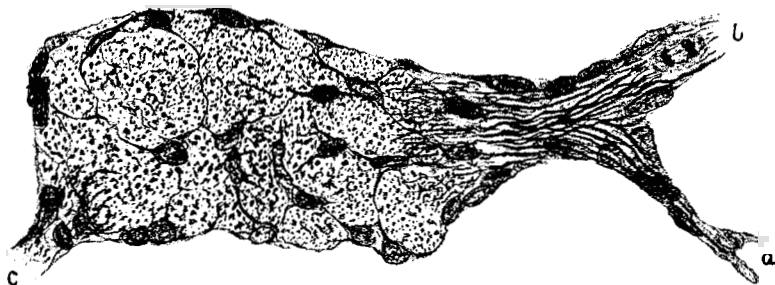


FIG. 4 Cross-section of the median ramus of the dorsal division of a thoracic nerve of a pig-embryo 18 mm. long. *a, b, c* branches arising from the main trunk. 720 diam.

The processes taking place after the formation of the primary embryonic nerves may be conveniently divided into two groups, those underlying the distribution of nerves during development and those underlying the histogenetic changes taking place within the growing nerves. We shall now consider each of these groups in turn.

II. NERVE DISTRIBUTION DURING DEVELOPMENT.

After the formation of the primary trunks of the cerebro-spinal nerves growth towards the areas which they are to supply becomes very active. In case of the sixth cranial nerve in man the nerve-trunk extends directly to the anlage of a single peripheral organ. In most instances, however, the conditions of nerve growth are far more complex. The primary nerve trunks give rise to primary branches each of which is destined for a more or less complex area of the body. Thus the fifth cranial nerve gives rise to its three chief branches, the cervical and

lumbo-sacral nerves give rise to the main branches destined for the limb-plexuses, and the thoracic nerves give rise to dorsal and ventral divisions and to branches for the sympathetic system. Frequently the primary branches extending outward from the main nerve-trunk of a given cerebral or spinal nerve become combined with certain branches derived from another nerve, and thus give rise to a common peripheral trunk containing fibres derived from two or more sources. Thus in man, a branch from the tenth joins the trunk of the ninth cranial nerve and the fibre-bundles of the eleventh cranial nerve, themselves derived from a series of anlagen, become to a greater or less extent bound up with the main nerve-trunk derived from the anlage of the tenth cranial nerve. In the region of the limbs, by the processes of anastomosis and of plexus-formation, fibre-bundles immediately connected with the large areas of the spinal cord become united into a few nerve-trunks which can grow forward with a minimum expenditure of energy towards a region of ultimate distribution.

The forces which direct the early embryonic nerves toward the anlagen for which they are destined are at present shrouded in mystery. There is much, however, which indicates that to a certain extent the nerves take a path of least resistance offered by the surrounding tissues and that they are guided to a considerable extent by the more fixed structures lying in the line of their general growth. The nature of these paths of least resistance can best be studied, however, in connection with the development of specific nerves. Thus the intercostal nerves are, to a considerable extent, guided by the costal processes of the embryonic vertebral column and by the myotomes. There is certainly the possibility, however, that given regions may exert a specific directive attraction on the nerves which are destined to supply them.

As a result of the primary nerve distribution certain nerves are directed toward various cutaneous areas and certain nerves are directed into the anlagen of the muscular apparatus. It is convenient to take up separately the distribution of these two sets of nerves.

The distribution of the cutaneous nerves may be followed with comparative ease during the early stages of embryonic development. Up to the period when a human or a pig-embryo reaches a length of two centimeters the cutaneous nerves may be followed readily in serial sections of embryos hardened in Zenker's fluid and stained in iron hæmatoxylin followed by Congo-red or in other intense stains. The relations of the nerves may best be understood by making reconstructions in wax or projection drawings. Thus in a previous article in this Journal, Lewis and I have pictured the peripheral cutaneous nerves of the body-wall and

limbs in several early human embryos. Grosser and Fröhlich have made a valuable special study of the development of the thoraco-abdominal cutaneous nerves in man and have compared the conditions found in early embryos with those found in the adult.

After pig-embryos have reached a length of from two to three centimeters it is possible to get very instructive pictures by means of impregnation with gold-chloride. My best preparations have been obtained by placing a portion of the embryo first in lemon juice for ten or fifteen minutes, then in a one per cent solution of gold-chloride for an hour, finally reducing in a twenty per cent solution of formic acid in the dark. Separate layers of tissue may be isolated and spread out in glycerine, or the specimen may be embedded and cut. By these methods the various stages in the formation of the peripheral plexuses may be most readily followed.

From the primary cutaneous rami extending toward the skin from the main nerve-trunks in the deeper parts branches are given off which run in various directions parallel with the epidermis but some distance below it. From these branches the main subcutaneous nerve-plexuses directly arise. The formation of these subcutaneous plexuses seems to be due to the tendency of branches sent by two nerves into a common region to be attracted toward the same area and to fuse into a common trunk on reaching it.² In Fig. 5 the larger, darker nerves here represented as the most superficial, form a portion of the main subcutaneous plexus arising from branches of the lateral rami (Tr.) of the dorsal divisions of two thoracic nerves of a pig embryo 4 cm long. While this plexus is being formed branches are given off from the nerves forming it. These extend towards the epidermis, just below which another plexus with finer meshes arises. In Fig. 5 this second plexus is shown in process of formation. The nerves entering into this latter plexus stain much lighter than the main nerve-trunks. As development proceeds from the stage illustrated in Fig. 5, more and more branches are given off from the plexuses there shown, finer plexuses are formed, and ultimately nerve-fibrils are distributed to the various end-organs and structures characteristic of the skin and subcutaneous tissue. The details of these latter processes I have not attempted to follow.

² Nerves growing into a given region from two or more directions do not always thus anastomose to form plexuses. Thus Mertens has shown that although the lateral cutaneous branches of the fourth and fifth intercostal nerves supply overlapping areas, anastomoses between the nerve branches revealed by dissection are infrequent. A bundle of fibres constituting a nerve may cross over or through another nerve without real anastomosis of the two trunks.

A study of the development of the peripheral cutaneous nerves leads to the belief that some sort of stimulus to growth is exerted upon the nerves by areas lacking innervation. In Fig. 5 numerous areas may be seen into which nerve branches proceed from several directions.

Kühn's interesting experiments on the regeneration of nerves in the dorsal cutaneous region of the frog have an important bearing on the mode of forward growth of nerves. When an area of the skin was deprived of its nerve supply by cutting two or three main nerve-trunks distributing branches to it, he found that from nerves in the neighboring regions new nerve-fibres extended into the deprived area. In this inner-



FIG. 5. A portion of the nerve-plexus formed by the lateral cutaneous branches of the dorsal divisions of two thoracic nerves of a pig embryo 4 cm. long. Tr. Main trunk of each nerve. $\frac{1}{38}$ diam.

vation new nerve-paths and peripheral plexuses not corresponding with the old were often formed, or the growth of the new fibres showed no regular order. This last was especially true of nerve-fibres arising from the central ends of the cut nerves.

During embryonic development nerve-fibres derived from very different sources and destined for a different ultimate distribution often take common paths for a part of their course. Good examples of this may be seen in the distribution of the vaso-motor nerves and in the relations entered into between the trigeminal nerve, the facial nerve and the cervical nerves. Popowsky has called attention to the development of anastomoses between the fifth and seventh cranial nerves.

A given cutaneous area with the nerves distributed to it may during

development be shifted extensively with respect to neighboring parts. Grosser and Fröhlich have called attention to this phenomenon in the dorsal and thoraco-abdominal regions of man.

Having thus briefly considered the mode of distribution of cutaneous nerves during development, we may take up the development of the nerves distributed to the voluntary motor-apparatus. In order that a description of this process may be clear, it is necessary to consider briefly the general features of the development of the musculature.

The muscles are differentiated from a mass of premuscle tissue which is variously derived in different parts of the body in the mammals. Thus the dorsal and thoraco-abdominal musculature arises from tissue derived from the myotomes, and the musculature of the leg from the mesenchyme of the limb-bud.

At the time when differentiation begins in this premuscle tissue two distinct groups of cells may be distinguished, myoblasts and embryonic connective-tissue cells. The former in part multiply rapidly by indirect division and in part become elongated into spindle-shaped muscle-fibres.

These muscle-fibres are usually grouped into bundles, new fibres being constantly added at the periphery of the bundles by elongation of myoblasts. Meanwhile, the skeletal tissue of the muscle becomes differentiated from the connective-tissue cells. The latter grow into and break up the primitive bundles of muscle fibres into smaller bundles. Each muscle fibre finally becomes surrounded by a certain amount of connective tissue, but usually groups of fibres become surrounded by a denser connective tissue than that surrounding any individual fibre.

In the simplest muscles the muscle fibres are parallel, of about equal length, and are attached at each end to a tendon running transverse to their course. In mammals these conditions may be seen in an anterior segment of the *rectus abdominis* muscle of a small rodent.

In most muscles the arrangement of the bundles of muscle fibres is far more complex. They run in various directions, interdigitate, and are so complexly combined that it is difficult to get an accurate idea of the internal architecture. Very often each muscle fibre is innervated about midway between its extremities. In the segment of the rectus mentioned above, gold-chloride specimens show a band of motor-endings running across the muscle midway between the two transverse tendons which limit it. In Fig. 6 there is represented a portion of the nerve-plexus distributed to the *M. transversus abdominis* of a guinea-pig embryo 8½ cm. long. A few bundles of muscle-fibres are shown in out-

line. The centre of these bundles may be seen to correspond closely to an area of distribution of motor-endings, here represented as black dots.³

The most important problem connected with the development of the nerves belonging to the muscles is the mode of distribution of the nerves within the muscle. Mainly on theoretical grounds Fürbringer, Eisler and others have supported the view that the nerve and muscle cells are

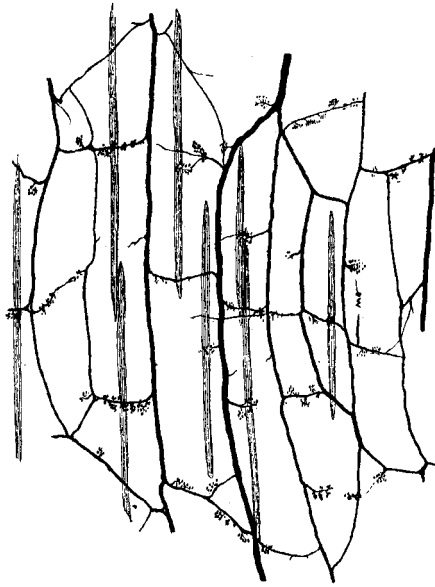


FIG. 6. A portion of the nerve-plexus distributed to the transversalis musculature of a guinea-pig embryo $8\frac{1}{2}$ cm. long. 11 diam.

closely associated from a period of development long preceding the differentiation of the permanent muscles. No good histological evidence has been brought forward in support of this view. On the contrary, the independent development of the nerves belonging to the muscles may be followed step by step from the primary embryonic nerves up to the union

³ Mays (1884), in his splendid contribution to the subject of the intramuscular distribution of nerves, has called attention to the fact that muscle-fibres are often innervated approximately midway between their extremities. Bardeleben and Frohse have made valuable studies of the nerve distribution in human muscles, but the methods used did not enable them to expose the full richness of the intramuscular nerve distribution. Frohse was able to trace nerves to muscle bundles a millimeter in diameter. Nussbaum has lately given a good review of the more recent work on the relation of nerves to muscles-

of nerve and muscle fibres at a comparatively late period of muscle development.⁴

As the mass of premuscle tissue in a given region becomes differentiated into the individual muscles characteristic of that region, paths for nerve growth are offered in the loose, vascular mesenchyme which separates the various muscles. The nerves extend out rapidly toward the various muscles and the various parts of each muscle which they are to supply. When the area to be supplied by a given nerve or set of



FIG. 7. A portion of the nerve-plexus formed on the surface of the *transversus abdominis* muscle of a pig-embryo 4 cm. long. 11 diam.

nerves is considerable, the nerves, as they are spread out, may branch and give rise to coarse plexuses. Fig. 7 shows such a plexus in process of formation in the area between the internal oblique and the transversalis muscles of a pig-embryo 4 cm. long. From the intermuscular nerves branches are given off which enter the

muscle substance and make their way, rapidly ramifying, toward the middle of each bundle of muscle fibres constituting the muscle (see Fig. 8). In simple muscles, like the segment of the rectus above mentioned, there is little or no plexus formation during the period of intramuscular distribution. But in complex muscles where the bundles of muscle fibres interdigitate, plexus formation is active during this period. As in the skin, so here, this plexus formation seems to be due to an attraction which causes branches to grow from several sources toward a given area.

In the course of development of the muscles new fibres may be differentiated in one or more directions, and toward these the intrinsic nerves of the muscle extend to be distributed. This may be beautifully fol-

⁴In many of the lower vertebrates there is good evidence that the motor roots of the spinal nerves become associated at an early period with the musculature of the myotomes. But in these vertebrates the myotome musculature is functional. In mammalian embryos, on the other hand, I have been able to find no good evidence of union of motor-root fibres with the myotome cells. Branches from the spinal nerves do not begin to enter the dorsal and the thoraco-abdominal musculature until the latter begins to be differentiated from the myotomes. This I have previously shown in describing the development of the thoraco-abdominal musculature of the pig. The myotomes are probably no more functional in mammals than are the branchial clefts.

lowed in muscles like the *latissimus dorsi*. Nussbaum has shown that the distribution of nerves belonging to a muscle indicates the direction of development of that muscle. Popowsky's interesting work on the development of the facial nerve illustrates this principle well. In addition to this active extension of nerve distribution on or within a muscle, the bundles of muscle-fibres comprising it may shift their relative posi-

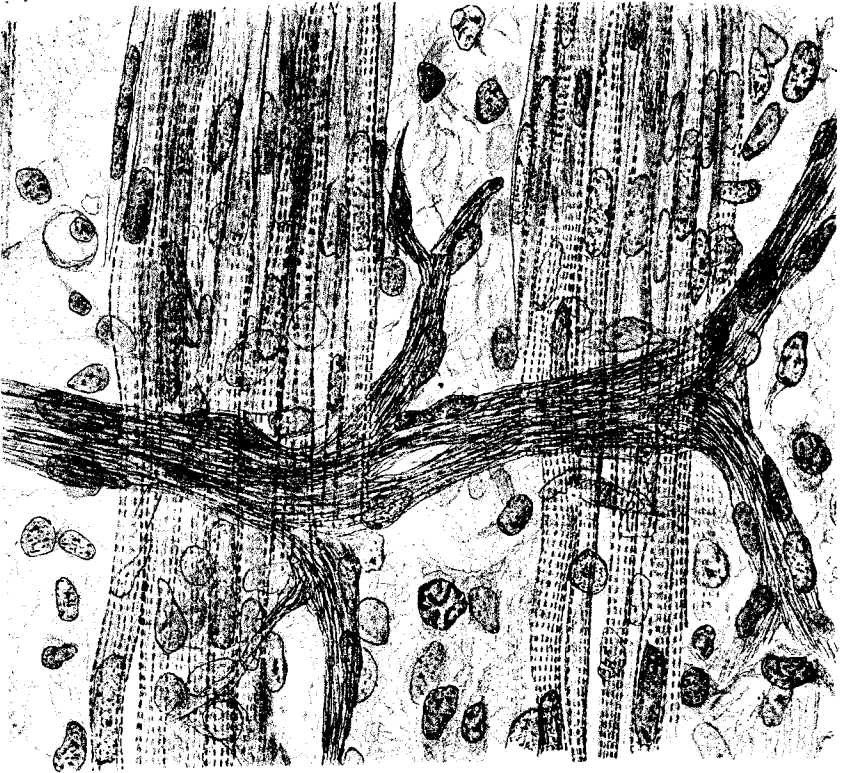


FIG. 8. A portion of a segment of the rectus muscle of a pig embryo 39 mm. long. An intramuscular nerve may be seen distributing branches in the loose mesenchyme separating the bundles of muscle-fibres. 720 diam.

tions, as in certain segments of the *rectus abdominis* of the larger mammals, and the muscle as a whole may shift its position in relation to other parts of the body, and thus the nerves may be passively altered in their relations.

In addition to the nerves furnished to the muscle-fibres during development, nerves are also furnished to various sensory endings lying in the muscle, to its associated skeletal apparatus and to the blood-vessels.

The development of this sensory and vaso-motor apparatus can only be followed with satisfaction when better methods for differentially staining developing nerves have been devised. Methylene-blue, which is so good for adult tissues, seems to act much less specifically on developing nerve-fibres. Gold-chloride used on fresh tissues is excellent for the study of the grosser tissue relations, but the tissues are so altered by the acids used in reducing the gold that the finer histological details are many of them lost. The Apáthy method of using gold-chloride on fixed tissues does not seem to give good results with mammalian embryos. In young embryos Congo-red gives an excellent stain for nerve-fibrils, but it is not a stain sufficiently specific for the complex tissue relations of later embryonic development. The changes, however, taking place in nerve-trunks may be traced both in teased specimens and in sections, by means of the usual methods of technique, and the development of the nerve-endings on the striated muscle-fibres may be followed, although less perfectly than one might wish, by the use of the gold-chloride method combined with other methods of technique. We shall now consider certain of the histogenetic phenomena disclosed by these methods. It is convenient to describe first the processes connected with the forward growth and secondly those connected with the internal differentiation of the nerves.

III. HISTOGENESIS IN THE DEVELOPING NERVES.

a. Processes Taking Place at the Growing Tip.

A description has above been given of the histological structure of the primary embryonic nerve-trunks. Each nerve is composed of bundles of embryonic fibres and fibril-groups. The nerve as a whole is fairly completely ensheathed, while the constituent fibre-bundles are supported and partially ensheathed by anastomosing cells. When a nerve-trunk gives rise immediately to large primary branches, as is the case with the spinal and most of the cranial nerves, these branches are similar in structure to the primary nerve-trunks. The nerves which arise by direct forward extension of the primary branches, such, for instance, as the ventral tip of each intercostal nerve and the nerves which arise from the fusion of two or more primary branches, such as the nerves arising from the brachial and lumbo-sacral plexuses, send forward a few fibrils closely accompanied by, perhaps preceded by, sheath-cells. Behind this tip the ingrowth of bundles of fibrils and the multiplication of these fibrils by branching causes the nerve-trunk rapidly to increase in thickness toward the central nervous system (see Fig. 2). When the nerves

first formed give rise to secondary branches, the peripheral cells of the nerve multiply in the vicinity of the future branch and give rise to a tube-like projection into which the nerve-fibrils extend. In Fig. 4 at "a" may be seen a branch of this kind in process of formation. Whether a few nerve-fibrils first extend outward and then sheath-cells multiply so as to cover them, or the tube-like process is first formed, or the two processes are simultaneous, it is difficult to decide. Perhaps all three modes occur.

The forward growth of the nerves takes place by essentially the same processes both when the growing tip represents the extremity of an original nerve and when it represents the extremity of a branch. In the cutaneous nerves, branching and forward extension is so rapid that the growing extremities of the nerves soon become small in calibre. In Fig. 9 is shown the extremity of a branch of a lateral cutaneous ramus of the ventral division of a spinal nerve of a pig 14 mm. long. The nerve is composed of a small bundle of fibrils inclosed by flat endothelial cells.



FIG. 9. Tip of a branch of the lateral cutaneous ramus of the ventral division of a spinal nerve of a pig 14 mm. long. 720 diam.

At "a" a few of the fibrils branch off into another level.

At "b" what appears to be a naked axis-cylinder process extends outward into the surrounding mesenchyme. This process is enlarged at the end like the two fibres pictured in Fig. 3, *d*. This enlargement corresponds to that described by Cajal as the growing extremity of a nerve-fibre. Unless we assume that the sheath-cell belonging to this fibre was cut off in sectioning, we must take the fibre to be a naked branch of the nerve. At *c* a few nerve-fibrils are also extending forward, but they are covered by a process of a sheath-cell. The more the nerves branch the finer the branches become. The ultimate branches consist of a few fibrils either naked or ensheathed by a series of greatly elongated sheath-cells.

Leontowitsch has made an extensive study of the nerves in the adult human skin. His results, obtained by methylene-blue methods, are of great value, although it is probable that certain of his conclusions will not be accepted without further support. Leontowitsch divides the nerves of the skin into two groups, the medullated nerves and their branches, and the non-medullated nerves, or nerves of Remak. The latter he again divides into two groups, Type I and Type II. The most primitive portion of the nervous apparatus of the skin, according to Leontowitsch, is composed of the "Remak" cells of Type I. These are cells

which give rise to true plexuses by the anastomosis of long, narrow branches which extend outward from the cell-body in various directions. Four such plexuses may be distinguished: a deep one in the corium, a middle and a subepithelial in the papillary layer, and an intra-epithelial. The Remak nerves of Type II likewise are composed of anastomosing cells which give rise to a true plexus. The protoplasm of the processes of these cells, however, is much more developed than in those of Type I, and there seems to be much less branching of the processes. Often the protoplasm of these cells shows a distinct fibrillation. Certain cells seem to be in a state of transition between Type I and Type II. The medullary nerves terminate in arborizations, telodendrites, in which plexus formation is slight or does not exist. The ramifications of the medullary nerves may resemble the Remak fibres for a distance, or they may at once pass over into naked processes which are marked by plate-like varicosities and terminate either "free" or in sense-organs. Leontowitsch thinks that there is constant physiological regeneration going on in the skin and that this takes place by a transformation of the "Remak" cell-plexuses into the peripheral portion of the "neurite" of the central ganglion cell. He can explain his findings only on the hypothesis advanced by Schwann, Balfour, and numerous recent investigators that the nerves arise by differentiation from a chain of anastomosing cells.

Although Leontowitsch makes the statement that the tyro can distinguish between connective-tissue cells and the cells he figures as belonging to Remak nerves Type I, I do not think that he makes the distinction clear either in his text or figures. The cells of the Remak nerves Type II may well be taken for sheath-cells surrounding small bundles of fibrils such as are known to exist in the sympathetic system, the intestinal plexuses, and in the whole peripheral nervous system during early embryonic development. Gold-chloride and methylene blue both show an affinity for the sheath-cells as well as for the nerve-fibrils. Although I have not had an opportunity to repeat Leontowitsch's work extensively, my own studies on the nerves of the skin lead me to believe that there is nothing there to disprove the hypothesis that the sensory nerves of the skin are developed from nerve-fibrils which have grown out from central ganglion cells and which multiply greatly in number by branching. The growing fibrils are closely accompanied by sheath-cells until near their ultimate termination. The ensheathed paths, but not the contained fibrils, may either anastomose freely or to no considerable extent, according to the region in which the branching takes place.

Galeoti and Levi in a recent valuable contribution have described

the growing extremities of nerves on their way to muscle cells in the regenerating tail of a lizard. The nerve-fibres they describe are composed first of a chain of cells within which later the axis-cylinder processes become differentiated. They used gold-chloride impregnation in their studies. In many respects the growing nerves which they picture resemble those which I have seen in mammalian embryos and have described above as the "sheath cells." I have found that as a rule in early embryos the gold-chloride is precipitated, not in the axis-cylinder fibrils, but in the stroma of the nerves and in the sheath-cells.⁵ It seems, therefore, possible that the cells described by Galeotti and Levi were cells which ensheath nerve-fibrils not revealed by the methods they used. What they describe as developing axis-cylinders appears somewhat like a beginning deposit of myelin about axis-cylinder processes. For myelin in the early stages of development, gold-chloride has an especial affinity.

In general the growing extremities of the nerves within the developing muscles resemble those of the skin except that, as a rule, larger bundles of fibrils are contained within the nerve-sheaths until the final branchings take place which serve to distribute fibrils to the individual muscle cells composing the muscle bundles. Fig. 8 shows the branching of a nerve growing out to supply several muscle bundles of the rectus muscle of a pig 39 mm. long. Up to this stage the growing tip of the nerves may be easily distinguished in well stained sections.

The final union of the growing tip of the nerve with muscle-cells seems, in the rectus muscle of the pig, to begin in embryos of 8 cm., but definite endings are few until considerably later. The formation of nerve-endings cannot be satisfactorily followed in mammalian embryos, owing to the great number and the small size of the cells. So far as I have been able to determine, the steps in the formation of the end-plate are as follows:

An ensheathed bundle of nerve-fibrils extends transversely across a number of bundles of muscle fibres. As each bundle is reached an ensheathed nerve branch is sent into the midst of the muscle-fibres.

⁵ Kaplan has shown that his excellent ink-stain for axis-cylinder processes does not stain these in embryos before the appearance of the myelin sheath. The same thing seems to be true of the aniline stains for axis-cylinders. In staining Auerbach's and Meisner's plexuses with gold-chloride, as a rule the individual fibres composing the fibre-bundles are not distinct. The gold seems to be distributed in the stroma of the bundles. Apáthy also has called attention to the fact that gold-chloride usually stains the stroma of axis-cylinders, but not the contained fibrils when used before fixation. For Apáthy's haematein and gold-chloride stains the nerves of mammalian embryos do not seem adopted.

From this latter branch nerve-fibrils are sent out in company with sheath-cells to each muscle-fibre. A sheath-cell becomes applied to the surface of the muscle-fibre, while the protoplasm of the latter in the vicinity of the sheath-cell becomes granular and nuclei collect about the granular area. At the time of the formation of the end-plate, the muscle-fibre possesses both peripheral and central nuclei. I have elsewhere (1900) given pictures of cross-sections of muscle-fibres which seem to show a wandering of central nuclei toward the surface of the cell. The nuclei which collect about the granular area of the muscle-fibre seem to arise from the surface nuclei. They represent probably the nuclei of the sole of the adult muscle-plate while the nucleus of the cell which accompanies the nerve-fibre to the muscle-fibre, probably represents a nucleus of the sheath, or possibly a nucleus of arborization. Fig. 10 shows an early stage in the union of nerve-fibres and muscle-cells.

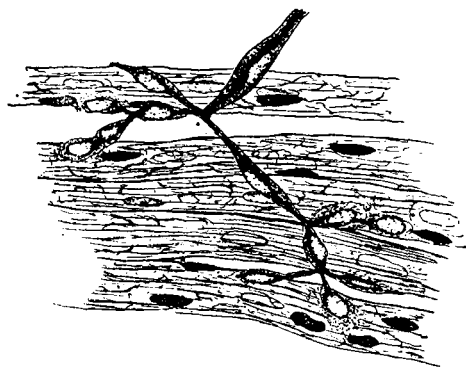


FIG. 10.

FIG. 10. An early stage in the union of nerve and muscle-fibres. From a gold-chloride specimen of the rectus muscle of a pig 19 cm. long. 720 diam. For the sake of contrast the surface nuclei are represented very much darker than they appear in the specimen.

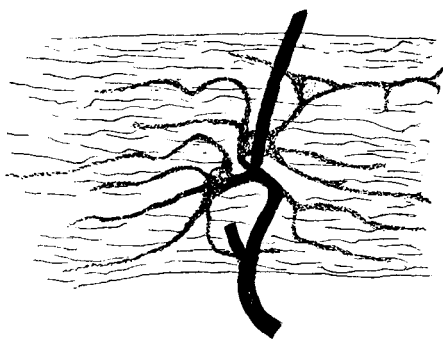


FIG. 11.

FIG. 11. Nerve, nerve-ending, and sarcolemma from a portion of the rectus muscle of a frog. 720 diam.

Union of nerve-fibres and muscle-cells does not begin to be common until the embryo has reached a length of 15 cm., and it continues for a considerable period after this time. So far as I have been able to determine, a definite sarcolemma forms after the nerve-fibre and sheath-cell have joined the muscle-cell. By the digestion of specimens hardened in osmic acid I have been able to isolate delicate sarcolemmata from muscle-cells of the rectus of pig embryos 20 cm. long, but not from younger embryos. This valuable digestion method of Chittenden also enables one to show the relation of the sarcolemma to the sheath of

Schwann. If muscle-fibres with the terminal nerves running to them be hardened in osmic acid or impregnated with gold-chloride and then digested in pancreatin, the protoplasm of the muscle-fibre disappears, but the sarcolemma, the sheath of Schwann, the myelin sheath, and some portions of the nerve-endings, probably the stroma, are left undigested. With care these digested specimens may be stained and mounted in glycerine or in balsam.⁶ In all successful specimens which I have prepared in this way, I have found the sheath of Schwann inseparably connected with the sarcolemma and the undigested portion of the end-plate closely united to and apparently on the under surface of the latter. The most satisfactory specimens are obtained with the muscles of frogs. See Fig. 11. It seems probable that the sarcolemma is formed through the action of the surface nuclei of the muscle-fibre, in response, possibly, to stimuli arising from the union of the nerve with the muscle-fibre; and it is so formed that it incloses that portion of the nerve which is spread out over the protoplasm of the muscle.

Various stages in the development of end-plates in muscle-fibres of mammals were described in 1892 by Mays. To the descriptions in Mays' most valuable paper I have nothing to add. In a study of the development of end-plates in pigs, mice, and guinea-pigs, I have seen specimens corresponding to many of Mays' pictures. In certain respects what I have seen corresponds to the description given by Galeoti and Levi of the formation of end-plates on the regenerating muscle-fibres of the lizard. This last paper and that of Mays both contain good summaries of the literature on the subject of the development of nerve-endings.

b. Internal Differentiation of the Nerves.

From the neuroblasts and from the spinal ganglion cells processes of considerable thickness are sent out into the peripheral nerves. It has been mentioned above that these processes soon begin to give rise at their extremities to groups of fibrils. During the early stages of development these fibrils may either be gathered in small compact groups, each of which represents an axis-cylinder process or they may be so scattered within the nerve that it is impossible to distinguish definite groups of fibrils corresponding to axis-cylinder processes. It seems probable that these embryonic fibrils increase in thickness as well as in length and in turn give rise at their extremities to new groups of fibrils. It is possible

⁶ The capillary cells and the white fibrous tissue are also left undigested. By staining heavily in Delafield's haematoxylin and then counter-staining in Congo-red, the sarcolemma, the capillary cells and the sheath of Schwann take a blue tint, and the fibrous tissue a red.

that the fibrils may increase in size and multiply by dividing longitudinally somewhat in the manner which Heidenhain has described for the fibrils of muscle-cells. When a nerve branches certain of the fibrils belonging to a given axis-cylinder process may be diverted into the branch while others may continue in the main trunk. Mays showed that in the frog a given nerve-fibre gives rise to a large number of peripheral branches. Dunn has recently shown that in the sciatic nerve of the frog there is a constant increase in the number of fibres as the periphery is approached. Although there is at the same time a decrease in area of cross section of the individual fibres, this is more than offset by the increase in number of fibres. All this goes to show that the fibrils connected with a given ganglion cell must increase very greatly in number and mass as one passes from the central toward the peripheral areas. The growth and longitudinal division of fibrils therefore probably constantly increases in amount as the fibrils extend outwards. The fibrils of embryonic nerves seem to be larger than the "primitive" fibrils described by Apáthy and by Bethe in adult nerves. The relations of the latter to the former can only be determined when methods have been devised which will stain primitive fibrils in young embryos.

The relations of the fibrils to the sheath-cells are difficult to determine in very small peripheral branches. The sheath-cells are so closely applied to the fibrils that it becomes mainly a matter of judgment to decide whether the fibrils are surrounded by or are embedded within the sheath-cells. In the large nerve-trunks first formed near the central nervous system sheath-cells appear scattered within the nerve as well as about the periphery (Fig. 2), and this, too, may lead to dispute as to the relation of the cells within the nerve to the nerve-fibrils. It is in peripheral nerves of a moderate size found during the earlier stages of embryonic development that the absence of genetic continuity between nerve-fibrils and the intrinsic cells of the nerve becomes most clear. Thus in Fig. 12 is shown a cross section of the median branch of the dorsal division of a thoracic nerve of a pig embryo 14 mm. long. Some of the surrounding mesenchyme is also shown. This nerve is composed of a large bundle of fibrils surrounded by a single layer of flat anastomosing cells. No cells are to be found among the fibrils, although the nerve may be followed for a considerable distance through a series of sections.

After a nerve of this kind has become considerably distended by ingrowth of new fibrils from behind, cells begin to wander from the investing sheath in among the fibrils. These cells give rise through anastomosis of their membranous processes to a skeletal framework similar

to that previously described in page 234. Kolster has described the invasion of cells of the sheath in among the nerve-fibrils which it surrounds in the nerves of *Salmo trutta*.

During the growth of the nerves the elementary bundle or bundles of fibrils of which it is composed become further broken up into secondary bundles by the invasion of fibrous tissue from the investing sheath. Thus, each intercostal nerve of the pig is at first composed of but a single funiculus, but later it becomes divided into two main bundles of fibrils, and these again are further subdivided. Fig. 13, *a*, shows a cross-section of an intercostal nerve of an embryo 8 cm. long. Above is a rather small compound bundle of fibrils, and below is a much larger one. Each of these is in turn divided into several funiculi. The septa sepa-

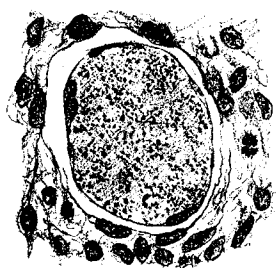


FIG. 12.

FIG. 12. Cross-section of the median branch of the dorsal division of a thoracic nerve of a pig embryo 14 mm. long. 720 diam.

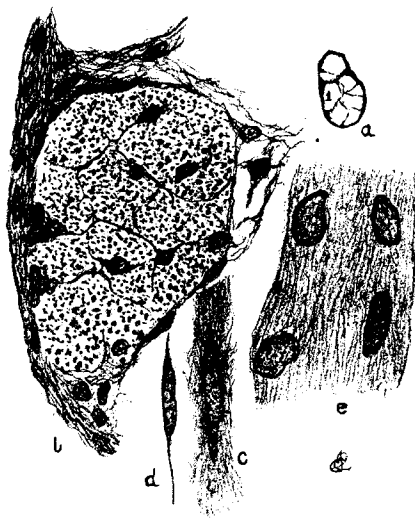


FIG. 13.

FIG. 13. *a*, Cross-section of an intercostal nerve of an embryo 8 cm. long; *b*, cross-section of the bundle of nerve-fibres designated "1" in "*a*"; *c*, An isolated cell corresponding to the cells shown in section in the bundle of nerve-fibres in "*b*"; *d*, longitudinal section through one of these cells; *e*, small portion of the membranous sheath surrounding the bundle of fibres shown in "*b*." *a*, 76 diam.; *b*, *c*, *d* and *e* 720 diam.

rating the funiculi last formed are delicate. This is shown in the septum at the lower margin of the funiculus pictured in Fig. 13, *b*, an enlargement of "1" in Fig. 13, *a*. But these septa are always lined toward the funiculus by a layer of flat, anastomosing cells. In Fig. 13, *e*, is shown a portion of such a membrane isolated from a funiculus of an intercostal nerve of a pig 8 cm. long and stained in hæmatoxylin and safranin.

The invasion of tissue which breaks up the elementary bundles into

secondary funiculi, seems to accompany the blood vessels which penetrate the nerve at various places along its course. In consequence of the irregular manner in which the septa arise, the funiculi of the embryo, like those of the adult, branch and anastomose freely along the course of the nerve. New funiculi may also be added to the nerves during the process of plexus formation.

In the further development of the nerve-trunks the most interesting questions are those concerned with the development of the myelin sheath and the sheath of Schwann. The work of Vignal is of most fundamental importance in this connection. In general the observations which I have made correspond with Vignal's. Certain details in the development of the sheath of Schwann can, however, be better studied in sections than by the teasing method employed by Vignal. Gurwitsch in studying this question made use of the Apáthy gold-chloride method, which he found a specific stain for the membranous sheaths of the developing nerve-fibrils. By the use of the Van Gieson stain I have been able completely to confirm the valuable work of Gurwitsch.

In Fig. 13, *b*, at the left, two projecting masses of cells seem to be on the point of contributing new cells to the interior of the nerve bundle. By the anastomosis of the flattened lateral processes of these intra-funicular cells, a membranous framework is formed which serves to divide the nerve-fibrils into groups. The membranes run parallel with the nerve-fibrils and thus appear as fine lines in cross-section (Fig. 13, *b*) and usually so in longitudinal sections (Fig. 13, *d*). They may be stained in sections, when Zenker's fluid has been used, by the Van Gieson or by the Mallory connective-tissue stain. The processes extending from the intra-funicular cells have been taken by some investigators to be nerve-fibres. The longitudinal section shown in Fig. 13, *d*, might suggest a bipolar nerve cell. But the processes take a purple stain in the Van Gieson mixture and a blue stain by the Mallory method, while the nerve-fibrils take on quite a different color in each case. Owing to the great delicacy of the membranous process of the cell, one is apt to obtain in teased preparations only the nucleus and a bit of the more granular entoplasm immediately about it. With care, however, specimens may be obtained like that shown in Fig. 13, *c*, in which a considerable portion of the membranous exoplasm is retained intact. The membranous processes appear to have a slight fibrillation. Before the intrinsic cells of the developing nerve have become differentiated into sheath-cells they multiply by indirect division (Figs. 3, *b*, and 4). After they become thus differentiated, direct cell division takes place. The line of division may be in the long or in the transverse axis of the nucleus

of the cell. After the sheath of Schwann is formed, cell division does not take place in its component cells. In regeneration, however, according to most observers, indirect division takes place in the cells which arise from the cells of the sheath of Schwann. These processes remind one of those taking place in muscle-cells. Myoblasts divide by indirect division. The central nuclei of the elongated spindle muscle-fibres multiply by direct division and so do also the peripheral nuclei which later appear. In regeneration, on the other hand, the myoblasts which arise from the muscle-fibres multiply by indirect division.

After they begin to appear the intra-funicular cells increase rapidly in number and give rise to membranous septa which divide the fibrils into smaller and smaller bundles and finally surround small groups of fibrils with a septum, the sheath of Schwann. Fig. 13, *b*, shows the membranous septa of bundles of fibrils in a pig-embryo 8 cm. long. Considerable groups of fibrils are enclosed. Fig. 14, *a*, shows a cross-section of a small portion of a nerve of an embryo 15 cm. long. The septa here form sheaths of Schwann about small dense bundles of fibrils, although not all the fibrils are thus inclosed.

Gurwitsch suggests the possibility that the membranous septa which he describes might be taken to represent endoneurium. In that case one would expect to find two sorts of cells within the funiculus, one set belonging more intimately to the nerve-fibrils, the other giving rise to the membranous septa. Gurwitsch, however, shows that stage by stage the development of membranes within the fasciculus may be followed until the sheaths of Schwann appear. A study of a large number of sections and teased preparations has served to convince me that Gurwitsch is correct in his interpretations. The endoneurium develops comparatively late. It is very slight at the time of the formation of the sheath of Schwann.

There is a vast amount of literature connected with the genetic origin of the cells of the sheath of Schwann and of the relations of these cells to the axis-cylinder. Those who assume that the latter structure arises by fusion of parts derived from a chain of cells, usually consider that the cells of the sheath have an ectodermal origin. In the mammals, as well as in the lower vertebrates, a certain number of cells wander out from



FIG. 14. *a*, Cross-section from the centre of an intercostal nerve of a pig-embryo 15 cm. long; *b*, Cross-section of several fibres near the margin of an intercostal nerve of a pig-embryo 20 cm. long. 720 diam.

the spinal ganglia and cord along with the bundles of axis-cylinder processes. These cells may contribute in part to the origin of the cells of the sheath of Schwann, but my observations lead me to believe with Vignal and Gurwitsch, that the latter arise in mammals, in the main at least, from the mesenchyme. I agree with Gurwitsch that the sheath of Schwann is an exogenous structure which has quite a different genetic origin from that of the nerve-fibre. When once the sheath of Schwann is formed it does, however, undoubtedly enter into intimate physiological relations, "symbiosis," with the axis-cylinder process which it incloses.

Indeed, throughout the period of the development of the nerve-fibrils as well as in the adult life, the elaboration of nerve-sheaths shows that they must play a vital part. In the embryo, at least, their function is doubtless mainly nutritional. The early nerves are composed of nerve-fibrils within a sheath of anastomosing cells. In addition to the fibrils there is contained within the sheath some substance either fluid or semi-fluid in nature, which serves to "float" the growing fibrils and to furnish them with nutrition. Vignal has described this substance as homogeneous. It deserves much more careful study from the micro-chemical standpoint than it has yet received. The perineural sheath in nerves before the sheath of Schwann has appeared has, in all likelihood, specific action in determining the physical and probably also the chemical characteristics of this stroma.

For the formation of a medullary sheath a special cellular sheath about each nerve fibre is not absolutely necessary. As Gurwitsch has pointed out, formation of myelin about an axis-cylinder process may begin before the corresponding sheath of Schwann is complete. I have observed this also in the pig. Kolster in a valuable study on the development of the nerve-fibres in *Salmo trutta* has shown that a medullary sheath may develop about fibres in a nerve in which no cells have as yet passed from the perineurium into the midst of the fibres which it surrounds. Apathy has paid special attention to the interfibrillar substance of nerve-fibres and has shown that it may present many characteristics of myelin.

As a rule, however, in mammals the sheath of Schwann, as shown by Vignal, completely incloses the axis-cylinder fibrils before formation of the medullary sheath begins. In Fig. 15, *a*, is shown a fibre of this kind dissected from the intercostal nerve of a pig 15 cm. long. The cells comprising the sheath here shown are nearer together than is common at the period when the sheath is first formed. The average distance between nuclei at the period under discussion seems to be about a tenth of a milli-

meter, though variation is great. Fibrils free from cells for a distance of over a millimeter can be dissected from pig embryos six to eight millimeters long. It therefore seems highly improbable that the cells of the sheath of Schwann, which form segments of a tenth of a millimeter or less in length, could have anything to do with a segmental formation of the corresponding axis-cylinder.

Within the sheath of Schwann the axis-cylinder lies at first apparently surrounded by a fluid, judging from the space which intervenes between it and the thin wall of the sheath.⁷ Only in the vicinity of the nucleus is the wall thick. Here there is a mass of granular protoplasm. About the axis-cylinder myelin is deposited, owing apparently to the action of the axis-cylinder on the surrounding fluid. At first the deposit of myelin seems usually to be fairly evenly distributed (Fig.

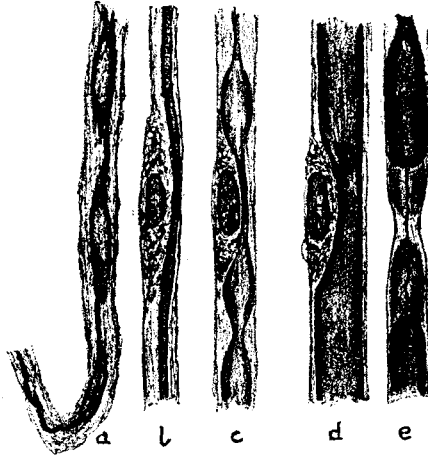


FIG. 15. Nerve-fibres illustrating various stages in the development of a medullary sheath. 720 diam.

15, *b*), but soon it is much more active in some areas than in others, giving rise to the beaded appearance shown in Fig. 15, *c*; and finally, the sheath becomes filled out (Fig. 15, *d*). The formation of myelin seems to continue in the vicinity of the nodes of Ranvier after the sheath has become completely filled out elsewhere. Fig. 15, *e*, shows a fibre in which a "segment" of lighter myelin is being formed on each side between a node of Ranvier and the darker, more fully formed myelin of the nerve-fibres. This suggests that the internodal segments of the nerve-fibres grow at their extremities by addition there of new material. Whether the "beads" and the secondarily-formed segments of myelin of the nature just described have anything to do with Lantermann's segments, is open to question. The various stages in the formation of myelin pictured by Vignal may be readily confirmed.

The changes taking place in the nerves and nerve-fibres after birth have been described at length by Westphal. What he describes as free axis-cylinders seem to be fibres in which the sheath of Schwann is com-

⁷ In osmic acid specimens the sheath usually shrinks down against the axis-cylinder process. This may be prevented by the use of formalin before the nerves are subjected to osmic acid treatment.

pletely filled with a substance resembling embryonic myelin, that is, of fibres approximately like that shown in Fig. 15, *d*. Often when myelin is being very actively formed, it is difficult to distinguish the axis-cylinder from the surrounding myelin. Differentiation of the embryonic myelin into the adult form begins at the periphery of the fibres and proceeds toward the centre. This process has been carefully studied by Westphal. I may mention here again that, while neither by the Weigert method nor by the use of osmic acid is embryonic myelin stained so dark as that of adult fibres, gold-chloride has an especial affinity for embryonic myelin.

The intercalation of new segments of the sheath of Schwann at the nodes of Ranvier during the later stages in the development of the fibres has been described by Vignal and has deserved more attention than it has received. The processes he describes may be readily confirmed. The progressive myelinization of nerve-fibres from the center towards the periphery may also be readily confirmed. In pig embryos 20 mm. long the nerve-fibres in the main trunk of an intercostal nerve are most of them covered with embryonic myelin. In the peripheral nerves distributed to the anterior segments of the rectus muscle, on the other hand, one finds only rarely a fibre in which the myelin has been formed up to the final branches distributed to the individual muscle-fibres. None of these final branches seem to be medullated.

SUMMARY.

In the development of the peripheral nervous system it is convenient to recognize several stages, although it is difficult to draw a sharp line of demarcation between them. The first stage is that of the differentiation of the motor nuclei and sensory ganglia; the second includes the period of outgrowth from the region of the central nervous system to various peripheral anlagen; the third, the development of branches from the primary nerves to the various parts differentiated from these anlagen; and the fourth, the development of functional unity between the nerve-fibres and the structures to which they are distributed. During the second period the proximal nerve-plexuses are formed, during the third the coarser peripheral plexuses, and during the fourth the finer terminal plexuses. During the second, third and fourth stages there may take place considerable shifting in relative position of the structures to which the nerves are distributed.

The axis-cylinder fibrils of the nerve grow out by continuous extension from central cells. They divide and branch extensively as they proceed from the region of the central nervous system outwards. They

leave the central nervous system and spinal ganglia in naked bundles (Fig. 1) but soon become intimately related with sheath-cells which accompany them closely throughout the period of growth. At the growing tip of a nerve it is difficult to decide whether the axis-cylinder fibrils or the sheath-cells proceed (Figs. 2, 4 and 9). Posterior to this the nerve gradually becomes distended with fibrils by ingrowth from behind and by multiplication due to division. In an early embryonic nerve of moderate size one finds many hundred fibrils inclosed by a sheath of flattened cells, but with no cells among them (Fig. 12). In such nerves one can most easily see that the fibrils are not differentiated parts of cells lying in the nerve. In addition to this it is possible to isolate from embryos one to five or six centimeters in length unensheathed nerve-fibril bundles from one-half to a millimeter long. When the sheath of Schwann is formed in embryos ten to twenty centimeters in length, the nuclei of the sheath are about a tenth of a millimeter apart. There is, therefore, no segmentation in the axis-cylinder fibrils corresponding to the cells of the sheath of Schwann.

Union of nerve and muscle fibres takes place before the formation of the sarcolemma. The latter membrane becomes so closely united to the sheath of Schwann that no line of demarcation can be seen between them in specimens from which the muscle substance has been digested. The terminal apparatus of the nerve is more resistant to digestive fluids than the muscle substance, and is closely attached to the under surface of the sarcolemma (Fig. 11).

The sheaths of the nerves serve to maintain the stroma in which the axis-cylinder fibrils grow. At first large numbers of fibrils are ensheathed within the main trunks of the nerves, but by proliferation of sheath-cells smaller and smaller bundles are inclosed until finally but a small group of fibrils is inclosed within each sheath of Schwann. The work of Gurwitsch on the formation of the sheath of Schwann is confirmed. Myelinization is due to influences exerted by the axis-cylinder fibrils on the surrounding stroma.

During development the nerve funiculi may be broken up into smaller funiculi by invasion of tissue of the investing sheath.

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