A PHYSIOLOGICAL, HISTOLOGICAL AND CLINICAL STUDY OF THE DEGENERATION AND REGENERATION IN PERIPHERAL NERVE FIBRES AFTER SEVERANCE OF THEIR CONNECTIONS WITH THE NERVE CENTRES.

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The interest which the degeneration and regeneration of peripheral nerve fibres has aroused, especially among physiologists and surgeons, is fully shown by the extraordinarily voluminous literature bearing upon the subject. The changes which take place during these processes have been observed and described with more or less completeness over and over again, so that it would seem almost impossible to add any strikingly new observations to those already recorded. In our investigations we have borne in mind the leading questions[1] as to the possibility of a "union and return of function in severed nerves without degeneration of the distal portion," and, furthermore, the "possibility of union with return of function between the central portion of any one spinal nerve and the distal portion of any other." It may be said that to both of these questions our work, physiological and histological, has given, for the animal experimented upon, the most satisfactory and unmistakable answers. With reference to the more difficult undertaking of a complete description of the histogenesis of the regenerated fibres, which we have attempted to include in this research, our results are in the nature of the case far less convincing. In this, as in other histological problems, the facts may bear several interpretations, and the piecing together of the whole story from many isolated observations is necessarily attended with many chances of error. We believe, however, that our work in this field has given results of positive value,

[1] It should be stated that this paper was written in competition for a prize offered by the American Physiological Society for the best research bearing upon the points stated.

though some points of importance have been left untouched. In order to make as complete a presentation of the whole subject as possible, we have divided the paper into three portions. In the first a brief review of the previous experimental work upon degeneration and regeneration is given in order that the points most in dispute may be clearly brought forward. In the second an account of our own experimental work upon the physiology and histology of degeneration and regeneration is presented. In this, of course, all the original work of the investigation is found. In the third portion an attempt has been made to collect the most important of the surgical cases of primary and secondary suture for the purpose of a critical study of them as a whole, with special reference to the possibility of union by first intention with immediate return of function, and also to show statistically the failures and successes attending such operations.

PART I.

Review of previous Experimental Work.

The possibility of a reunion of the two ends of a divided nerve seems to have been a subject of discussion in the earliest medical writings. At first, apparently, the discussion was to determine whether a severed nerve can grow together. But after the establishment of this fact, it became further a matter of investigation to determine whether on the one hand there was a return of function to the nerve, and on the other whether the cicatrix uniting the severed ends was composed of true nerve tissue. Accordingly the problem was treated both from a physiological and a histological standpoint. Microscopic examination, according to some, proved that the cicatrix was composed of genuine nerve elements or tissue, while others obtained different results. Mayer[1] treated the two ends of the nerve and the uniting tissue with nitric acid, and thought from the behaviour of the tissue toward this reagent that he had demonstrated the true nervous nature of the cicatrix. Modern experimental examination by physiological methods seems to have begun with the ingenious experiments of Cruikshank and of Haighton, published in the same year in the *Phil. Trans.*, 1795. Cruikshank divided the vago-sympathetic trunk, in the neck of the dog, on both sides and proved that death invariably followed within a short time. He then cut the nerve on one side alone, and found that the animal survived without apparent injury. Finally he divided the

[1] The authors quoted are given in the list of references at the end of this section, arranged in alphabetical order.

nerve on one side, and after an interval of three weeks cut the other side also. The animal lived, proving that the nerve first severed must have united and become functional. Haighton performed the same experiment, but allowed longer intervals between the successive sections, and obtained better results; the animal in one case living nineteen months after the second operation. He further showed that in this last animal, which had completely recovered from the first operation, section of the nerve on both sides,—below the place of the original section,—was followed by death within two days. It is rather curious that similar experiments have been made within quite recent times by Gluck and by Bakowiecki without knowledge, apparently, of the older results. The experiment is a difficult one to interpret clearly, especially as Gluck states that though he obtained, like the older investigators, an apparently successful union of the divided vagus (in rabbits), since section of the second nerve eight days after the first did not result in the usual rapid death of the animal, nevertheless stimulation of the vagus which had united did not give inhibition of the heart. We must believe, then, either that the united nerve was no longer irritable to artificial (electrical) stimuli, though it could conduct normal impulses, or that the cardiac fibres had failed to unite. Obviously the argument is not entirely satisfactory, and it may be that some other explanation of the efficacy of the long interval between the two successive sections will be found. A more direct and satisfactory proof, on the physiological side, that united nerves may become functional was given by the celebrated experiments of Flourens. This distinguished physiologist cut the two principal trunks of the brachial plexus in the fowl, going one to the upper and one to the lower surface of the wing. The ends were then cross sutured. After some months the bird regained the use of its wing and, more than that, mechanical stimulation of the central end of the upper trunk caused movements of the muscles of the lower surface of the wing and *vice versa*. We have not seen the account given by Flourens of this experiment. From the account given by Milne Edwards the nerves divided and cross sutured were the N. brachialis longus superior (musculo-spiral or radial) and the N. brachialis longus inferior, which gives off branches corresponding to the median and ulnar in mammalia. No more satisfactory experiment than this could be desired. Since Flourens' time similar results on lower animals and surgical operations on the human being have demonstrated beyond question that a severed nerve may become functional again, if the two ends succeed in making union. Whether

on the other hand the distal end of one severed motor nerve will unite with the central end of another has not been so satisfactorily shown. The experiment of Flourens, quoted above, would seem to prove that this is possible, but there has been a lack of corroborative experiments on other motor nerves. It may be said, however, that our own experiments on the median and ulnar nerves in the dog have given us the clearest proof that such union is possible. With reference to the still wider and more difficult question of the possibility of the union of the central end of a sensory nerve with the peripheral end of a motor nerve, or the reverse, the results of the numerous experiments made are conflicting. In fact the question is a complicated one and the results of the experiments are difficult to understand, largely because the whole interpretation turns on the fate of the peripheral nerve. The older experimenters, Bidder, Schiff, Philippeau and Vulpian, *et al.*, used chiefly the hypoglossal and the lingual nerves for experiments, and, as a rule, sutured the peripheral end of the hypoglossal to the central end of the lingual. The idea that lay behind the experiments was to determine whether the motor and sensory fibres can conduct impulses in either direction. Now in the peripheral end of the nerve in the case under consideration it is a question in dispute whether the axis cylinders are outgrowths from the central stump of the lingual, or whether they belong to the peripheral hypoglossal end. In the latter case, moreover, they may be either the old axis cylinders of the hypoglossal which have escaped degeneration, or they may be newly formed by processes of regeneration in the peripheral end. If the axis cylinders are outgrowths from the lingual fibres, then the original purpose of the experiment fails, in part at least, since the peripheral fibres are then in reality sensory fibres. If we leave this point aside and ask simply whether it is possible to unite a sensory nerve to a motor, e.g., the central end of the lingual to the distal end of the hypoglossal, and get union and a functional nerve, the results of the experiments made give a partially satisfactory answer. Philippeau and Vulpian, Bidder, and Rosenthal have been able to show that the two nerves unite, that stimulation of the peripheral end gives sensation and reflex movements, and that stimulation of the central end gives movements of the tongue. The establishment of the sensory paths may be due to the direct union of the old sensory fibres in the hypoglossal (it is known that the hypoglossal normally contains some sensory fibres) with those in the lingual, or to the secondary union of new fibres formed in the peripheral end of the hypoglossal with the sensory fibres of the lingual, or to the

outgrowth of sensory fibres from the lingual end,—the physiological experiments throw no light upon these possibilities. As to the motor phenomena, the movements of the tongue from stimulation of the lingual end of the sutured nerves,—whether the fibres of the peripheral end are outgrowths from the central end, or whether they belong to the peripheral end, and in the latter case whether there is primary union or union after regeneration,—the experiments would seem to prove that a sensory impulse can be transmitted centrifugally, since under the conditions given it must travel peripheralward for some distance over the lingual fibres. But, unfortunately, any conclusion which we might wish to draw from such experiments is rendered uncertain by the discovery made by Philippeau and Vulpian, and afterwards confirmed by Cyon, Bleuler and Lehmann, and by Heidenhain, according to which stimulation of the uninjured lingual nerve after previous section of the hypoglossal will cause movements of the tongue.

This so-called pseudo-motor activity of a sensory nerve seems to be caused, according to the explanation given by Heidenhain, by an increased secretion of lymph, which acts as a stimulus to the partially destroyed end plates of the muscle fibres. The chorda tympani fibres of the lingual are responsible for this action. It is, therefore, open to question whether, in the case of union of central end of lingual with peripheral end of hypoglossal, it is not these efferent fibres in the lingual which grow out, and, making their usual terminations, produce upon stimulation a movement which has been mistaken for a genuine muscular contraction. The experiments of Reichert and Schiff on union of the vagus and hypoglossal are probably open to the same objections. This possibility seems to throw some doubt upon the deductions which have been made from these experiments, so that, as was said in the beginning, the interpretation of the results is at present unsatisfactory. We might sum up this side of the subject by saying that it has been demonstrated that a spinal nerve when severed can reunite with return of functional activity; that with the evidence we have to offer, it may equally be asserted that the peripheral end of one spinal nerve can unite with the central end of another with return of function; but that there are no completely satisfactory experiments to show that a pure motor trunk can unite with a pure sensory trunk with return of function to the peripheral portion.

Although the physiological fact is very clear that a severed spinal nerve can reunite, with or without suture, and become functional, the

series of histological changes through which this is effected is not at all clearly made out. The problem to the older histologists was confined only to the cicatrix that united the two ends. It was soon demonstrated, however, by Schwann, by Steinruck, by Nasse, by Guenther and Schoen and others, that this intermediate piece contains true nerve fibres. Nasse also described with accuracy the degenerative changes which take place in the peripheral end after section. He was apparently the first to observe the breaking-up of the myeline sheath into segments and afterwards into irregular masses and small balls. Nasse seems to have believed, however, that these degenerative changes affect the peripheral end only when functional union with the central end does not take place. Though he and others, therefore, knew that when a peripheral nerve is separated from the nerve centres the portion separated undergoes degeneration, it is to the insistence of Waller, in his numerous publications, that we owe the general acceptance of this, the most fundamental fact in connection with the subject of nerve injuries. Waller, indeed, believed that in every case of section of a nerve trunk, whether union is made with the central end or not, the entire peripheral portion undergoes degeneration, and, moreover, that this degeneration is complete, not only the myeline but the axis cylinder and the sheath of Schwann as well going to ground.

How far Waller's views are accepted at present it is hard to say. Everyone believes that when a nerve is sectioned and the two ends are not brought together the peripheral end undergoes degeneration. But even under these circumstances there is no agreement as to the extent of the degenerative changes. All admit that the myeline disappears. Indeed this phenomenon is too evident to permit of any discussion. The descriptions given by the various observers of the degeneration of the myeline are in general the same, and are as follows. The continuous sheath of myeline first breaks up into cylindrical segments, corresponding, according to the observations of Ranvier, Colasanti and others, to the segments of Lantermann. These gradually disintegrate into smaller fragments and balls, and are eventually absorbed, though the process is slow, especially towards the end. Even after regeneration (in cases of union) has set in, scattered balls of myeline are found in the sheaths of the old fibres. Neumann, Eichhorst, Mayer, *et al.*, speak of the myeline as simply undergoing a transformation, not a genuine absorption, of such a character that at the time of regeneration it can be reconverted into a new myeline sheath. That the myeline undergoes a

chemical change during the degeneration has been noticed by Ranvier, Neumann, and others, and is shown by the difference in its staining with osmic acid and other reagents as compared with the normal fibre. The great majority of writers, however, believe that this is only a step in the process of complete absorption. According to some accounts (Erb, Tizzoni, Neumann) the degenerative changes begin at the wound and spread centrifugally, but most observers (Schiff, Hertz, Lent, Engelmann, Weir Mitchell, Colasanti, Benecke, Eichhorst) state that the segmentation of the myeline begins simultaneously throughout the peripheral end. The belief of Erb and Neumann is based upon the study of severed nerves in frogs where the changes are slower than in mammals. Perhaps their view is explained by the observations of Engelmann and of Colasanti upon the differences between the immediate and the secondary effect of section. Engelmann states that immediately after section (in frogs) degeneration of the myeline takes place in the central and the peripheral ends in the internodal segments of the fibres directly involved in the section. This immediate degeneration spreads only as far as the 1st node of Ranvier. Some days afterwards there comes on a simultaneous fragmentation of the myeline throughout the whole peripheral trunk, owing to the nutritive changes resulting from the separation of the fibres from their centres.

With regard to the fate of the axis cylinders in the peripheral end there has been a greater divergence of opinion, among the earlier observers at least. Many (Schiff, Philippeau and Vulpian, Erb, Weir Mitchell, Remak, Bidder, Korybutt-Daskiewicz, Wolberg) assert that the axis cylinder remains intact during the degenerative changes. In his book on electro-therapeutics when speaking of nerve degeneration Erb describes the degeneration of the axis, from which we may infer that he has changed his view since the publication of his paper. Neumann and Eichhorst advocate the view that the substance of the axis cylinder undergoes a metamorphosis during degeneration, but does not actually disappear. Its substance becomes fused with that of the altered myeline to make the so-called protoplasm of the new fibre during the first period of regeneration. This apparently homogeneous protoplasm afterwards differentiates with the formation of a new axis cylinder and medullary sheath. Most of the recent papers, however, agree in stating that the axis cylinder goes to ground along with the myeline. Ranvier, Waller, Benecke, Leegaard and others take this view. Indeed it is difficult to understand, with the

modern methods of staining, how anyone can doubt that the axis cylinder is destroyed. Its fragmentation and gradual absorption are certainly as plain as the simultaneous changes in the myeline. Nevertheless Wolberg in a very recent paper states his belief that the axis cylinder persists, and in Vol. II. of the *Handbuch der Physiologie* Hermann, after summing up the various views, states his belief that the axis cylinder remains unchanged during the degeneration. According to Ranvier, the first actual disruption of the axis cylinder takes place at the level of the internodal nuclei. At these points the active growth of the protoplasm surrounding the nucleus breaks the myeline and the axis cylinder at about the fourth day after the section (in dog), at the time, in fact, when physiological examination first shows a loss of irritability. This mechanical explanation of the beginning of the fragmentation of the axis cylinder and the myeline is not supported by later observers,—Colasanti, Leegaard and others. In fact the immediate cause of the breaking-up of the myeline as well as of the axis does not show itself to the microscope. According to the best descriptions the axis breaks up along with the myeline and gradually disappears during the absorption changes which take place, though for many days it is visible in the blocks and balls of myeline, first as a central thread, oftentimes coiled more or less, and later as irregular fragments. The modern view of the axis cylinder as an enormously elongated process of a nerve cell,—a view which is accepted both upon embryological and physiological grounds,—almost makes it necessary for us to believe that after separation from its nutrient cell it should undergo destruction and absorption; though independently of any theory the disappearance of the axis cylinder will be accepted as demonstrated by anyone who reads the recent literature upon the subject.

The changes, if any, undergone by the primitive sheath during peripheral degeneration are not so well known. It is agreed by all (with the exception of Engelmann, Schiff and Wolberg) that the nuclei of the sheath undergo proliferation, but this is so intimately associated with the process of regeneration that it will be best to speak of it in that connection. As to the sheath itself, it was formerly thought by most writers to remain intact as the sheath of the new fibres formed during regeneration. Waller, it is true, believed that it disappeared along with the myeline and axis. Ranvier seems to hold the same view. Neumann and Benecke believe that after the new nerve fibre is formed within the sheath of the old one, this

sheath is gradually displaced outward and becomes a part of the general endoneurium. Leegaard, on the other hand, seems to believe that the sheath persists. Perhaps it may be fairly said that no one has really been able to satisfactorily determine what becomes of the old sheath. That a nerve fibre or several nerve fibres are formed within it in the beginning of regeneration is stated by many, but the later history of the sheath has not been traced. This is partly due to the fact that it is not possible to stain it differentially from the surrounding connective tissue, and partly no doubt arises from the feeling that it is a comparatively unimportant part of the fibre.

Though all are agreed that a degeneration of some or of all of the elements of the fibres takes place in the peripheral end when union is not made with the central end, there is no such unanimity of opinion as to what follows if connection between the two ends is made shortly after division. Up to the time of Nasse and Waller it was generally believed that regeneration consisted in the formation of new fibres between the cut ends, and that the peripheral end suffered no degeneration, union taking place *per primam intentionem.* At the present day the possibility of such a union is still discussed, especially among the surgeons. A number of cases in human surgery are on record, in which, apparently, after suture of the ends of the cut nerves, there was an immediate return of motion or of sensation or of both. It cannot be said that any of these cases are entirely satisfactory. In none of them, naturally, could there be any histological examination of the peripheral end; in some the cases are so badly reported that it is not possible to accept them as they stand, and in the best of them there is always the possibility that the so-called return of sensation or of motion may be in reality an example of supplementary or substituted sensibility or motion. The extent to which supplementary or vicarious sensibility and motion may be developed has been admirably shown for the human being by Letievant, Weir Mitchell and others, and for dogs by the well-known experiments of Arloing and Tripier. The surgical and anatomical criticisms we hope to take up in the section devoted to that part of the subject. At present it will be necessary to consider only the evidence for union by first intention derived from experiments on lower animals. The term, union by first intention, when applied to nerve trunks means, when taken literally, an immediate attachment end to end of the severed fibres without degeneration and with immediate or rapid return of function. But the meaning of the term is extended by some, Wolberg for example, to include the union of the fibres of the

peripheral end, without previous degeneration, with those of the central end, by means of new fibres formed in the cicatricial tissue between the cut ends. The essential idea of union by first intention is, therefore, in this case, a union of the two ends without degeneration in the peripheral portion. Bruch reports a single case of this kind upon a young kitten. The details of this we have not been able to obtain, and cannot therefore attempt to criticise it. Bruch himself seems to have considered it very exceptional. He states his belief that in the adult, at least, peripheral degeneration must always take place. Gluck is quoted most extensively as having shown the possibility of union by first intention. His experiments are of two kinds. In the first place he repeated the old experiment of Cruikshank of cutting and suturing the vagus nerve of one side (in rabbits), and after a certain interval cutting the nerve of the other side. The argument is that if the sutured nerve on the first side unites and becomes functional, the section of the nerve on the other side will not cause death. In the rabbit section of the vagus on both sides causes death, according to Gluck, in 30 to 36 hours. If he left an interval of only 8 days between the operations he found that the animal lived from 80 to 90 hours after the second operation. He considers this result a proof that the first nerve was partly functional, and since the time was very short, he concludes that union must have taken place by first intention. This conclusion is entirely unjustifiable unless there is at the same time histological proof that no degenerative changes had taken place in the peripheral end. But Gluck does not seem to have made a histological examination of any kind. Even after an interval of 30 days between the two operations section of the vagus eventually proved fatal, and, after that long interval, he could not obtain inhibition of the heart by direct stimulation of the vagus. The mere result that leaving 8 days between the two operations enabled the animal to live some 40 to 50 hours longer than if both nerves had been cut at the same time, does not prove that functional union had taken place. If this short respite from death is not accounted for by a degree of adaptation acquired by the animal within that time, it is still within the range of probability that degeneration had taken place in the peripheral end up to the point of the production of "embryonic fibres" which, as the experiments of Erb, Leegaard, *et al.*, indicate, and as our experiments seem to prove, are at least capable of conducting impulses. It must also be borne in mind that the vagus contains a large number of non-medullated fibres, and the degeneration and regeneration and the union by first

or second intention of this kind of fibre are subjects which as yet have not been investigated. It seems evident that this old experiment on the vagus cannot be used to demonstrate union by first intention without corroborative histological work. Bakowiecki reports a similar but more successful experiment on the vagus in which also an interval of 8 days was left between the two operations. Naturally the same objections apply to it. The experiment of Gluck to which most importance must be attached is the following: The left sciatic nerve of a fowl was cut and sutured. At first there was complete paralysis of the limb, but movements began to return within 50 hours, and were perfect in 86 hours. At this time also there was a return of sensation to the foot. Gluck then dissected out the sciatic and found that stimulation, mechanical as well as electrical, of the nerve above the wound caused contractions of the muscles supplied by the sciatic. He obtained similar results after 70, 80, and 90 hours, and 6, 8, and 11 days. The results in these cases are positive and unmistakable, the only apparent omission is the failure to make a thorough histological examination of the peripheral nerves. Yet when we remember how many sections and sutures of peripheral nerves have been made by other observers with contrary results, we are justified in hesitating to accept these experiments unreservedly until they have been corroborated by others, especially as Wolberg, who is a firm believer in union by first intention, was unable to obtain similar results in seven experiments also made upon fowls, peripheral degeneration occurring in all of his cases. Wolberg reports one experiment made upon the sciatic of a cat which he claims to be a case of union by first intention, but this experiment is incomplete and open to obvious objections. The sciatic nerve was exposed and with a sharp knife one bundle was cut, the others being untouched. The gap in the nerve was sutured and the animal, after 94 hours, was killed and examined. No trace of degeneration could be found in either trunk, though previous experiments had shown him that in a cat degeneration is apparent in the peripheral end after 44 hours. It must be remembered that different animals of the same species show considerable variation as to the time when degeneration begins,—the experiments of others as well as our own (see "Experimental Work") give striking proof of this. Moreover the peculiar nature of the operation, the incomplete interruption of the lymph and blood supply, may have made the changes of degeneration slower in appearing. The experiment is not convincing, and it is difficult to see what mechanical or operative advantage it

had over a complete section or crushing of the nerve that should have led to such an exceptional result.

In addition to the degenerative changes which take place in the peripheral end most authors describe similar changes as occurring in the central end, in the immediate neighbourhood of the wound. Usually these changes extend only to the first or second node of Ranvier above the wound. There has been some discussion as to whether the first node forms an impassable boundary to this ascending degeneration. The general opinion seems to be that the first node does not form a necessary limit, though this is obviously a difficult and perhaps unimportant point to settle. The degeneration in this case seems to be essentially a traumatic effect and therefore might vary with the extent of the injury. Most authors state that the degeneration in the central end takes substantially the same course as that in the peripheral end. Ranvier, however, ascribes the change to a very peculiar action of leucocytes. These leucocytes migrate into the nerve fibres and devour the myeline as far as the first node, and may, in addition, cause the destruction of the axis and sheath. As far as we are aware these observations have not been supported by recent investigations.

Following upon the degeneration there comes a period of regeneration in cases of successful union, during which new nerve fibres are built up. The greatest difference of opinion exists as to the nature of these changes. Almost everyone who has published anything upon the subject differs to some degree at least from other writers, so that to present completely all the theories could only be done by briefly reviewing each of the important papers. If we consider only the general character of the regenerative changes, the various theories may be grouped more or less satisfactorily. We have, in the first place, the somewhat peculiar view of Neumann, which is supported by Eichhorst and, apparently, by Mayer. According to Neumann's description, the degenerative changes affect both myeline and axis in such a way as to cause a chemical alteration, resulting in the production of a common protoplasmic substance, lying in the old sheath and representing the material of the old myeline and axis. The process of regeneration consists in a differentiation of this common substance into a new axis and a new myeline. The new fibres appear within the protoplasmic substance as narrow, pale fibres with, at first, a delicate layer of myeline surrounding a relatively large axis. They appear first near the central end and grow toward the periphery. Nevertheless this growth is discontinuous. Each internodal segment of the new fibre

originates separately and becomes united afterwards to the segment preceding; thus giving an idea of the meaning of the nodes in the adult fibres. As to the immediate cause of this differentiation of the protoplasmic substance of the fibres he ascribes it to the influence of impulses originating in the centre and passing over the fibres from centre to periphery. This seems too indefinite an hypothesis to be satisfactory, yet it expresses a fact which Neumann was the first to clearly demonstrate, namely, that regeneration can only take place after union with the centre and always proceed centrifugally. The peculiarity of this theory seems to lie chiefly in the view that the new myeline and axis are formed directly from the transformed substance of the old, and are not newly made *ab initio*. Ranvier designates the theory as *bizarre*, but as a matter of fact no paper published on the subject contains such a complete and accurate description of the changes of degeneration and regeneration as that of Neumann.

Those who believe that the axis cylinder persists throughout peripheral degeneration need to explain in the regeneration only the appearance of new myeline and possibly of a new sheath. Remak thought that the persistent axis cylinders underwent longitudinal division with the formation of several new fibres within the old sheath, but he says nothing of the origin of the myeline. Erb describes the myeline as forming first near the wound as a thin continuous tube round the axis cylinder, but gives no explanation of its origin. Wolberg describes the formation of entirely new fibres in the cicatricial tissue from the round or oval cells of the perineurium. By means of these fibres connection is established between the central and peripheral ends and then, when the union is not by first intention, a deposition of myeline begins around the axis cylinders in the peripheral fibres. He thinks that in some way the nuclei of the sheath are connected with the production of myeline, but the details he was not able to discover.

Those who believe in the destruction of axis and myeline during peripheral degeneration must explain the origin of both in regeneration. The most fundamental difference of opinion appears with reference to the axis cylinder. Waller and Ranvier have taught that the new axis is an outgrowth from the central end, and Ranvier's special contribution to the subject is his description of how the axis cylinders in the central end branch and send down two or more processes which penetrate the cicatrix, reach the peripheral end, and then start the formation of new fibres. This view is so thoroughly in accord with our

present conceptions of the axis cylinder as a process of the nerve cell in which it originates that it has come to be generally accepted. Nevertheless the best of the recent papers which have treated the subject from a purely histological standpoint agree in stating that the axis is developed anew in the peripheral end and is connected secondarily with the central end. This, for instance, is the view of Neumann as described above. Leegaard also describes the formation of a new axis and myeline from the protoplasm which comes to fill the old sheaths after the absorption of the old myeline and axis. The new axis and myeline are formed discontinuously in this protoplasm and afterwards fuse to a continuous fibre. He claims to have followed these changes, but can give no explanation of what initiates them. Benecke describes regeneration in the peripheral fibres as follows: A few days after degeneration has set in the nuclei of the sheath begin to multiply. They increase rapidly, and, after the absorption of the old myeline and axis, are the only structures found in the sheath. These nuclei, or the cells to which they belong, elongate, become spindle-shaped, and fuse to form slender threads lying within the sheaths. Eventually they are transformed to young fibres in this way. The new myeline develops first in the neighbourhood of the nuclei and gradually forms a continuous sheath on the periphery of the fibre. The central core persists as the axis cylinder.

Those who maintain that the new axis cylinders are sprouted from the old ones of the central end do not claim to have seen the process. As Ranvier says, the fibres in process of regeneration, on account of their intricacy, are very difficult objects of study, especially in the neighbourhood of the wound at the junction with the old fibres. Nothing is easier than to find places where old fibres pass into new ones, but the closest study with the methods of staining heretofore used, such as osmic acid, shows nothing of the axis cylinder at the point of transition.

The theories of the origin of the axis cylinder though fundamentally different are at least clearly stated, but the development of the new myeline is usually described in the vaguest terms. One is justified in believing that the origin of the myeline in regeneration must be essentially similar to its development in the embryo, but the accounts in the latter case are just as unsatisfactory. Waller, Bruch, and others thought that the myeline in regeneration, as well as the axis, is formed from the central stump. Ranvier, in the papers to which we have had access, speaks of the development of the new nerve

fibres from the central end, but apparently means to include in this only the axis cylinders. In his Pathological Histology (Cornil and Ranvier) he states that "The membrane of Schwann, the cellular elements which lie in it, and the medullary sheath are alone formed from the proliferating cellular elements which have accumulated in the interior of the old tube." In his "Traité Technique" Ranvier speaks of each internodal segment of myeline as corresponding to a single cell, and this conception is quite generally prevalent at the present time. According to Vignal the myeline cells are of epiblastic origin, they apply themselves to the axis cylinder during its outgrowth, and form in this way a continuous sheath in which the internodal segments are at first much shorter than in the adult. This view of the origin of the myeline is difficult to reconcile with what is seen during regeneration. A more satisfactory hypothesis is given by Koelliker in a recent article. He makes the statement that in the spinal nerves the myeline is undoubtedly developed in the periphery, but not from connective tissue cells. The nerve fibre in the embryo starts as a protoplasmic outgrowth from a nerve cell. In this process a central core becomes differentiated from a circumferential layer; later the core becomes the axis and the outside layer the myeline, thus making the myeline a part of the original process of the nerve cell. The formation of the myeline is however influenced in some way by the nuclei scattered along the fibre though he is not able to state in just what manner this influence is exerted. Koelliker's work was done upon the batrachian embryo, but his theory has many points of resemblance to those of Neumann, Leegaard and Benecke given above. It is worthy of special remark that Koelliker in the developing fibre, and Neumann, Benecke, Bruch, and Wolberg in the regenerating fibre attribute an action of some kind to the nuclei, the so-called nuclei of the sheath, in the production of the myeline. The older theories of Laveran, Robin, and others, according to which the liquid myeline was formed from the cells of the sheath as a sort of secretion need not be described in detail.

Finally in the regeneration of severed nerves we have to explain the formation of new fibres in the cicatricial tissue uniting the two ends as well as in the peripheral and central ends. Those who believe with Waller and Ranvier that the new fibres grow out from the central end, suppose that the new fibres simply penetrate the cicatrix on their way to the peripheral end. Those who believe in a development of new fibres in the peripheral end within the old sheaths, either

say nothing of the events taking place in the cicatrix, or suppose that the newly formed fibres penetrate the intervening tissue from the central end, or from both central and peripheral ends. Others, as Gluck, Laveran, Wolberg, suppose that new fibres are formed within the cicatricial tissue itself from leucocytes or from connective tissue cells.

One other hypothesis needs a brief mention before completing this review. Philippeau and Vulpian asserted that new nerve fibres may be formed within the peripheral end of a severed nerve, even if no connection is made with the central end. They spoke of this process as autogenetic regeneration, but in their latest work acknowledged that the regeneration required a much longer time under such conditions, and was never so complete as when union with the central end was made. For instance, in young animals autogenetic regeneration did not begin after 40 days, while in successful sutures it was far advanced at 28 days. In adults regeneration after suture was apparent in 60 days, but, when union was not made, was not perceptible after several months. No one seems to have supported this view within recent times except Bowlby in his book "Injuries and Diseases of Nerves." He gives in this book a brief and very incomplete statement of a microscopic examination of the peripheral end of the divided nerve in three cases of secondary suture, the times between the injury and the operation being 7, 9 and 24 months respectively. He says that he was able to trace all stages of the development of the new fibres, and gives several illustrations which "show these conditions very plainly." If we are permitted to make a judgment from these cuts and from the incomplete description which is given of the methods of examination, there would seem to be little doubt that Bowlby is in error. The only cut which is intelligible (Fig. 10, Cross section of nerve 7 months after injury) will be recognized by anyone who has made transverse sections of peripheral nerves after degeneration, as showing the cross sections of nuclei with central nucleoli and not of nerve fibres with central axes. Teased preparations of the nerve give the clearest proof of this. We feel justified in making this statement because of the similarity of this drawing to many preparations we have made of the peripheral end of divided nerves in lower animals and also in the human nerve in a case of secondary suture nearly 7 months after injury. Teased preparations taken together with cross sections show that the appearance in cross section is due to the nuclei and nucleoli, the nuclei, as everyone has described, being very abundant in the

tissue of the peripheral end under these conditions. It will be noticed that in Bowlby's figure no nuclei are represented. We shall have something further to say of this and of what we conceive to be the explanation of the statements of Philippeau and Vulpian when we describe our own observations upon regeneration[1].

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[1] This paper was completed and sent to the Secretary of the American Physiological Society, September 1891. Since that time a new paper on the subject has been published by Büngner (*Ziegler's Beiträge z. pathol. Anat. u. z. allg. Pathol.* x. 4, S. 321). We have seen only the abstract of this paper which appeared in the *Centralbl. f. Physiol.* v. No. 23, Feb. 13, 1892. Büngner's experiments were made upon dogs and guinea-pigs. He was also unable to get union by first intention. His histological account of the processes of regeneration agrees in some points with our own, but with regard to the development of the new axis and myeline in the peripheral fibres, his results are fundamentally different from those given in this paper.

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PART II.

Experimental Work.

Our experimental work has been partly physiological and partly histological; it will make the presentation of results easier if we describe them, to a certain extent, separately.

Physiological Experiments.

The animals used, with one exception, were dogs. The nerves experimented upon were the ulnar on the two sides, and in the case of cross sutures the median and the ulnar. Our operations were all made with antiseptic precautions, the instruments being sterilized with carbolic acid 5%, and the wounds carefully washed during and after the operation with corrosive sublimate solutions 1 to 8000, or with sterilized water. For suturing the nerves carbolized catgut or catgut in juniper oil was used. In almost every case two sutures were employed to bring the ends of the nerve together, one upon each side, and the needle was passed through the epineurium, wounding the nerve itself as little as possible. The skin wounds, which, on account of the superficial position of the nerves, were always slight, were sewed with catgut or sterilized linen thread. With one or two exceptions the wounds healed nicely, though not always by first intention. In the exceptional cases there was a little suppuration, but not enough to cause any trouble, or to interfere, as far as we could ascertain, with the results. In destroying the nerve to bring about degeneration we employed three methods. In most cases the nerve was lifted with a thread and cut with sharp scissors, and, in order that there should be as favourable an opportunity as possible for immediate union, the two ends were sutured at once. In other cases the nerve was crushed by tying tightly around it a single stout ligature of catgut. The ligature was immediately loosened and the completeness of the destruction at the point of ligation was tested by stimulating the nerve above the wound with moderate induction currents. If the block was complete no flexion of the paw or digits was obtained. Lastly, in order to vary the conditions as much as possible, the nerve was coagulated over a small area, an eighth of an inch or more, by circulating round it a stream of water heated to 80° C. The circulation was continued until stimulation above gave no movements of flexion in the foot. At first we used a silver

Tabular Synopsis of the Records of the Operations and Physiological Examinations.

Number of Experiment.	Operation.	Time between operation and examination.	Results of Examination.
XX	Ulnar, left side sutured „ right side sutured	L. S. 4 days R. S. 2 „	L. S. Complete absence of irritability R. S. Irritability not completely gone
XXIII	Ulnar, l. s. cut, but not sutured „ r. s. „ „ „	L. S. 3 days R. S. 1 „	L. S. Irritability preserved R. S. „ „
XII	Ulnar, l. s. cut, not sutured „ r. s. „ „	L. S. 4 days R. S. 4 „	L. S. Irritability preserved R. S. „ „
XIII	Ulnar, l. s. cut, not sutured „ r. s. „ „	L. S. 4 days R. S. 4 „	L. S. Irritability completely absent R. S. „ „ „
III	Ulnar, l. s. sutured „ r. s. crushed	L. S. 7 days R. S. 7 „	L. S. Irritability completely absent R. S. „ „ „
VI	Ulnar, l. s. sutured „ r. s. crushed	L. S. 7 days R. S. 7 „	L. S. Irritability completely absent R. S. „ „ „
X	Ulnar, l. s. coagulated „ r. s. crushed	L. S. 7 days R. S. 7 „	L. S. Irritability completely absent R. S. „ „ „
XI	Ulnar, l. s. coagulated „ r. s. „	L. S. 9 days R. S. 9 „	L. S. Irritability completely absent R. S. „ „ „
XV	Ulnar, l. s. coagulated „ r. s. cut, not sutured	L. S. 12 days R. S. 12 „	L. S. Irritability completely absent R. S. „ „ „

VII	Ulnar, l. s. sutured	L. S.	14 days	L. S.	Irritability completely absent
	„ r. s. crushed	R. S.	14 „	R. S.	„ „ „
VIII	Ulnar, l. s. sutured	L. S.	22 days	L. S.	Beginning of return of irritability near wound
	„ r. s. crushed	R. S.	22 „	R. S.	More distinct return of irritability near wound
IX	Ulnar, l. s. sutured	L. S.	21 days	L. S.	Distinct return of irritability near wound
	„ r. s. crushed	R. S.	21 „	R. S.	„ „ „ „ „
XIV	Ulnar, l. s. coagulated	L. S.	21 days	L. S.	Irritability completely absent
	„ r. s. cut, not sutured	R. S.	21 „	R. S.	„ „ „
XVII	Ulnar } l. s. double cross suture of median } ulnar and median	L. S.	28 days	L. S.	No return of irritability
	Ulnar r. s. sutured	R. S.	28 „	R. S.	Partial return of irritability
XVI	Ulnar } l. s. double cross suture of median } ulnar and median	L. S.	35 days	L. S.	Beginning of return to sensory fibres near wound
	Ulnar, r. s. coagulated	R. S.	35 „	R. S.	Partial return of irritability
XVIII	Ulnar, l. s. sutured after 1 hour	L. S.	35 days	L. S.	Partial return of irritability
	„ r. s. sutured immediately	R. S.	35 „	R. S.	More complete return, proceeding centrifugally
XIX	Ulnar } l. s. cross suture of median } to ulnar	L. S.	49 days	L. S.	Unsatisfactory evidence of any return
	Ulnar, r. s. sutured	R. S.	49 „	R. S.	Quite complete irritability far as middle of forearm
XXII	Ulnar } l. s. cross suture of median } to ulnar	L. S.	75 days	L. S.	Very complete return to both motor and sensory fibres
	Ulnar, r. s. sutured	R. S.	75 „	R. S.	Almost normal conditions of irritability
Rabbit XXVI	Ulnar, l. s. cut, not sutured	L. S.	70 days	L. S.	Complete absence of irritability
	„ r. s. crushed	R. S.	70 „	R. S.	Almost normal conditions of irritability

cannula especially made for the purpose, but later a piece of glass tubing conveniently bent to encircle the nerve was found to be more satisfactory. In all cases the animals were narcotized with morphia and ether during the operations. After a certain time the animal was submitted to examination. He was narcotized as usual, the nerve was laid bare, and its irritability tested in various ways by direct stimulation. For stimulating we used ordinarily induction shocks from a Du Bois Reymond coil, and generally the unipolar method was employed in order to minimize the danger of radiation of stimulus. In this case the indifferent electrode was applied to the skin over the sternum, after shaving off the hair, while the nerve was touched on different sides with the slender stimulating electrode. In some cases the nerve was stimulated also in the ordinary way with catheter electrodes. In addition a small hammer of hard rubber was made for mechanical stimulation. In using this a small block of hard rubber was slipped under the nerve and then light blows were struck with the hammer. This form of stimulation often proved more efficacious than the electrical, as the records of the experiments will show. In examining the nerve we began usually above the wound, and tested its irritability from that point downward with more or less completeness, according to the indications, as far as the wrist. Where it was thought advisable the flexor carpi ulnaris and flexor profundus muscles were exposed in order that their contractions might be observed directly. In some cases the irritability of the flexor muscles to direct stimulation was also tested, though this idea was not carried out with sufficient thoroughness to make the results valuable. In the beginning some attempt was made to observe the loss and return of sensation and motion on the living animal before the final examination, but the results were so unsatisfactory that this method was afterwards abandoned. For the purpose we had in view the direct stimulation of the nerve seemed to be all that was necessary. We give (pp. 358, 359) a description of all the experiments made as they were recorded at the time. Afterwards we shall discuss the conclusions which seem to be justified by these experiments.

A careful examination of the facts recorded in the preceding tables taken in connection with the histological study of the nerves, the details of which will be given later, enable us to draw the following conclusions:

1. In none of our experiments was there a union of the nerve by first intention. In all cases the peripheral end degenerated completely throughout its whole length. Whether the nerve was cut and sutured,

or crushed, or coagulated, made no difference in this respect. Histological examination showed the degeneration of the fibres in every case, and direct physiological examination proved that after the first four days there was complete loss of irritability and conductivity in both motor and sensory fibres which lasted until the regenerative changes—as shown by histological examination—were well under way. We used very young dogs as well as those that were full grown, and our experiments, like those of others who have made similar observations, prove that in the dog reunion and return of function in severed nerves without degeneration of the distal portion is not possible.

2. With reference to the time after the section is made before loss of irritability comes on, our experiments show that it varies greatly with the animal. The record shows that in experiment XX., which was upon a very young bull pup, there was complete loss of irritability in 4 days and a partial loss in 2 days. In experiment XXIII., which was upon an apparently full-grown dog, the irritability of the peripheral end had not disappeared in 3 days. In experiment XII., recorded as a young dog, irritability in the peripheral end was perfectly normal 4 days after section. While in experiment XIII., recorded as full grown, the irritability at the end of 4 days had completely disappeared. It is usually stated that the younger the dog the more rapid the loss of irritability in the peripheral end, but it seems certain that other individual differences in the animals, which cannot be definitely determined, also influence the result. Guènther and Schoen, Landry, Ranvier and others have found also that in dogs the average time at which irritability disappears is the fourth day.

3. The return of irritability and conductivity to the peripheral end after union with the intact central end is made takes place readily, in primary sutures, but gradually. The first certain indications of a return of function, when treated by our method of direct excitation of the nerve, were noticed at the end of 21 days, in experiments VIII. and IX. Experiment VIII. was made upon a young bull-dog and experiment IX. upon a small mongrel which, however, seemed to be full grown. In each of these animals the ulnar was cut and sutured on the left side and crushed on the right. The return of irritability was imperfect, but it will be noticed that it was slightly better on the side crushed than on the side sutured, showing that the more normal coaptation of the ends of the fibres secured by the former method does make a difference in the rapidity of regeneration. It is surprising that the difference is not more marked. With reference to the third method of interrupting

the physiological continuity of the fibres, the method of coagulation, the time necessary for return of function was much longer. At the end of 21 days there was no sign of a return of irritability, experiment XIV., and even at the end of 35 days, experiment XVI., the return of function was very imperfect and was manifest only in the neighbourhood of the wound. This result is easily explained. The coagulation caused a dead area of an eighth of an inch or more in which, of course, the nerve fibres were completely destroyed, so that the experiment was equivalent to removing a piece of nerve equal in extent to the coagulated area, except that a perfect union of the ends was secured.

A study of the cases in which functional activity had begun to return to the peripheral end, viz., experiments VIII., IX., XVII., XVI., XVIII., XIX., XXII., and XXVI., develops the following interesting points, the connection of which with the corresponding histological changes will be referred to briefly, though for fuller histological details the reader is referred to the following section.

4. The irritability in all cases appeared first, after union was made, in the neighbourhood of the wound, and from that point spread centrifugally, apparently with great slowness. In the later stages, when return of function could be detected as far as the middle of the forearm or farther, the irritability was found to be greatest at the wound and to grow less and less as one passed toward the periphery (see experiments XIX. and XXII.), indicating that the return of irritability at any one level was at first imperfect and gradually increased to normal. Histological examination of these same nerves showed that irritability had only returned to those portions of the peripheral nerve in which the regeneration had proceeded so far that some completely formed though small medullated fibres could be found. By completely formed fibres is meant those possessing both myeline sheath and axis cylinder. Moreover the gradual increase in the irritability at any one level seemed to go hand in hand with the increase in the number of these newly-formed fibres. The increase in irritability, therefore, noticed in going toward the wound was probably not due in the motor fibres, at least, to any progressive change in the individual fibres as they matured, but to the greater number of fibres which were capable of excitation and the greater muscular response thereby produced. This statement, however, is not meant to exclude the fact that in the first stages of regeneration the new fibre possesses a lower degree of irritability than the fully formed fibre; proof for this is given under the next heading. But the gradual betterment in the irritability of

the nerve trunk as a whole seemed to keep pace histologically with the increase in the number of the completely formed fibres rather than with a progressive regeneration in the single fibres.

5. In many cases it was distinctly seen that a return of function to the sensory fibres preceded the return to the motor fibres (experiments VIII., IX., XVII., XVI., XVIII., R. S. especially, XIX., XXII.). Whether this means that the sensory fibres regenerate more quickly than the motor, it is impossible to determine histologically, in the experiments as we made them at least. But we are inclined to give to it another explanation. Either the form of stimulation generally used in these cases (strong mechanical) was more effective upon imperfectly regenerated sensory fibres than upon imperfectly regenerated motor fibres, or possibly there was a greater difficulty in making proper connections on the part of the motor fibres. We may imagine that once a functional connection at the wound has been established the path for the sensory fibres will be open, but for the motor fibres connection with the muscle fibres by end plates must also be made, and this may require a longer time. The only safe statement we can make, however, is that in going down the peripheral trunk from the wound, the return of irritability to the sensory fibres is in advance of that to the motor fibres.

6. Another point of interest to the physiologists brought out by the experiments is the difference in the action of the various forms of stimuli, particularly the mechanical and electrical. This was noticed distinctly in the reactions of both the motor and the sensory fibres, and most clearly at the beginning of the return of irritability. As shown by the records of experiments XVII. and IX. especially, light mechanical stimulation was often more effective in exciting the fibres than quite strong induction shocks. As regeneration advanced and more and more of the fibres assumed their normal structure this difference became less noticeable. The efficacy of mechanical over electrical stimulation was most strikingly shown in the stimulation of sensory fibres, a fact which has already been mentioned. During the course of regeneration from the 3rd to the 7th week, at least, the lower portion of the ulnar nerve from the middle of the forearm to the wrist was quite unirritable to the strongest induction shocks. Yet somewhere in this stretch, varying with the stage of regeneration, cutting the nerve or crushing it with a ligature would arouse sensory impulses, as shown by the violent reflex movements of the trunk and limbs. Our histological examinations showed that at these levels the vast majority, if not all of the nerve fibres, were in that first stage of regeneration which we have

called the embryonic stage, since at this time the fibres resemble in structure those of early embryonic life. These embryonic fibres are described and figured in the histological portion of the paper. It is sufficient to say here that they contain no axis cylinder and no myeline sheath, but consist simply of a membranous sheath filled with a protoplasmic material in which are numerous nuclei occurring at more or less regular intervals. Our stimulation experiments taken in connection with the histological examinations have made us believe that such fibres possess a low degree of irritability, and that the violent mechanical stimulation used was sufficient to arouse in them a form of nerve impulse.

7. Perhaps the most surprising physiological result which came out of our experiments was the marked difference in the time of return of the two important properties of the fibres, namely, irritability and conductivity. The separation of these two properties has frequently been made by physiologists. Schiff has called especial attention to it[1], and Erb makes mention of it in his work upon the regeneration of severed nerves. Erb states that the regenerating fibres will conduct impulses originating above, before an impulse can be aroused in them by direct electrical stimulation. He was under the erroneous impression that the axis cylinder persisted in the peripheral fibres during degeneration and that the chief phenomenon of regeneration was the appearance of a new myeline sheath. He was, therefore, led to make the untenable hypothesis that the presence of the myeline is necessary for the action of electrical stimuli. Our experiments have shown in the clearest way that, for instance, stimulation of the motor branches to the flexor profundus or the flexor carpi ulnaris might be entirely ineffective, while stimulation of the central end of the sutured nerve, of the wound or of portions below the wound at distances varying with the stage of regeneration, would cause a contraction of these muscles. The fibres of the motor branches to these muscles undoubtedly conducted impulses started above, at a time when impulses could not be directly aroused in them. Subsequent histological examination showed that the great majority of the fibres of the motor branches, and in some cases, as far as our examinations could determine, all of the fibres, were in the condition of embryonic fibres described in the preceding paragraph,—fibres, that is, in which neither axis cylinder nor myeline sheath was present. It would seem, then, that the embryonic fibres, though unirritable to electrical stimulation, can conduct nerve impulses

[1] *Physiologie* and *Zeit. f. rat. Med.* III. Reihe, XXIX. 221.

originated above. It seems to us moreover that it is quite within the range of possibility that the paths to the muscles innervated by a cut and sutured nerve may be opened to the passage of a volitional or normal impulse some time before direct electrical stimulation of the central end can cause any contractions. Possibly some of the very rapid returns of the power of movement reported in surgical cases may be due to this difference. It did not occur to us to test the hypothesis while making the experiments, though it could easily be done by laying bare the muscles and attempting to stimulate them reflexly. In the preceding paragraph we have given reasons for believing that the "embryonic fibres" possess a low form of irritability, since they react to strong mechanical stimulation, in addition to the power of conductivity just described. Physiologically then they function as nerve fibres though they contain no axis cylinders. We may conceive that the undeveloped nerve fibres of the young embryo possess similar properties.

8. The return of function in the sutured nerve which began to appear at the 21st day was not perfect at the end of 7 weeks, though nearly so at the end of 11 weeks. We have not attempted to construct a curve of this return of irritability with reference to the time interval, as our experiments were not sufficiently numerous. It seemed to us, however, that the return of function after it first put in an appearance did not improve with the regularity that one would have supposed. After the 3rd week there was apparently a slowing of the regenerative activity and subsequent to the 5th or 6th week a more rapid improvement. But it is more than probable that this irregularity was due to differences in the animals used or in the success of the operations.

9. Another curious fact worth a word of mention is that although functional activity returns first to the fibres near the wound and spreads centrifugally, it does not necessarily follow that the fibres which first branch off from the trunk and have the shortest route to travel are the first to regenerate. In experiment VIII., L. S., the first return was apparently to the fibres distributed to the dorsal interossei of the digits. In experiment XIX., R. S., contractions of the flexor profundus were obtained when the flexor carpi ulnaris remained unstimulated. While both the physiological and histological examinations show that the regeneration begins first at the wound, and travels slowly peripheralward, so that the shorter the route of the fibre, other things being equal, the quicker it will be entirely regenerated, nevertheless, it is apparently true that regeneration does not necessarily begin first in the shortest

fibres. On the contrary, there seems to be a certain amount of irregularity as to the fibres in which regeneration starts first,—an irregularity which will depend doubtless upon variations in the perfection with which the two ends are brought into coaptation by the sutures.

10. In answer to the question whether it is possible to get union with return of function between the central portion of any one spinal nerve and the distal portion of any other, our experiments, though not numerous, are conclusive. In making the experiments we chose the ulnar and median nerves and sutured the central end of the median to the peripheral end of the ulnar. In experiments XVI. and XVII. we also united at the same time the central end of the ulnar to the peripheral end of the median, thus making a double cross suture. This turned out to be unfortunate, as the four ends of the nerves became imbedded at the point of suture in a mass of new cicatricial tissue, so that it was not possible to dissect out the median-ulnar tract from the ulnar-median tract. In these two experiments the time between operation and examination was 4 weeks in experiment XVII. and 5 weeks in experiment XVI. In the first one stimulation of the peripheral end of the ulnar below the median-ulnar wound and above the elbow gave no response whatever. Stimulation of the peripheral median at the same level gave flexion of the paw, but this was afterwards explained by the discovery that in dogs the median receives a motor branch from the musculo-cutaneous a short distance below the place of operation (middle of arm). See notes. In experiment XVI., electrical stimulation of the peripheral ulnar at the same level as in XVII. gave no response at all, but cutting with the scissors caused reflex movements, indicating a beginning of a return of function to the sensory fibres. No safe conclusions, however, could be drawn from this experiment, owing to the condition of the nerves at the point of suture.

In experiment XIX. this error was avoided. The central end of the median was sutured to the peripheral end of the ulnar, and long pieces were then removed from the peripheral stump of the median and the central stump of the ulnar, to prevent the possibility of union. This animal was examined after seven weeks. As the notes record he was a large vigorous hound-pup. Stimulation with electricity of the peripheral ulnar above the elbow but below the wound gave negative results. The results of strong mechanical stimulation, if made, were not recorded. Histological examination of the peripheral ulnar at and immediately below the wound showed an active regeneration of nerve fibres similar in all respects to what was found at the wound of an

ulnar suture. At the wound many newly-formed medullated fibres were found, but at the level of the elbow regeneration had only reached the stage of formation of embryonic fibres.

In experiment XXII., made upon a young healthy spaniel, the same operation was performed as in the preceding experiment. The dog was kept for 75 days and was given especially good treatment in the way of food and exercise. At the examination, the details of which are given in the table of experiments, the union of the two nerves was found to be exceptionally good,—scarcely any swelling being perceptible. Electrical stimulation of the ulnar from the wound down to the wrist gave movements of flexion easily. Stimulation (electrical) of a small cutaneous branch at the level of the wrist and tying a ligature round the trunk of the ulnar at the same level gave reflex movements, showing a return of function to the sensory fibres even to that point. Physiologically, then, there was complete proof that the median and ulnar had united and that the peripheral ulnar had regained its functional activity, both motor and sensory. As stated in the notes, both the flexor carpi ulnaris and the flexor profundus digitorum muscles were exposed during the stimulation of the ulnar and were seen to contract. Histological examination of the peripheral trunk of the ulnar from the wound downward gave specimens practically identical with those from the ulnar of the other side in which an ordinary ulnar suture had been made. In the neighbourhood of the wound very many newly-formed fibres were found, having both medullary sheath and axis cylinder. Lying in among these were many fibres in the "embryonic stage," without myeline or axis. Similar specimens were obtained from the motor branches to the flexor carpi ulnaris and flexor profundus digitorum. As we passed down toward the wrist the newly-formed fibres became less and less numerous, and more of the embryonic fibres were found. At this level were found also capital examples of fibres in which a medullary sheath was just appearing, such as will be described and figured in the histological portion of the paper.

Our experiments prove the possibility of the functional union of two spinal nerves, but apparently the time required for the regeneration of nerve fibres is longer than in the union of two ends of the same nerve. Why this should be is difficult to see from a histological standpoint, and it may be that it was only apparently the case in our experiments because the point of operation was higher up in the arm by two inches, in consequence of which, though the regeneration might begin as quickly, it would be slower in showing its effect physiologically in

causing contractions in the flexor muscles. In this, as in some of the similar experiments, the animal was closely watched to see if there was any paralysis or awkwardness in his movements. We found that at the second day after the operation, with both median and ulnar cut on the left side high in the arm, and with the ulnar cut on the right side at the level of the elbow, there was very little evidence of any paralysis or even awkwardness. The dog jumped in and out of his cage over a board two feet high without any difficulty, though occasionally the jar seemed to give him pain. Stimulation of the left foot with a needle showed sensitiveness to pain everywhere. Before the end of the first week the animal was running a round in perfect freedom, and the closest scrutiny could detect no awkwardness of movement, except possibly in running rapidly upstairs he would frequently stumble with his front feet, but whether this was due to the unusual innervation of the muscles or was caused by the over-zealous activity characteristic of young dogs generally could not be determined. The median and ulnar nerves are closely related in their origin and in their muscular distribution. A more interesting suture would probably be one between the musculo-spiral and ulnar in which centres of origin of extensor fibres would be obliged to innervate flexor muscles. Our experiments show that there is no histological or physiological obstacle to such a union, though the functional use of the nerve by the animal might be attended with considerable awkwardness of movement in the beginning.

Histological.

Methods:—In studying the degeneration and regeneration of the nerve fibres we have used a number of different histological methods some of which proved valuable, while some were almost useless. The nerves taken from the animals after the physiological experiments were, as a rule, preserved with a view to teasing as well as sectioning. In the latter case they were hardened in Mueller's solution for 3 to 6 weeks, being kept meanwhile in a state of physiological extension by the suspension of a weight of 20 grammes or more to one end. The hardened specimens were imbedded in paraffine, cut both longitudinally and transversely, and stained in duplicate in the picric fuchsin stain of Van Giessen, and by the potash gold method of Freud. The former of these stains, while it gives rapid and satisfactory results upon the normal nerve, was found to be less valuable for the degenerating nerve. During the degeneration both axis cylinder and myeline undergo

chemical changes, of such a character that the former instead of taking the fuchsin readily, stains a greenish yellow so that it can be distinguished from the surrounding myeline only with great difficulty. The Freud method proved more satisfactory, but the method of sectioning the nerve was found to be less instructive than teasing so that toward the end of the research it was abandoned except on occasions for purposes of corroboration. In studying the teased nerve we made use of three methods. First, the fresh nerve while pinned out was hardened and stained in osmic acid for 24 hours, and then washed in water for 24 hours, or, in some cases, to facilitate the subsequent teasing for from 6 to 7 days. Next it was partially teased, stained in Boehmer's haematoxylin, and finally teased on the slide in a drop of glycerin or Farrant's solution. This method is chiefly valuable for following the changes in the myeline, it gives poor results for the axis cylinder. The second method was to preserve the nerves in Mueller's solution as described above, then to partially tease the bundles and stain by the potash gold method of Freud. After staining in gold the preparations were still farther treated with Boehmer's haematoxylin to bring out the nuclei, and were then teased out on the slide and mounted in glycerin or Farrant's solution. This method gave very useful preparations. The gold stains the myeline almost, if not quite as well as the osmic acid, and in addition brings out the axis cylinder often with great distinctness. In some cases the axis cylinder would escape staining by this method, although the conditions remained the same, and unfortunately at the time of the first appearance of the axis cylinder in the newly regenerated fibres it was particularly uncertain in its results. The third method which was put into use only toward the end of the work proved of the greatest value in tracing the development or, at least, the first appearance of the new axis cylinders. The method was very simple. The nerves while pinned out were hardened and stained in a saturated picric acid solution for 48 hours. They were then washed in water for 5 or 6 hours and subsequently in 33 % and 50 % alcohol, and finally put in 95 %. They were next partially teased, and then stained somewhat deeply in Böhmer's haematoxylin (exposure of 10–15 minutes); the stained bundles of fibres were next carefully teased and mounted in glycerin or Farrant's solution. The picric acid in connection with the Böhmer's seems to eat out the myeline, and thus allows the axis cylinder to be stained easily with the haematoxylin. In all the cases operated upon and reported in the table of physio-

logical experiments, and in some not there reported, the nerves were treated by one or all of the above-described methods. Usually portions of the nerve were taken from above the wound, at the wound, below the wound at different distances down to the wrist, from the motor branches to the flexor carpi ulnaris and the flexor profundus digitorum, and in some cases, from the long cutaneous branch of the ulnar. The object in taking so many specimens was to trace the course of the degeneration and regeneration, an intention which we have carried out with partial success. It was found afterward that in studying regeneration the instructive specimens were obtained along the line of advancing regeneration; portions proximal or distal to this showed chiefly, on the one hand fully developed fibres and on the other fibres still in an embryonic condition, so that a single piece taken at random from the peripheral end might show almost nothing of what was most characteristic of that stage.

Degeneration in the Peripheral End:—After the interruption of the connection between a nerve fibre and its centre, whether the interruption be by actual section, by crushing, or by coagulation, the peripheral end of the fibre undergoes degeneration, the changes affecting first the myeline and the axis, and subsequently the sheath and its nuclei. This degeneration extends throughout the whole peripheral end, it is accompanied by a total loss of the characteristic properties of nerve fibres, conductivity and irritability, and, according to our experiments, it is an inevitable result of the interruption of connections with the nerve centres. In no case were we able to get return of function without a previous degeneration of the entire peripheral end. The degenerative changes of the myeline have been described so often, that it would seem useless to speak of them again, except to give our evidence upon one or two points in dispute. The degeneration begins in the dog at a variable time after section of the nerve, though the average time is about four days. Up to that time the nerve is apparently normal histologically as well as physiologically. Then the continuous myeline sheath breaks up into a number of segments, and according to most observers this fragmentation takes place at the segments or intersegmental lines of Lantermann. Certainly the first segments formed correspond in shape and general appearance to the segments of Lantermann. Ranvier attributes the cause of this phenomenon to the growth of the protoplasm, especially in the neighbourhood of the internodal nucleus, and speaks of the segmentation as an active phenomenon. The increase in the protoplasm, which in the normal

fibre according to his view spreads over the whole myeline, cuts the myeline and axis into two at the nucleus, and possibly at the same time at several other points in the internodal segment. This explanation seems to us to be erroneous. According to our observations the myeline breaks into fragments, beginning apparently at the intersegmental lines, before there is any marked increase in the protoplasm surrounding the nuclei.

If we could accept the view that the intersegmental lines are filled in with protoplasm which is in connection with the nucleus through the peripheral layer of protoplasm lying under the sheath, then the most simple explanation of the fragmentation would be that it is caused by the hypertrophy, as it were, of this protoplasm. But this view of the normal structure of the fibre has not been demonstrated, it is at best an hypothesis. We are inclined to look upon the fragmentation, after the manner of the older histologists, as a process of coagulation brought on by the metabolic changes necessarily resulting from the interruption of connections with the trophic centres. If the hypertrophy of the protoplasm at the intersegmental lines were the originating cause in the segmentation, one would expect some regularity in the subsequent rounding off and absorption of the pieces of the myeline, for a time at least, since they would be acted upon from each end; but the contrary is true.

The segments first formed in degeneration, unlike the normal appearance of the segments of Lantermann seen in fibres after the action of osmic acid, have a complete envelope of the myeline, as shown in Fig. 1, and the small interspaces between them are filled with a colourless material. In each of these segments is a piece of the original axis cylinder, at this time apparently unchanged. Many of the older histologists have asserted that the axis remains unaffected during the degeneration. On the contrary, even at this early stage the breaking of the myeline into segments is accompanied by, or causes a simultaneous breaking of the axis. As Ranvier pointed out, this interruption of the axis is the immediate cause of the loss of irritability of the nerve to artificial stimulation.

There has been some discussion as to whether this first segmentation of myeline (and axis) is simultaneous throughout the entire peripheral end or whether it is progressive, starting either from the distal or from the proximal end. Those who have studied the phenomenon upon mammalian nerves are nearly unanimous in believing that it is simultaneous throughout the entire peripheral end.

Our observations have convinced us also that the change takes place substantially at the same time throughout the whole length of a nerve fibre. If it is a progressive change it must pass over the fibre with great rapidity. We could detect no difference in the time of the appearance of the incipient changes in the nerve at the elbow and at the wrist or in the small motor branches. In the immediate neighbourhood of the wound, on the contrary, degenerative changes of a traumatic origin undoubtedly occur before they appear in the rest of the nerve, as Engelmann and Colasanti have described.

Shortly after the first cylindrical segments of myeline and axis are formed an irregular fragmentation occurs in these segments in most parts of the fibre. The segments break up into smaller or larger irregular pieces or balls, an extreme case of which is shown in Fig. 2. Very frequently in these irregular masses of myeline remnants of the axis cylinder can be clearly distinguished in all stages of disintegration as shown in Figs. 3, 4, 5. It should be stated that some of the large segments frequently persist in different portions of a fibre long after the other segments have broken into small pieces and become partially absorbed,—the process is very irregular. It is at the time that this breaking up of the cylindrical segments into smaller fragments becomes apparent that the increase in the size of the nuclei of the sheath and the growth of the protoplasm surrounding these nuclei become clearly marked. In fact this secondary fragmentation is always visible first in the neighbourhood of the nuclei as shown in Figs. 6, 7, 8. The large rounded nuclei lie in the middle of the fibre, and close to them are the small drops or balls of myeline. For this reason we believe that one cause of this secondary fragmentation is to be found in the absorption which takes place under the influence of the nucleus and its surrounding protoplasm. At this time, and in fact before this, an evident chemical change has taken place in the substance of the myeline and the axis. Other observers have noted this in connection with the difference in staining with osmic acid. The change was even more marked with the picric fuchsin stain. In this stain the normal fibre has its myeline coloured yellow and its axis red, but at the time the secondary fragmentation begins both structures take a curious greenish yellow hue which is very characteristic. As evidence of the actual absorption which is beginning to take place one finds, at this time, say the seventh day, in the fibres stained with osmic acid or gold, some of the balls of myeline in the neighbourhood of the nuclei left colourless by the stain, see Fig. 6. In the later stages the same fact may

frequently be noticed at different parts of the fibre and always most clearly near the nuclei. See Fig. 11.

By the 7th day a very active proliferation of the nuclei of the sheath has begun. The increase in their number is very striking. Our methods of hardening were not such as to show the method of multiplication of the nuclei, though we often found nuclei showing a dumb-bell form, i.e., an elongated nucleus constricted in the middle as though multiplying by direct division. There can be little doubt that the division is indirect, as we sometimes found, even after hardening in Mueller's solution, nuclei like that pictured in Fig. 9 which evidently represents a badly preserved mitosis. However, we made no special study of this point. An interesting fact in connection with the multiplication of the nuclei is the way in which they migrate. In the beginning, of course, there is a single nucleus to each internode. At the time the secondary fragmentation of the myeline is fully under way one frequently finds a number of nuclei in the space which an internode would occupy. Sometimes they are in pairs as though from a recent division, but in other cases one or more large masses of myeline will be found between. See Figs. 7 and 8. This latter appearance has been used to support the theory that there are several nuclei present in the internode in a normal fibre, but that they are hidden by the myeline. Such a view as this it is not necessary to consider at present. The only explanation of the appearance described that seems reasonable is that after division the nuclei migrate or may migrate to some distance and start the process of absorption at a new place. The physical characteristics of the fibre while in the living animal cannot be such as to oppose this movement of the nuclei.

From the 7th day to the 14th day the process of absorption of the balls of myeline with their contained fragments of axis cylinder goes on actively, yet quite irregularly. Fig. 10 from a nerve after 7 days shows very well the breaking-up of the myeline and the formation in between, especially at the nuclei, of an apparently liquid substance in which are contained numerous fragments of the old myeline. As the absorption progresses and the fragments of myeline become smaller and less numerous the direct participation of the nuclei in the process becomes more evident. The nuclei are much more numerous and are found clustered in and about the remaining balls of myeline as shown in Figs. 11 and 12, representing a degeneration of 9 and 14 days respectively. One often sees bits of the myeline partially imbedded in a nucleus, and this appearance is found from this time on well into the later stages of

regeneration, as long, in fact, as any of the myeline remains unabsorbed. After 14 days absorption has gone so far that long stretches may be found, as shown in Figs. 12 and 13, in which only small fragments of myeline are present. At such places the fibre consists of a homogeneous, apparently liquid substance lying in the old sheath, and of many nuclei, often in pairs or groups, the latter giving indication of an active proliferation. Yet at this time, 14 days, and even later, one sees many fibres in which the absorption has lagged behind the condition of what may be considered a typical fibre of this period. In one and the same fibre places will be found in which absorption has made rapid progress in spots, all the myeline having disappeared, while in other spots the large cylindrical segments have suffered scarcely any change. Examples of this are pictured in Figs. 14 and 15. However irregularly the process may go on the final outcome is the complete absorption of the remnants of the old myeline and axis; though as we have said before, balls of the myeline may be found in certain fibres long after this period, even at the time when fully formed new fibres have been produced. As the absorption proceeds the old sheath collapses more or less. It seems at first to contain a liquid material with some *débris* of the old myeline, but this too finally disappears and the beginning of the actual process of regeneration is inaugurated by the formation of new protoplasmic material around the numerous nuclei contained in the fibres.

Regeneration in the Peripheral End:—The increase of protoplasm round the nuclei is shown in its different stages in Figs. 16, 17, 18. At first, where the nuclei lie singly, the new protoplasmic formation gives the appearance of spindle-shaped cells or fibres such as have often been described as taking an active part in the formation of the new fibres,—Fig. 16. Later the increased formation of new material results in filling the old sheath with a continuous band or fibre of protoplasm in which the nuclei are imbedded, as shown in Figs. 19, 20, 21, 22, 23, 24. The fibre as now formed represents the completion of the first stage in regeneration. This stage has been described more or less carefully by many observers. Adopting the nomenclature of Neumann, we will speak of such fibres as "embryonic fibres" because of the great similarity in structures between them and the fibres in the early embryo. We have spoken of the substance of the fibre, at this time, as being composed of protoplasm. The word is used in want of a more definite term, and it must be added, as was clearly stated by Neumann, that the material of the fibre shows an almost perfectly homogeneous appearance, quite unlike the granular structure of ordinary cytoplasm.

Moreover, it stains very slightly with haematoxylin and the other stains which we used; only a long exposure to such reagents will make it take up any of the colouring matter. In this respect also the fibre appears to resemble the fibres in the early embryo before myeline and axis have been formed. The whole series of changes up to this point has taken place within the old primitive sheath. After the formation of the complete embryonic fibre a new sheath is made by a differentiation of the peripheral layer of the protoplasmic thread. The appearances which have led us to this view are shown in Figs. 23 and 24, in which the newly-formed fibre with its sheath lies within the old sheath. The old sheath is displaced outward and one may suppose that it eventually forms a part of the endoneural connective tissue which is at this time abundant. It must be said, however, by way of reservation, that the fate of the old sheath was not actually followed. The new sheath seems to be formed in the way described, the fate of the old one is a matter of inference.

It will be seen by reference to the physiological experiments that there is considerable probability that these "embryonic fibres" have some of the properties of mature nerve fibres. They can certainly conduct impulses after they have made connections with the normal fibres of the central stump, and there is a probability that they possess a low degree of irritability to strong mechanical stimulation. We have suggested the possibility that these considerations may explain the very rapid return of sensation in some of the reported surgical cases. The embryonic fibres, it should have been stated, often appear in the hardened specimens as flattened ribbons, instead of cylindrical threads, as shown in Fig. 21. Whether this is true of the fresh specimens we did not ascertain.

We have described the formation of a single embryonic fibre as taking place within the sheath of one of the old fibres, and we believe that where union is made with the central end this is the rule. But two other possibilities forced themselves upon our attention while studying the preparations. There is some reason for believing that two or more embryonic fibres may arise within an old sheath by a process of longitudinal division after the fibre is formed, or during its formation. We have been able to demonstrate this point in the fibres of the central end, especially where union with the distal end has not been made, but for the peripheral fibres we can only suggest the possibility of its occurrence. In the second place there is a strong probability that many of the old fibres do not succeed in regenerating,

but go to ground completely. One often meets specimens such as are represented in Fig. 23, in which large balls of myeline are found on the sides of new fibres, engulfed in the cytoplasm of the wandering cells. Either an old fibre has gone completely to ground, or else in the formation of a new sheath some of the unabsorbed balls of myeline have been cut off and left to the mercy of the phagocytes. In addition, after the myeline is absorbed, one sometimes sees, even in later stages, entirely empty, collapsed sheaths, as though the formation of new protoplasm had miscarried. It may be that the regenerative activity of the nuclei of the sheath is not always successful in producing an embryonic fibre.

The Extent of Regeneration in the Peripheral End when it is not reunited to the Central End: As far as our experiments go they give unmistakable proof that when the peripheral end does not reunite with the central end the degenerative changes proceed in much the same way as when a suture is made. But the regeneration, beginning with the formation of the embryonic fibres, proceeds more slowly than in the case of suture, and never gets beyond the embryonic stage. Our longest experiments upon a nerve cut but not sutured, and in which reunion was prevented by removing a piece of suitable length, were experiment XXVI., upon a rabbit examined 70 days after the operation, and XXII., central ulnar of dog, 75 days. In addition, however, we have had the opportunity of examining a portion of the peripheral end of a human ulnar nerve which had been cut accidentally six and a-half months previously. We owe the opportunity to the kindness of Dr T. A. Mc Graw, who in an operation for secondary suture removed small portions of the central and peripheral ends and sent them to us in Mueller's solution. Figs. 25, 26, 27, 28 are from drawings of specimens prepared from the peripheral end of this nerve after treatment with the potash gold stain of Freud and Böhmer's haematoxylin. They show that regeneration had advanced to the stage of the formation of embryonic fibres, but that there were no signs of an axis cylinder or myeline sheath. Similar preparations were obtained from the animals upon which section without suture was made. Cross sections of the peripheral end of the human nerve are shown in Fig. 29, stained with gold and haematoxylin and in Fig. 30, stained in haematoxylin alone. The drawing in Fig. 29 was made from a portion of the nerve in which some of the nuclei show a central nucleolus in cross section, simulating the appearance of new nerve fibres with a central axis. This is better shown in Fig. 31, taken from the ulnar nerve of a

dog after suture but before regeneration. We call particular attention to these figures because they seem to us to explain Fig. 10 in Bowlby's recent book on "Injuries and Diseases of Nerves" which he uses to prove the autogenetic production of nerve fibres in the peripheral end of ununited nerves. We feel confident that if Bowlby had teased the peripheral end, and stained with the usual reagents for nerve fibres, he would not have been able to demonstrate either a new axis or a new myeline. It is difficult to judge from the description alone whether what Philippeau and Vulpian have described as fibres produced autogenetically in the peripheral end correspond to what we have called embryonic fibres or not. Our own results are so clear that we feel justified in explaining their observations in that way, unless as has been suggested, the two ends of their nerves had reunited through cicatricial tissue which they had disregarded. Furthermore, specimens like that represented in Fig. 24 may explain what Schiff and others have mistaken for a persistent axis cylinder. Certainly our modern methods of staining prove that after the first week or ten days there are no distinguishable fragments of the old axes remaining in the fibres of the peripheral end of the nerve. We have said that in our examinations of the peripheral end when not united with the central end, we found no newly-formed myeline or axis. Yet, as we will presently show, the new myeline in regenerated fibres, after connection with the central end is made, is formed in the peripheral end of the fibres. In accordance with this fact we found in some of the peripheral fibres of a rabbit's ulnar, which was prevented from reuniting with the central end and was examined 70 days after section, what seemed to be abortive attempts at the formation of new myeline in the embryonic fibres in the neighbourhood of the nuclei.—See Figs. 32 and 33. Such appearances were rare, and it may be that they are to be otherwise explained. We will come back to them in speaking of the formation of the myeline.

Regeneration in the Peripheral End after Reunion with the Central End:—After the formation of the embryonic fibres, if reunion with the central end has been made, the regenerative changes go on to the formation of complete nerve fibres having myeline sheaths and axis cylinders. Certain general facts with reference to this complete regeneration are capable of easy demonstration. In the first place it occurs more rapidly the smaller the intervening space between the two ends of the nerve, and in the case of suture the quicker the suture is made after injury, and the more perfect the coaptation of the two ends. These

results came out clearly in our study of the regeneration after suture, after crushing, and after coagulation. It was slowest in the last case, where there was a comparatively large dead area between the two ends, and was most rapid after the crushing experiments in which the epineural sheath was not ruptured. So in experiment XVIII., regeneration was more rapid on the side on which the cut ulnar was immediately sutured than on the side on which an hour was allowed to pass before the suture was made. In the second place it is easy to demonstrate that the formation of new myeline and new axis begins at the proximal end, at the wound, and proceeds centrifugally. Neumann has already called attention to this. Our experiments showed that along with this downward growth of myeline and axis there was a corresponding return of irritability to direct stimulation, the details of which have already been described.

After the formation of the embryonic fibres the additional steps necessary for a complete regeneration consist in the production of a new myeline sheath and a new axis cylinder. The explanation of the origin of these structures has been found peculiarly difficult by all writers. The various theories proposed have been mentioned in the review of previous work given at the beginning of this paper. With reference to our own work, one result has seemed to us especially clear, and that is that the myeline is formed in the peripheral fibres, after connection with the central end has been established, and moreover is formed in a discontinuous way. It is quite easy to find in the peripheral trunk newly regenerated fibres showing a delicate continuous layer of myeline, but to demonstrate how this myeline is deposited is more difficult; to ascertain this point one must obtain his specimens from just that portion of the nerve in which the process is actively going on. Unless one is fortunate in finding such places he will almost inevitably be led to a conclusion like that of Erb, that the myeline is formed as a continuous sheath along the length of the fibre. In two animals we were particularly fortunate (experiments XXVIII. and XXII.) in getting the incipient stages. Figs. 36, 37, 39, 40 and 41 give an idea of how the myeline first appears. As shown in these figures, it appears first as irregular deposits in the protoplasm of the embryonic fibres, and usually first in the immediate neighbourhood of the nuclei. Delicate prolongations of the myeline are often seen running from one small mass of the myeline to another, and eventually these latter become connected together, forming a varicose tube, shown in various stages in Figs. 36 to 51. With reference to the immediate origin of

these deposits, we must confess ourselves in doubt. In many cases we obtained specimens such as are shown in Figs. 34 and 35, in which clear bead-like spaces are seen forming in the protoplasm. The general arrangement of these spaces strongly suggests that they mark the beginning of the transformation of the protoplasm to myeline; a step farther and the chemical change will be such as to give the usual staining with osmic acid or gold as seen in Figs. 36–38, etc. On the other hand, the first deposits of myeline, especially when stained in osmic acid, often bear a striking resemblance to the nuclei, both in shape and in the fact that they show a rather distinct staining with the haematoxylin. (See Figs. 38, 39, 40, 42, etc.) There is no doubt that as the myeline forms many of the numerous nuclei scattered along the fibre disappear. It is a tempting hypothesis to suppose that the superfluous nuclei suffer a myeline degeneration and directly or indirectly give rise to the myeline tube. We have hunted long and faithfully for intermediate stages which might support such a view, but have been unsuccessful. We are inclined to believe, therefore, that the pale violet staining of the interior of the myeline drops which gives them the appearance of nuclei partially changed is due either to the thinness of the myeline layer surrounding them causing a colour contrast, or is one result of the chemical differentiation which is taking place. As the myeline layer becomes thicker, the deposits stain a deeper black, as seen in the drawings. This leaves us the alternative hypothesis that the myeline is formed within the substance of the protoplasm by a process of chemical differentiation. If we ask what starts this chemical alteration, we can only connect it with the fact that it seems to depend upon a physiological connection with the normal fibres of the central end, and we are therefore forced to fall back upon the trophic action of the nerve centres, or rather the trophic nature of the impulses sent along the fibres from these centres, however indefinite such an hypothesis may appear. The connection of the nuclei with the first formation of myeline is to be expected, perhaps, upon this hypothesis, since it may be assumed that it is through them that the nutritive influence of the centres is exerted. In reaching this conclusion we are partially in accord with Neumann, though we differ from him fundamentally in our views as to the origin of the protoplasm composing the embryonic fibres. Whatever theory of the immediate cause of the formation of the myeline may be the true one, there can be no doubt that it is first found as disconnected drops. These may afterwards become united by slender processes to form a bead-like string which sooner or

later elongates to an even tube, or the drops may first elongate to form cylindrical segments which eventually unite to form continuous, delicate tubes of myeline. Both of these processes, with the intermediate stages, are shown in Figs. 36 to 55 better than we could describe them in words. What becomes of the numerous nuclei scattered along the embryonic fibre it is not possible to say, other than that they disappear. In some cases, as in Figs. 38, 39, 42, they disappear rapidly as the myeline tube is formed, while in other cases they persist for a much longer time, the newly-formed myeline tube winding in and out among them in a very beautiful manner (see Figs. 37, 51, 55). We can only suppose that they disappear by absorption, as their nutritive relations with reference to the protoplasmic contents of the fibre become less and less important. With reference to the nodes and internodes of Ranvier, it is evident that no simple hypothesis, such as the development of each internode from a single cell, will fit the facts as they appear in regenerating fibres. The developing internodes and nodes are plainly shown in Figs. 51 to 55, but why the ends of the internodal tubes do not fuse together is difficult to explain. The internodal nucleus must, throughout life, play an important part in the nutrition of the protoplasm in connection with it, and of the myeline sheath. In an indefinite way we may suppose that this nutritive influence on the myeline can extend only over a limited area,—the distance of an internode,—but to connect this with the formation of these internodes takes us into the field of speculation, though it seems to us that the true explanation lies along this line of thought. The origin of the segments of Lantermann may doubtless be traced directly to the primitive, disconnected deposits of myeline which we have described.

Axis Cylinder:—The origin of the axis cylinder is more difficult to trace than that of the myeline sheath. The difficulty is largely caused by the want of a perfectly reliable method of staining this structure. The myeline sheath round the axis seems to prevent the penetration of the usual stains, so that we got our best results by the picric acid and haematoxylin method in which the myeline was first dissolved out. With the gold stain beautiful preparations of the new medullated fibres can be obtained, and in some cases the axis cylinder within can be clearly distinguished. But when the new myeline is evidently just formed no definite axis can be demonstrated by the gold stain. With the picric acid and haematoxylin stain, on the contrary, the newly-formed axis is clearly seen, even at that early stage in the formation of the myeline tube when it exists as a string of bead-like swellings (see

Figs. 56 and 57). It follows then that though the myeline sheath probably begins to form before the axis cylinder can be distinguished, the latter appears shortly afterward, before the new fibre has gone far in its development. The point of greatest difficulty is to make out the origin of the axis. The myeline sheath as first formed consists of a delicate circumferential layer of true myeline, staining dark with osmic acid, and enclosing a core which as yet does not stain with the osmic. Benecke, Leegaard, Neumann and others make the axis arise from this core, or what in their descriptions would correspond to it. That is, they believe that the axis forms *in situ* in the peripheral fibres. Ranvier, on the other hand, believes that one or more axes grow out from the axis of each intact fibre of the central end, and this view is practically implied in the almost universally accepted belief that the axis is an outgrowth from the central nerve cell. As far as the microscopic appearances of the axis in the newly regenerated fibres are concerned, in our work at least, they might be explained equally well on either hypothesis. The strong reasons for believing that the axis is an outgrowth from the nerve cell have made us search carefully at the central end for some evidence of the sprouting of the axis cylinder in the old fibres. Our best results in this direction have been obtained from the study of the central end of the divided nerve (experiment XXII., ulnar) when union with the peripheral end was not made. When union with the peripheral end is made it is very easy, with the picric acid and haematoxylin method, to obtain specimens showing the junction of the old and the new fibres, and the connection of the old axis with the new. But there is nothing in such specimens to indicate whether the new axis has grown out from the old, or is simply fused with it. In the central stump, in cases where union was not made, the old fibres, as will be described more fully presently, frequently end in a bundle of small new fibres. If the axis grows out from the central end, one ought to find at such places, when stained by the picric acid-haematoxylin method, a branching of the old axis cylinder to pass into the new fibres, such as was described by Ranvier, though not actually seen by him. In the only specimen of an ununited central end which we were able to stain by this method we found several specimens which showed this branching, the best of which is drawn in Fig. 60. The axis in the old fibre ends in a bulbous enlargement from which the new axis arises by a slender process,—after entering the new fibre it can be seen to branch, and one of the branches again breaks into two smaller axes. The three new medullated fibres to which these

three axes belong are coiled round one another in a way not shown well in the picric acid specimens (and not represented in the drawing). Very many apparent examples of this branching of the axis were found in this specimen, but owing to the intricate way in which the fibres were twisted and the possibilities of deception arising therefrom, the connection of the old and new axes could not be satisfactorily followed. The example given in the drawing, and some others, were, however, quite distinct and seem to us to give fairly satisfactory histological proof that in regeneration the new axis cylinders are outgrowths from the axes of the uninjured fibres of the central end. Fig. 61 gives an apparent example from another experiment of the outgrowth of the axis. In the dog, at least, where union is rapidly established between the two ends, each old fibre, as a rule, unites with only one new fibre so that there is no branching of the axis cylinder but a simple outgrowth. When union is not made there may be a branching of the axis to grow into the several new fibres, which, in some cases, are formed, as we shall describe, in connection with the old fibre.

Degeneration and Regeneration in the Central End:—According to most of the accounts, the degeneration in the central end after section or injury is substantially the same as in the peripheral end, with the exception that it extends centrifugally for only a limited distance,—through one or two nodes of Ranvier. The peculiar account of the degeneration in the central end given by Ranvier, according to which large leucocytes enter the ends of the tubes and devour the myeline and axis as far as the first node, has not been confirmed by other observers. We have not followed all the stages of degeneration and regeneration in the central end with the same care as in the peripheral end; but the stages we have examined have convinced us that the processes are practically identical in the two ends. The myeline and axis disintegrate and are absorbed for a certain distance; an embryonic fibre is formed from the new protoplasm arising from the nuclei, and in this a myeline sheath is first formed into which an axis cylinder penetrates as an outgrowth from the end of the old axis. Various examples of this formation of a new fibre within the sheath of the old are shown in Figs. 62 to 72. In many cases, in the central end, when union was not made or when difficult union was made as in cross sutures, an old fibre was found to terminate in a bunch of two or more new fibres (see Figs. 62 and 63), usually coiled round one another so that they could not be disentangled. We can only explain these by supposing that during the formation of the embryonic fibre there is a

longitudinal division resulting in the production of several embryonic fibres lying in the old sheath; each of these afterwards may take on a myeline sheath and receive an axis cylinder process from above. It is not at all uncommon to find in such specimens some of the new fibres of the bundle without a myeline sheath and others with one newly formed, or with both myeline and axis. Cross sections of the central stump of the human ulnar nerve operated upon by Dr Mc Graw for secondary suture, six and a-half months after injury, confirmed the results which were obtained by teasing (see Fig. 69). The section was made through the bulbous enlargement of the central stump. At the level of the section no normal medullated fibres were found, though occasionally a cross section of a smaller fibre with some remnant of the axis was seen. In other places a bundle of small fibres was found of the same area as one of these enlarged fibres, and at still other spots intermediate stages were seen showing an enlarged fibre surrounded by small new fibres in the same sheath. In this case the bulbous enlargement was undoubtedly caused by an increase in the nerve fibres as well as in the epineural connective tissue. If the cross section described is compared with the teased preparations, Figs. 63, 64, 65, made from the central end of the same nerve the way in which an old fibre makes connection with a bundle of new fibres lying in the same sheath will be more readily understood. One can understand from the teased preparations how in the cross section a portion of the myeline of an old fibre may be obtained surrounded by a number of newly-formed fibres in the same sheath.

Still another interesting fact is shown by the teased preparations of the central end of the same nerve, and that is, that the degenerative changes in the central end, when union is not made, apparently keep on progressing centripetally at a slow rate. Figs. 63, 65 and 67 give good illustrations of this fact. The end of the myeline is swollen and opaque, and has fallen into large segments. The embryonic fibre formed distally to this can be seen continuing into the hypertrophied protoplasm surrounding the end of the myeline, and in this latter the nuclei are prominent and increased in number. The whole appearance indicates a slowly backward progressing degeneration and regeneration, and gives, moreover, a striking demonstration of the fact that the protoplasm of the embryonic fibres is a product of the activity of the nuclei of the sheath. Similar preparations were obtained from the central end of the ulnar, in a dog, which had been severed 75 days before the examination was made, and had not been allowed to unite with the

peripheral end. In some of the specimens from this latter nerve the mode of union of the axis cylinder in the newly regenerated fibre with the axis in the old fibre is clearly shown (Figs. 70, 71, and 72). Fig. 71 is particularly instructive when compared with Figs. 64 and 65. The new axis cylinder is seen to escape the swollen end of the old fibre and to penetrate the myeline some distance beyond this point in order to reach the old axis. Fig. 72 shows the end of an old axis cylinder enlarged and sending out a new axis.

Union of the Central End with the Peripheral End:—It will be seen from the above description that the formation of embryonic fibres and of new medullary substance goes on in the degenerated portion of the central end. Similar processes take place in the peripheral end preparatory to receiving the new axis cylinders from above. How is the connection between the two made? It is practically an impossible thing to witness the making of this connection, but we are strongly convinced that it takes place in the cicatricial tissue, and chiefly from the downgrowth of embryonic fibres from the central end. In the cicatricial tissue itself no independent formation of new fibres can take place if the theory of their origin which we have adopted is correct. It forms a tissue into which the actively growing embryonic fibres from the central and peripheral ends penetrate, and finally meet and unite. The fusion will naturally take place more quickly the smaller the distance between the two ends. Once this union is established the myeline sheath begins to form in the peripheral fibres. While in the new fibres of the central stump, as the specimens demonstrate, new myeline develops in a normal way whether connection with the peripheral end is established or not, since their union with the old fibres of the central end is assured.

In conclusion we wish to speak briefly of an appearance often seen in the central end of the nerve, whether or not union is made with the peripheral end, an appearance which has been frequently described by other observers, but which has not been explained, as far as we are aware. This phenomenon consists in the intercalation of a segment of one or even two new fibres between the ends of an old fibre which has not undergone degeneration. (See Figs. 73, 74, 75, 76.) The noteworthy thing is the apparent exception to the general rule that the distal portion of a severed fibre always degenerates. Here we have a distal piece which seemingly escapes degeneration. To our minds the explanation is simple. Such fibres are always found in the central end near the point of injury. It is probable that in cutting or crushing the

nerve, or in injuring it by any other method, certain spots on some of the fibres above the point of actual destruction are sufficiently injured, by compression or otherwise, to lead to a degeneration of the myeline and axis, but over the microscopic stretch thus affected the rapid formation of a protoplasmic material permits nerve impulses to be transmitted to the distal piece and thus preserves it from degeneration. This intermediate piece is sometimes very short, at other times nearly as long as an internode. The processes of degeneration and of regeneration in the injured piece go on in the same way as in the fibres of the peripheral end of a nerve completely severed. The phenomenon seems to us a confirmation of the view we have taken, that the initial cause of the degenerative changes in the cut end of a nerve fibre lies in the perverted metabolism resulting from severance from the nutritive cells.

We have stated our histological results as briefly as possible, relying upon the illustrations to take the place of a more extended verbal description. For the sake of clearness we may be permitted to recapitulate the more important conclusions.

1. After complete severance of connection with the nerve centres the peripheral end of a nerve suffers degeneration throughout its entire extent.

2. The degenerative changes and the subsequent regeneration take place as follows:

(*a*) Segmentation of the myeline and axis at the intersegmental lines.

(*b*) Proliferation and migration of the internodal nuclei.

(*c*) Secondary fragmentation and absorption of the myeline (and the contained *débris* of the axis), most active in the neighbourhood of the nuclei.

(*d*) Increase of protoplasm round the nuclei, forming finally a continuous band of protoplasm lying in the old sheath.

(*e*) Formation of a new sheath on the periphery of this band, thus making an "embryonic fibre."

(*f*) Union of the embryonic fibres of the peripheral end with those similarly formed in the central end—the union taking place in the intervening cicatricial tissue.

(*g*) Formation of myeline in the peripheral end as isolated drops —usually seen first near the nuclei. These afterwards unite

to form a continuous tube. The formation of the myeline proceeds centrifugally, starting from the wound.

(*h*) The outgrowth of new axes from the old axes of the intact fibres of the central end—the outgrowth following quickly upon the development of the myeline.

(*i*) In the central end, especially when connection with the periphery is not made—several new fibres may form within the sheath of an old one to take the place of the portion degenerated. Each of these may develop myeline and receive a branch from the axis cylinder above.

In addition to these conclusions which have been based upon observed facts capable of demonstration, we venture to suggest certain speculations as to the causes of the changes described. We may suppose that in the normal fibre the nutrition of each internode is directly controlled (with the exception probably of the axis) by the internodal nucleus, and that the metabolic activity of the nucleus in turn is influenced by trophic impulses received through the axis cylinder from the nerve centres. When the flow of impulses is interrupted the metabolisms of the nucleus and its dependent structures, myeline and internodal protoplasm, are altered. The first effect of this alteration is a coagulation of the myeline into segments corresponding to the segments of Lantermann, and this brings about mechanically a rupture of the axis into corresponding segments. The rapid growth and multiplication of the nuclei cause the absorption of the old myeline and axis, and result in the formation of a continuous band of protoplasm, the embryonic fibre. The active growth of this fibre soon establishes, under favourable conditions, a functional connection with the fibres of the central end, and brings the protoplasm and nuclei again under the influence of the trophic impulses from the nerve centres, in consequence of which there is a new formation of myeline, and the metabolisms of the internodal segments return to their normal condition. The interruption of the myeline sheath at the nodes of Ranvier may be connected with the dependence of the sheath for its normal nutritive control upon the internodal nucleus and its protoplasm, and the fact that this influence can only be extended over a limited area.

RECORDS OF OPERATIONS AND PHYSIOLOGICAL EXAMINATIONS.

TABLE OF EXPERIMENTS.

EXPERIMENT II.

Oct. 11, 1890. Small terrier, young. Hypodermic injection of $\frac{2}{5}$ gramme morphia sulphate.

Operation. Ulnar nerve on left side cut just above the elbow and immediately sutured with two catgut sutures—sutures passed through the epineurium.

Ulnar nerve on right side crushed by tying firmly a single catgut ligature round it just above elbow, the ligature removed at once. Stimulation (induction shocks) above the point of ligature, after the removal, caused no flexion of foot or digits.

Examination. Oct. 14 (3 days after operation). Right side. Skin wound united by first intention, no suppuration. Only a faint indication of point of ligature on the nerve. Portions of the central end, the wound, and of the peripheral trunk and branches to the flexor carpi ulnaris preserved in Müller's liquid for histological examination.

Left side. Skin wound open, but no suppuration; nerve found united. Portions removed as on right side for histological examination.

No physiological examination made in this experiment.

EXPERIMENT XX.

April 24, 1891. Very young Bull pup. Hypodermic injection of $\frac{1}{5}$ gramme morphia sulphate followed by ether.

Operation. April 24. Left side. Ulnar nerve cut just above elbow, immediately sutured with one catgut suture.

April 26. Right side. Same operation.

Examination. April 28. Left side. 4 days after section. Stimulation of the ulnar below the wound by induction shocks, unipolar stimulation, gave no contractions at all. Stimulation above the wound gave reflex movements and retraction of arm, but no flexion of foot or digits. Hence complete loss of irritability in the peripheral portion of ulnar.

Right side. 2 days after section. Stimulation of the ulnar below the wound, induction current and unipolar stimulation, gave slight movements of flexion in digits. Hence irritability of peripheral ulnar not completely disappeared.

Portions of the ulnar on both sides, above the wound, at the wound and below at different distances, and the branches to the flexor profundus and flexor carpi ulnaris preserved in Müller's solution and osmic acid for histological study.

EXPERIMENT XXIII.

May 19, 1891. Black and tan, small but full grown. Hypodermic injection $\frac{2}{5}$ grammes morphia sulphate followed by ether.

Operation. May 19. Left side. Ulnar cut and not sutured, section made in the arm several inches above the elbow. Median crushed by tying a catgut ligature round it at same level.

May 21. Right side. Same operation upon ulnar and median at same level.

Examination, May 22. Left side. 3 days after operation. Stimulation with induction currents of both ulnar and median peripheral to the wound gave flexion of foot. Hence irritability still preserved.

Right side. 1 day after operation. Stimulation with induction currents of both ulnar and median peripheral to the wound gave flexion of foot. Hence irritability still preserved.

Portions of ulnar and median on both sides, above the wound, at the wound, below the wound, and motor branches to the flexor profundus and flexor carpi ulnaris preserved in Müller's solution, in osmic acid and in picric acid for histological examination.

EXPERIMENT XII.

Feb. 16, 1891. Black and tan, not full grown. Hypodermic injection of $\frac{2}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Quarter inch of ulnar removed, just above elbow. Ends not united by suture.

Right side. Same operation.

Examination. Feb. 20 (4 days after operation). Left side. Ends of nerve not united, central end very sensitive. Stimulation of peripheral end with induction shocks and mechanically (blows of small hammer) showed that the nerve was, if anything, more irritable than normal. Direct stimulation (induction shocks) of motor branch to the flexor carpi ulnaris showed that the irritability of this branch was apparently below normal, required a much stronger induction shock to produce flexion, but irritability still preserved.

Right side. Results of stimulation practically the same, irritability of the peripheral end of the nerve still present.

Portions of the nerves above and below the section, and of the motor branches to the flexor profundus and flexor carpi ulnaris preserved in Müller's solution for histological examination.

Experiment XIII.

Feb. 21, 1891. Black and white setter apparently full grown. Hypodermic injection of $\frac{2}{5}$ gramme of morphia sulphate followed by ether.

Operation. Left side. Quarter inch of ulnar removed just above elbow. Ends not united by suture.

Right side. Same operation.

Examination. Feb. 25 (4 days after operation). Left side. Ends of nerve not united. Mechanical and electrical stimulation of the peripheral end gave no flexion whatever and no perceptible contraction of the flexor carpi ulnaris or flexor profundus when these were laid bare by dissection. Hence complete loss of irritability. Direct stimulation of flexor carpi ulnaris with induction shocks gave slight contractions, with the galvanic current stronger contractions.

Right side. Results of stimulation the same. Complete loss of irritability of the peripheral end of ulnar.

Portions of the peripheral trunk and the motor branches preserved as usual in Müller's solution.

Experiment III.

Oct. 11, 1890. Small mongrel. Apparently full grown. Hypodermic injection of $\frac{2}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Ulnar nerve cut just above elbow and immediately sutured with two catgut sutures through the epineurium.

Right side. Ulnar nerve exposed above elbow and crushed by tying a single ligature of catgut. Ligature removed. Stimulation of the ulnar above the point injured caused no flexion of foot or digits.

Examination. Oct. 18 (7 days after operation). Left side. Stimulation of the ulnar below the wound (induction currents and unipolar stimulation) gave no movements whatever. Stimulation of the nerve just above the wound caused signs of pain and reflex movements of the arm. Complete loss of irritability in the peripheral nerve.

Right side. Results of stimulation the same.

Portions of the nerves removed. Above wound, at wound, below wound and motor branches to the flexor muscles preserved for histological work.

Experiment VI.

Oct. 24, 1890. Brown and white bull-dog, young. Hypodermic injection of $\frac{2}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Ulnar nerve cut just above the elbow and the two ends immediately joined by two catgut sutures through the epineurium.

Right side. Ulnar nerve above elbow crushed by the tying of a catgut ligature. The ligature quickly removed. Stimulation above the point injured gave no movements of flexion.

Examination. Nov. 1 (7 days after operation). Left side. Stimulation of the nerve below the wound (unipolar induction currents) gave no movements of flexion. Above the wound reflex movements and signs of pain. Complete loss of irritability.

Right side. Results of stimulation the same.

Portions of the nerves above wound, at wound, below wound and motor branches to the flexor muscles preserved for histological study.

Experiment X.

Jan. 24, 1891. Black mongrel, apparently full grown. Hypodermic injection of $\frac{2}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Ulnar nerve coagulated just above elbow by stream of water 80° C. circulating in a double walled silver cannula round the nerve. The stream continued until stimulation of the ulnar above the point coagulated caused no movements of flexion.

Right side. Ulnar nerve crushed just above elbow by a ligature of catgut, ligature soon removed. Stimulation above the wound caused no movements of flexion.

Examination. Jan. 31 (7 days after operation). Left side. Nerve had separated at point of coagulation. Complete loss of irritability in the peripheral end.

Right side. Mechanical and electrical stimulation of the peripheral end gave no movements at all. Stimulation above wound caused reflex movements and signs of pain.

Portions of the nerves, above wound, at wound, and below wound, together with the motor branches to the flexor muscles preserved for histological study.

Experiment XI.

Feb. 7, 1891. Small woolly black dog, not full grown. Hypodermic injection of $\frac{2}{5}$ gramme of morphia sulphate followed by ether.

Operation. Left side. Ulnar nerve coagulated just above elbow by stream of hot water 80° C., circulated in a bent glass tube round the nerve until stimulation above with moderate stimuli caused no flexion.

Right side. Ulnar nerve coagulated just above elbow by heating a broad copper wire bent round the nerve, until moderate electrical stimuli above caused no flexion.

Examination. Feb. 16 (9 days after operation). Left side. Wound scarcely perceptible. Mechanical and electrical stimulation below the point

of coagulation gave no movements whatever. Stimulation above gave signs of pain with reflex movements, and some movement in the foot which disappeared upon section of the median. Complete loss of irritability.

Direct stimulation of the flexor carpi ulnaris with induction current gave slight fibrillar contractions, with the galvanic current feeble contractions of the muscle.

Right side. Wound plainly visible but nerve continuous; results of stimulation practically the same as on the other side.

In taking out the nerves for histological examination it was recorded that the violent mechanical stimulation of the nerve in cutting with the scissors anywhere peripheral to the wound caused no reflex movements, which is further proof that this portion of the nerve (sensory fibres) was not irritable. See later experiments for similar observations.

Experiment XV.

March 2, 1891. Large black hound, apparently not full grown. Hypodermic injection of $\frac{3}{5}$ gramme of morphia sulphate followed by ether.

Operation. Left side. Ulnar nerve coagulated just above elbow by circulation of hot water (80° C.) round the nerve in a bent glass tube, continued until moderate stimuli above gave no movements of flexion.

Right side. Piece of ulnar, $\frac{3}{4}$ in. long, cut out just above elbow, ends not united by suture.

Examination. March 14 (12 days after operation). Left side. Nerve swollen at point of coagulation. Mechanical and electrical stimulation of the peripheral trunk and of the motor branches to the flexor muscles caused no movements whatever. Section of the peripheral trunk at different levels with the scissors gave no reflexes or signs of pain; hence no conduction of sensory impulses, complete loss of irritability.

Right side. Mechanical and electrical stimulation of the peripheral trunk gave no movements whatever. Hence complete loss of irritability.

Portions of the nerves removed as usual for histological examination.

Experiment VII.

Nov. 5, 1890. Bull-dog, full grown. Hypodermic injection of $\frac{2}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Ulnar nerve cut just above elbow and sutured immediately with 2 catgut sutures through the epineurium.

Right side. Ulnar nerve crushed just above elbow with single ligature of catgut; ligature soon removed and stimulation above caused no movements of flexion.

Examination. Nov. 19 (14 days after operation). Notes of the physiological examination were mislaid. Known that stimulation, mechanical and electrical, of the peripheral nerves on each side caused no movements of flexion at all. Hence complete absence of irritability.

Portions of the nerves removed as usual for histological examination.

Experiment VIII.

Nov. 14, 1890. Small brown bull-dog, not full grown. Hypodermic injection of $\frac{2}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Ulnar nerve cut just above elbow and immediately sutured with 2 catgut sutures.

Right side. Crushed with catgut ligature until stimulation above, after removal of ligature, gave no movements of flexion.

Examination. Dec. 6 (22 days after operation). Left side. 1. Stimulation (unipolar induction) above wound caused signs of pain and reflex movements.

2. Stimulation just below wound. Strong current gave slight spreading movements of digits (abduction).

3. Stimulation below branch to the flexor carpi ulnaris gave same result.

4. Very strong electrical stimulation of the trunk of the ulnar in the forearm above middle gave no signs of pain and no reflex movements of limbs or respiration. But cutting with scissors gave reflex movements distinctly showing production and conduction of sensory impulses after strong mechanical stimulation.

5. Direct stimulation of flexor carpi ulnaris with induction currents gave no visible contraction. With galvanic current gave slight muscular contractions.

Conclusion. Apparent return of conductivity and irritability to some of the motor fibres of abduction of digits (dorsal interossei) and to sensory fibres of trunk of ulnar above the middle of forearm when strong mechanical stimuli were used.

Right side. 1. Stimulation above wound caused signs of pain, reflex movements of arm, and also flexion of foot.

2. Stimulation below wound (minimal currents) gave flexion of foot and of digits.

3. Stimulation below branch to flexor carpi ulnaris gave slight movements of flexion of the digits.

4. Stimulation (very strong induction currents) of the trunk of the ulnar below the wound caused no signs of pain or reflex movements. But strong mechanical stimulation, cutting with scissors, caused distinct reflex movements especially of respiration.

Conclusions. A return of irritability and conductivity to the motor fibres, or some of them, most marked near the wound. The sensory fibres above the middle of forearm irritable at least to strong mechanical stimulation.

Portions of the nerve on each side removed and preserved as usual for histological examination.

Experiment IX.

Jan. 5, 1891. Small mongrel, apparently full grown. Hypodermic injection of $\frac{2}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Ulnar nerve cut just above elbow and immediately sutured with 2 catgut sutures.

Right side. Ulnar nerve crushed just above elbow by catgut ligature. After removal of ligature stimulation just above gave slight movements of abduction of foot, but no movements of flexion.

Examination. Jan. 26 (21 days after operation). Left side. 1. Stimulation (unipolar induction) above wound gave distinct flexion when current was too weak to cause reflexes.

2. Stimulation $\frac{1}{4}$ in. below wound. With same weak current gave no contraction, with stronger current distinct flexion of foot.

3. Light mechanical stimulation $\frac{1}{4}$ in. below wound (light hammer of hard rubber) gave flexion of foot easily.

4. Stimulation of the trunk of ulnar at wrist, beyond the origin of the dorsal cutaneous branch, with strong induction shocks and blows of the hammer, gave no visible effect.

Conclusions. Return of irritability and conductivity to some at least of the motor fibres of the peripheral ulnar. Irritability of peripheral end greater the nearer to the wound, disappears near level of wrist to ordinary electrical stimulation. Conductivity returns apparently sooner than irritability. Strong mechanical stimulation of nerve near wrist not attempted in this experiment. Near the wound in the irritable portion of the nerve mechanical stimulation seemed more effective than electrical.

Right side. 1. Electrical stimulation above the wound even with very weak currents gave distinct flexion of foot.

2. Stimulation (mechanical) with hammer gave same result.

3. Stimulation immediately below wound (electrical and mechanical) gave same result.

4. Stimulation $\frac{1}{2}$ in. below wound with electrical currents gave flexion only on the application of much stronger current. Stimulation at same point by the hammer gave flexion very easily.

5. Stimulation (electrical and mechanical) above wrist of both the main trunk of the ulnar and the dorsal cutaneous branch gave no effect whatever.

6. Tying ligature round the ulnar at the middle of forearm, where electrical stimulation and the blows of the hammer had had no effect, gave strong reflex movements.

Conclusions. Same as for left side. But here, as in Experiment VIII., violent mechanical stimulation, crushing, had aroused a sensory impulse when the strongest electrical shocks had no effect. Here as on the left side mechanical stimulation of motor fibres in the peripheral end was apparently more effective than electrical. The regenerated fibres of the peripheral nerve seemed to be conductive before they were irritable.

Portions of the nerve on each side removed and preserved as usual for histological examination.

Experiment XIV.

Feb. 28, 1891. Large black and white shepherd. Hypodermic injection $\frac{3}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Ulnar nerve coagulated just above elbow by stream of hot water 80° C. circulated round the nerve in a bent glass tube, until stimulation above gave no movements of flexion.

Right side. $\frac{1}{2}$ in. ulnar removed just above elbow, ends not sutured.

Examination. March 21 (21 days after operation). Left side. 1. Electrical and mechanical stimulation above wound gave reflex movements and signs of pain, but no flexion.

2. Electrical and mechanical stimulation below wound gave no response.

3. Direct stimulation of flexor carpi ulnaris with induced currents gave no contraction, with galvanic current a feeble contraction.

Conclusion. Irritability and conductivity not returned in peripheral end, owing probably to much wider "dead area" caused by coagulation.

Right side. Peripheral nerve totally unirritable. Flexor carpi ulnaris irritable to galvanic but not to induced currents.

Portions of the nerve on each side removed and preserved as usual for histological examination.

Experiment XXVIII.

June 1, 1891. Small mongrel puppy. Hypodermic injection of $\frac{2}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Ulnar nerve crushed by catgut ligature just above elbow. After removal of the ligature stimulation above caused no movements of flexion.

Right side. Operation in middle of arm. Central end of median sutured to peripheral end of ulnar, using two catgut sutures through the epineurium. Long pieces (inch +) cut from the peripheral end of median and central end of ulnar to prevent union.

Examination. June 22 (21 days after operation). Dog found dead in his cage. Supposed to have been caused by drinking some solution for "tanning." Animal warm when found. Post-mortem examination of the nerves showed that the median-ulnar suture on the right side had not united well. On the left side scarcely a perceptible trace of the wound in the nerve.

Only portions of the ulnar on the left side were removed and preserved for histological study.

Experiment XVII.

March 23, 1891. Small bull-dog, apparently full grown. Hypodermic injection of $\frac{2}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Operation in middle of arm. Central end of median sutured to peripheral end of ulnar, and central end of ulnar to peripheral end of median. Two catgut sutures through the epineurium used in each case.

Right side. Ulnar cut just above elbow and sutured with two catgut sutures through the epineurium.

Examination. April 20 (4 weeks after operation). Left side. The nerves at the double cross sutures bound together by a large amount of new growth, so that it was not possible to separate the median-ulnar tract from ulnar-median tract. Attempt made at physiological examination :—

1. Stimulation of wound and above gave signs of pain and reflex movements but no flexion.

2. Stimulation of peripheral ulnar gave no response even with very strong induced currents.

3. Stimulation of peripheral median above elbow gave movements of flexion, found to be due to a motor branch received at this level by the median from the musculo-cutaneous. When this alone was stimulated gave flexion. This branch afterwards found in all the dogs examined.

Conclusion. No return of function to the peripheral ulnar or to the peripheral median as far as the fibres cut were concerned.

Right side. 1. Stimulation at and above wound gave signs of pain, reflex movements including movements of foot; these latter disappeared after section of the median.

2. Stimulation below the wound gave no response.

3. Mechanical stimulation of the motor branch to the flexor carpi ulnaris gave slight flexion of foot.

4. Violent mechanical stimulation of trunk of ulnar below branch to the flexor carpi ulnaris (cutting with scissors) gave distinct reflex movements showing the generation of sensory impulses. Cutting below this point had given no result.

Conclusions. Partial return of function to motor and to sensory fibres. As before the return of function came first toward the central end and proceeded centrifugally. Mechanical stimulation seemed to be more effective than electrical, and the return of function to the sensory fibres more rapid than to the motor.

Experiment XVI.

March 23, 1891. Large shepherd dog. Hypodermic injection of $\frac{3}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Operation middle of arm. Central end of median sutured to peripheral end of ulnar, and central end of ulnar to peripheral end of median. Two catgut sutures through the epineurium in each case.

Right side. Ulnar nerve coagulated just above elbow by stream of hot water (80° C.) through a bent glass tube; heating continued until stimulation above caused no movements of flexion.

Examination. April 27 (5 weeks after operation). Left side. Nerve fused together at the wound by large amount of cicatricial tissue so that it was not possible to separate the median-ulnar tract from the ulnar-median tract.

The physiological examination gave the following:

1. Stimulation of median and ulnar above wound gave signs of pain and reflex movements; but no movements of flexion could be certainly seen.

2. Stimulation of the peripheral ulnar below the wound and above the elbow gave no flexion.

3. Strong mechanical stimulation of the ulnar at same place (cutting with scissors) gave distinct reflex movements showing the generation of sensory impulses (cutting the nerve below the elbow had given no result).

4. Stimulation of the median just below wound gave no result. Stimulation a little further down above elbow gave strong flexion owing to the presence of the communicating branch from the musculo-cutaneous.

Conclusions. Beginning of a return of function to the sensory fibres in ulnar, appearing first toward the wound. Strong mechanical stimulation more effective than strong electrical stimulation.

Right side. 1. Stimulation just above wound and just below wound gave feeble flexion of foot even after section of median.

2. Mechanical stimulation (blows of the small hammer) at the same points gave the same result.

3. Stimulation (mechanical and electrical) at the level of elbow gave no result.

Conclusions. Beginning of a return of function to some of motor fibres, appearing first just below the wound, indicating, as in the other experiments, a centrifugal development of function.

Portions of the nerves on each side removed and preserved as usual for histological examination.

EXPERIMENT XVIII.

March 30, 1891. Large red dog, full grown. Hypodermic injection of $\frac{3}{5}$ gramme of morphia sulphate followed by ether.

Operation. Left side. Ulnar cut just above elbow; sutured after 1 hour with two catgut sutures through the epineurium.

Right side. Ulnar cut just above elbow, sutured immediately with two catgut sutures through the epineurium.

Examination. May 4 (5 weeks after operation). Left side. Nerve much swollen at point of suture.

1. Stimulation (electrical and mechanical) of nerve at wound and just below gave movements of flexion together with signs of pain (reflex movements). Same result after section of median though not so marked.

2. Stimulation just below wound gave flexion of foot after section of branches to flexor carpi ulnaris, but not after section of whole trunk below those branches, hence probably some return of function to the motor branches to the flexor profundus.

3. Stimulation of ulnar in forearm below branch to flexor profundus gave no result.

4. Stimulation directly of branch to flexor profundus gave no result.

5. Violent mechanical stimulation (cutting with scissors) of ulnar above branch to flexor profundus caused distinct reflex movements.

Conclusions. Some return of function (irritability) to motor fibres just below wound, but evident power of conduction as far down as branches to flexor profundus. Return of function to sensory fibres (violent mechanical stimulation) further towards the periphery, near origin of branch to flexor profundus, indicating a quicker return of function to sensory fibres.

Right side. Nerve nicely united without much swelling.

1. Stimulation (unipolar induction) at wound and below for a fraction of an inch gave signs of pain, reflex movements and also flexion of foot (flexor carpi ulnaris was seen contracting).

2. After section of branches to flexor carpi ulnaris stimulation of the trunk of the nerve below wound caused slight flexion of digits (mechanical as well as electrical stimuli were used).

3. Direct stimulation of the branches to the flexor carpi ulnaris and the flexor profundus gave no result.

4. After section of the median and of the motor branch to the flexor carpi ulnaris stimulation of the ulnar below the giving off of the latter still gave distinct flexion of digits, though the muscular response could only be obtained occasionally.

5. Stimulation of the trunk of the ulnar fully two inches below the wound, when strong currents were used, still gave reflex movements when no direct muscular movements of flexion could be obtained.

6. Violent stimulation of the nerve near the wrist gave no response at all.

Conclusions. More complete return of function to the motor fibres of the peripheral nerve, proceeding centrifugally from the wound. Here, as in previous experiments, indications that the conductivity to an impulse aroused above returns before the property of irritability to a direct stimulus. See (3) stimulation of branch to flexor carpi ulnaris.

The return of function to sensory fibres somewhat more rapid than to motor, that is, the fibres can be stimulated further away from the wound.

Portions of the nerve on each side removed as usual for histological examination.

Experiment XIX.

April 6, 1891. Large black hound, puppy. Hypodermic injection of $\frac{2}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Operation in middle of arm. Median and ulnar cut. Central end of median sutured to peripheral end of ulnar with two catgut sutures through epineurium. Long pieces of peripheral end of median and central end of ulnar removed to prevent possibility of union.

Right side. Ulnar nerve cut just above elbow and sutured with two catgut sutures through the epineurium.

Examination. May 25 (7 weeks after operation). Animal showed sores on ball of last digit.

Left side. Nerves had united well, very little swelling.

1. Stimulation (electrical and mechanical) of the ulnar above elbow but below wound gave no result.

2. Stimulation at wound and above gave signs of pain, reflex movements including an adduction movement of forearm.

3. Mechanical stimulation of wound gave slight abduction movements of paw (possibly from feeble and partial contractions of flexors of paw, though this was not ascertained).

Conclusions. Unsatisfactory evidence of any return of function in the peripheral ulnar.

Right side. Nerve swollen at point of suture.

1. Stimulation (unipolar induction) above wound with stimuli too feeble to arouse reflex movements or signs of pain gave flexion of foot.

2. Stimulation just below wound with same strength of current gave no effect; with increase in current got flexion of foot.

3. Same experiments repeated after section of the median, to throw out the possibility of a reflex, gave same results.

4. Stimulation of ulnar half an inch below wound with same strength of current as in Exp. II., gave no result; increasing the strength of current obtained good flexion of foot.

In these experiments contractions of the flexor carpi ulnaris could not be seen when the muscle was exposed, but contractions of the flexor profundus were distinctly seen.

5. Direct stimulation of the motor branches to the flexor carpi ulnaris gave no response. Cutting with scissors also no response.

6. Direct (unipolar induction) stimulation to the chief motor branch to the flexor profundus gave no response. On cutting with scissors got a distinct contraction of the muscle. From two smaller motor branches to same muscle no response could be obtained.

7. Stimulation (electrical) of ulnar below branches to flexor profundus gave no response. Cutting with scissors gave distinct reflex movements showing production of sensory impulses.

8. After section of all branches to the flexor carpi ulnaris and flexor profundus stimulation at and just below wound gave no flexion of digits that could be unmistakably seen (there was apparently a slight abduction of foot). But the nerve had been stimulated so often and exposed so long by this time that the results are to be considered uncertain.

Conclusions. More complete return of function to some of motor fibres, the more perfect the nearer to the wound. A return of function to the fibres of the flexor profundus before those to the flexor carpi ulnaris which are given off sooner from the trunk. The return of the property of conductivity before that of irritability as shown by the experiments on the branch to the flexor profundus. The return of function to sensory fibres had proceeded farther towards the periphery than in the case of the motor fibres.

Experiment XXII.

May 18, 1891. Small brown spaniel, not full grown. Hypodermic injection of $\frac{2}{5}$ gramme morphia sulphate followed by ether.

Operation. Left side. Operation in middle of arm. Median and ulnar cut. Central end of median sutured to peripheral end of ulnar with two catgut sutures through the epineurium.

Long pieces of central ulnar and peripheral median cut off to prevent possibility of union.

Right side. Ulnar cut just above elbow and immediately sutured with two catgut sutures through the epineurium.

Examination. Aug. 1 (75 days after injury). Left side. Union of the two nerves very complete, scarcely any perceptible swelling.

1. Stimulation (electrical and mechanical) of median above wound gave strong flexion of foot. Both flexor carpi ulnaris and flexor profundus were exposed and seen to contract.

2. Stimulation at the wound and just below gave the same result.

3. Stimulation of the ulnar at the elbow gave the same result, but required stronger electrical stimulation.

4. Stimulation of the ulnar at the giving off of motor branches to the flexor profundus gave a contraction of the muscle, but required an increase in the strength of the stimulus compared with (3).

5. Stimulation of ulnar at wrist gave movement of digits but required stronger current than in (4).

6. A small cutaneous branch near the wrist when stimulated electrically gave distinct reflex movements especially of inspiration, showing generation of sensory impulses.

The dorsal cutaneous branch of the ulnar was apparently abnormal in its origin in this dog on both sides, arising above the middle of the forearm together with a branch to the flexor carpi ulnaris. It was not stimulated therefore on either side, but specimens from the right side were preserved for histological examination.

7. Tying ligature round ulnar at wrist gave violent reflex movements.

Conclusions. Almost complete return of function to motor and sensory fibres as far as the wrist at least. The irritability (motor fibres) was greater the nearer to the wound the stimulus was applied.

Right side. Nerve united well but some swelling, more than on left side.

1. Stimulation of the nerve at different levels as on the left side gave identical results, with the exception that the small cutaneous branch arising near the wrist, as on the left side, gave no distinct reflex movements when stimulated.

2. Electrical stimulation of ulnar at wrist gave distinct reflex showing the production of sensory impulses at this level.

3. Tying a ligature round the ulnar at wrist gave violent reflexes and signs of pain.

Conclusions. Nearly complete return of function to both motor and sensory fibres as far as wrist at least. The irritability of the fibres diminished the greater the distance from the wound the stimulus was applied.

Portions of the nerve on each side were removed as usual for histological examination.

Post-mortem examination of the nerves on the left side showed that the central end of the ulnar ended in a slight enlargement which tailed off peripherally into a loose connective tissue-like strand. The peripheral end of the median was slightly enlarged and adherent to the brachial artery.

EXPERIMENT XXVI.

May 29, 1891. Rabbit. Anaesthetized during the operations by ether.

Operation. Left side. Ulnar cut just above elbow, piece $\frac{3}{4}$ in. long removed. Ends not united by suture.

Right side. Ulnar crushed by ligature just above elbow. After removal of ligature stimulation above gave no flexion.

Examination. Aug. 8 (10 weeks after operation). A very careful physiological examination was not attempted, as the experiment was made chiefly for histological work. The following observations were made.

Left side. Ends of nerve not united. Stimulation of the peripheral end at various points along the forearm showed that it was perfectly unirritable.

Right side. Wound not visible. Stimulation of the nerve along the forearm gave flexion of foot and digits easily with very weak currents, showing an apparently complete return of irritability.

In this animal the difference in the appearance of the peripheral ulnar on the two sides was very marked. On the left side where no union was made it was smaller and of a translucent grayish appearance. On the right side it had the normal size and opaque look.

Portions of the nerve on each side were removed and preserved as usual for histological examination.

EXPLANATION OF FIGURES. PLATES XII.—XVII.

The figures with one exception were drawn under a zeiss *D* and ocular 4. The outlines were filled in with the Camera Lucida and an attempt made to colour the drawings like the particular specimen under examination.

Fig. 1. To show the first segmentation of the myeline in degeneration. Each segment completely enclosed in myeline, with a central piece of the axis cylinder. Experiment XX. 2 days. R. S. middle of forearm. Osmic acid and haematoxylin.

Fig. 2. To show the secondary fragmentation of the myeline (extreme case). Exp. XX. 2 days. L. S. middle of forearm. Osmic acid and haematoxylin.

Fig. 3. To show the remnants of the disintegrating axis in the segments of myeline. Exp. XIII. 4 days. R. S. 3 in. below wound. Gold and haematoxylin. Nucleus stained blue in haematoxylin.

Fig. 4. The same. Exp. III. 7 days. R. S. Below wound 2 in. Picric acid and fuchsin stain.

Fig. 5. The same. Exp. III. 7 days. R. S. Below wound 2 in. Picric acid and fuchsin stain.

Fig. 6. To show the more rapid fragmentation of the myeline in the neighbourhood of the nuclei. Exp. III. 7 days. Gold and haematoxylin. Nucleus stained blue.

Fig. 7. To show the multiplication and migration of the nuclei of the sheath and greater absorption near nuclei. Exp. III. 7 days. Gold and haematoxylin.

Fig. 8. The same. Exp. III. 7 days. Gold and haematoxylin.

Fig. 9. An instance of apparent indirect division of nucleus. The progressing fragmentation of myeline. Exp. III. 7 days. Gold and haematoxylin.

Fig. 10. (Drawn under obj. C. oc. 4). To show fragmentation of myeline especially at nuclei, and the material containing fragments of myeline which fills the fibre. Exp. III. 7 days. Gold and haematoxylin.

Fig. 11. To show progressive absorption of myeline; the proliferation of the nuclei; the position of the nuclei with reference to the balls of myeline; and the collapse of the empty sheath. Exp. XI. 9 days. R. S. middle of forearm. Gold and haematoxylin.

Fig. 12. To show still further absorption of myeline, the increase in nuclei and the way they are grouped round the balls of myeline. The

collapsed tube between the nuclei. A bit of endoneural sheath lying at the side of the fibre. Exp. VII. 14 days. R. S. Gold and haematoxylin.

Fig. 13. To show the nearly complete absorption of myeline and the nuclei lying free in the tubes. Exp. VII. 14 days. R. S. Gold and haematoxylin.

Fig. 14. To show the irregularity of absorption. A portion of the tube free from myeline next to a portion still showing large segments. Exp. VII. 14 days. L. S. Gold and haematoxylin.

Fig. 15. The same. Exp. VII. 14 days. R. S. Gold and haematoxylin.

Fig. 16. To show the beginning of the formation of an embryonic fibre, the growth of protoplasm at the nucleus. Exp. XIV. 3 weeks. R. S. 3 in. below wound. Gold and haematoxylin.

Fig. 17. The same. Later stage. Exp. XIV. 3 weeks. L. S. 3 in. below wound. Gold and haematoxylin.

Figs. 18, 19, 20. To show stages in the formation of the embryonic fibres, 18 from Exp. IX. R. S. (3 weeks), 19 from Exp. IX. R. S. (3 weeks) and 20 from Exp. VIII. R. S. (3 weeks). Gold and haematoxylin.

Fig. 21. To show the flattened shape of the embryonic fibres often seen in the preserved specimens. Exp. VIII. 3 weeks. L. S. Below wound $\frac{3}{4}$ in. Gold and haematoxylin.

Fig. 22. To show the large balls of myeline in the plasma cells lying between the fibres. Exp. XVII. R. S. 4 weeks. 4 in. below wound. Osmic acid and haematoxylin.

Figs. 23, 24. To show fully formed embryonic fibres and appearance of new sheath. Exp. XXVIII. 3 weeks. Br. to flex.-profundus. Osmic acid and haematoxylin.

Figs. 25, 26, 27, 28. From the peripheral end of human ulnar $6\frac{1}{2}$ months after section, union with central end not made. To show formation of "embryonic fibres." Gold and haematoxylin.

Fig. 29. Cross-section of human ulnar peripheral end $6\frac{1}{2}$ months after section, union with central end not made. To show cross-section of bundles of embryonic fibres and the appearance of the nuclei (only one bundle filled in). Gold and haematoxylin.

Fig. 30. The same. Stained in haematoxylin alone.

Fig. 31. Cross-section of peripheral ulnar 4 weeks after division. After suture but before complete regeneration. To show the resemblance of the nuclei and their nucleoli to cross-sections of young nerve fibres. Exp. XVII. 4 weeks. L. S. Gold and haematoxylin.

Figs. 32, 33. Embryonic fibres in peripheral ulnar of rabbit, 7 weeks after section without union to central end. To show apparent attempt at the formation of myeline. Exp. XXVI. Osmic acid and haematoxylin.

Figs. 34, 35. To show the apparent beginning of a myeline transformation in the protoplasm of the embryonic fibres. Exp. XXVIII. 3 weeks. From branch to flexor profundus. Osmic acid and haematoxylin.

Figs. 36, 37. The first, to show the isolated drops of newly-formed myeline, the second, to show the varicose tube formed by their union. (Portions of the same fibre.) Exp. XXVIII. 3 weeks. Just below wound. Gold and haematoxylin.

Fig. 38. To show the isolated drops of newly-formed myeline and the processes which unite them. Exp. XXVIII. 3 weeks. Just below wound. Osmic acid and haematoxylin.

Figs. 39 and 40. To show the same and the staining of the balls of myeline in the haematoxylin. Each of the fibres shows a few fragments of old myeline unabsorbed lying near the nuclei. Exp. XXVIII. 3 weeks. Just below wound. Osmic acid and haematoxylin.

Figs. 41 and 41a. The same. To show discontinuous formation of the myeline tube. Exp. XXVIII. Below wound. Osmic acid and haematoxylin.

Fig. 42. The same. Exp. XXVIII. Below wound. Osmic acid and haematoxylin.

Fig. 43. To show varicose appearance of newly-formed tube of myeline. Exp. XXVIII. $\frac{3}{4}$ in. below wound. Osmic acid and haematoxylin.

Fig. 44. The same. Exp. XXII. R. S. near wrist. Osmic acid and haematoxylin.

Fig. 46. To show the formation of the myeline tube without varicosities. Exp. XXVIII. $\frac{3}{4}$ in. below wound. Osmic acid and haematoxylin.

Fig. 47. To show union of isolated drops of myeline to form a tube. Exp. XXVIII. Osmic acid and haematoxylin.

Fig. 48. To show discontinuous formation of myeline sheath. Exp. XXVIII. Osmic acid and haematoxylin.

Fig. 49. The same. Exp. XXVIII. Osmic acid and haematoxylin.

Fig. 50. To show the persistence of balls of old myeline as yet unabsorbed and their position with reference to the nuclei. Exp. XXVIII. Osmic acid and haematoxylin. Nuclei stained in haematoxylin.

Fig. 51. To show the formation of a node of Ranvier. Exp. XXVIII. Osmic acid and haematoxylin.

Fig. 52. To show the newly-formed myeline tube lying in the embryonic fibre. Exp. IX. 3 weeks. From dorsal cutaneous branch. Gold and haematoxylin.

Fig. 53. The same. Exp. IX. L. S. Dorsal cutaneous branch. Gold and haematoxylin.

Fig. 54. To show the formation of nodes of Ranvier. Exp. IX. L. S. Dorsal cutaneous branch. Gold and haematoxylin.

Fig. 55. To show newly-formed myeline tube with node of Ranvier and persistent nuclei. Exp. IX. L. S. 3 weeks. At wound. Gold and haematoxylin.

Figs. 56, 57, 58, 59. From nerve stained in picric acid and haematoxylin. Exp. XIX. 7 weeks. R. S. $\frac{1}{2}$ in. below wound. 59. An embryonic fibre. 58. Newly-formed fibres with myeline and axis. 57 and 56. Newly-forming fibres, myeline as a varicose tube. Axis also present but not stained in the interior of the swellings of myeline. Shows how quickly after the formation of the myeline the axis grows down from above.

Fig. 60. To show the branching of the axis cylinder where an old fibre passes into several new fibres, central end of ulnar. Exp. XXII. Cut for 75 days and union with peripheral end prevented. Picric acid and haematoxylin.

Fig. 61. To show what seems to be the outgrowth of an axis cylinder from an old fibre toward a new one. New myeline lies in the embryonic fibre as a continuous delicate sheath. Exp. XVII. 4 weeks. R. S. Gold and haematoxylin.

Fig. 62. To show the formation of several new fibres in the degenerated portion of a single old fibre at the central end of wound. Exp. XVII. 4 weeks. L. S. wound of median ulnar suture. Osmic acid and haematoxylin.

Fig. 63. The same. Human ulnar nerve. Central end of nerve $6\frac{1}{2}$ months after injury. No union with peripheral end. Gold and haematoxylin.

Fig. 64. To show junction of old with new fibre at the central end. Exp. XVIII. R. S. wound. Osmic acid and haematoxylin.

Fig. 65. The same. Human ulnar. Central end $6\frac{1}{2}$ months after section and no union with peripheral end. The continuation of the protoplasm of the "embryonic fibre" into the hypertrophied protoplasm surrounding the nuclei of the old fibre, and the fragmentation of the end of the old myeline to be specially noted. Gold and haematoxylin.

Fig. 66. To show junction of old and new fibres, and the new fibre with its thin myeline sheath lying in the protoplasm of the "embryonic fibre." Exp. XVII. R. S. wound. Gold and haematoxylin.

Fig. 67. To show junction of old and new fibres. Central end of human ulnar, $6\frac{1}{2}$ months after injury and no union with peripheral end. The fragmentation of the myeline and the hypertrophy of the protoplasm round the nuclei of the old fibre to be noted. Gold and haematoxylin.

Fig. 68. The same.

Fig. 69. Cross-section through the bulbous enlargement of central end of human ulnar, $6\frac{1}{2}$ months after injury and no union with central end. Shows the increase in the endoneural connective tissue as well as in the

nerve fibres. The fibre marked + shows cross section of new fibres in same sheath with old. Compare with figs. 63, 65.

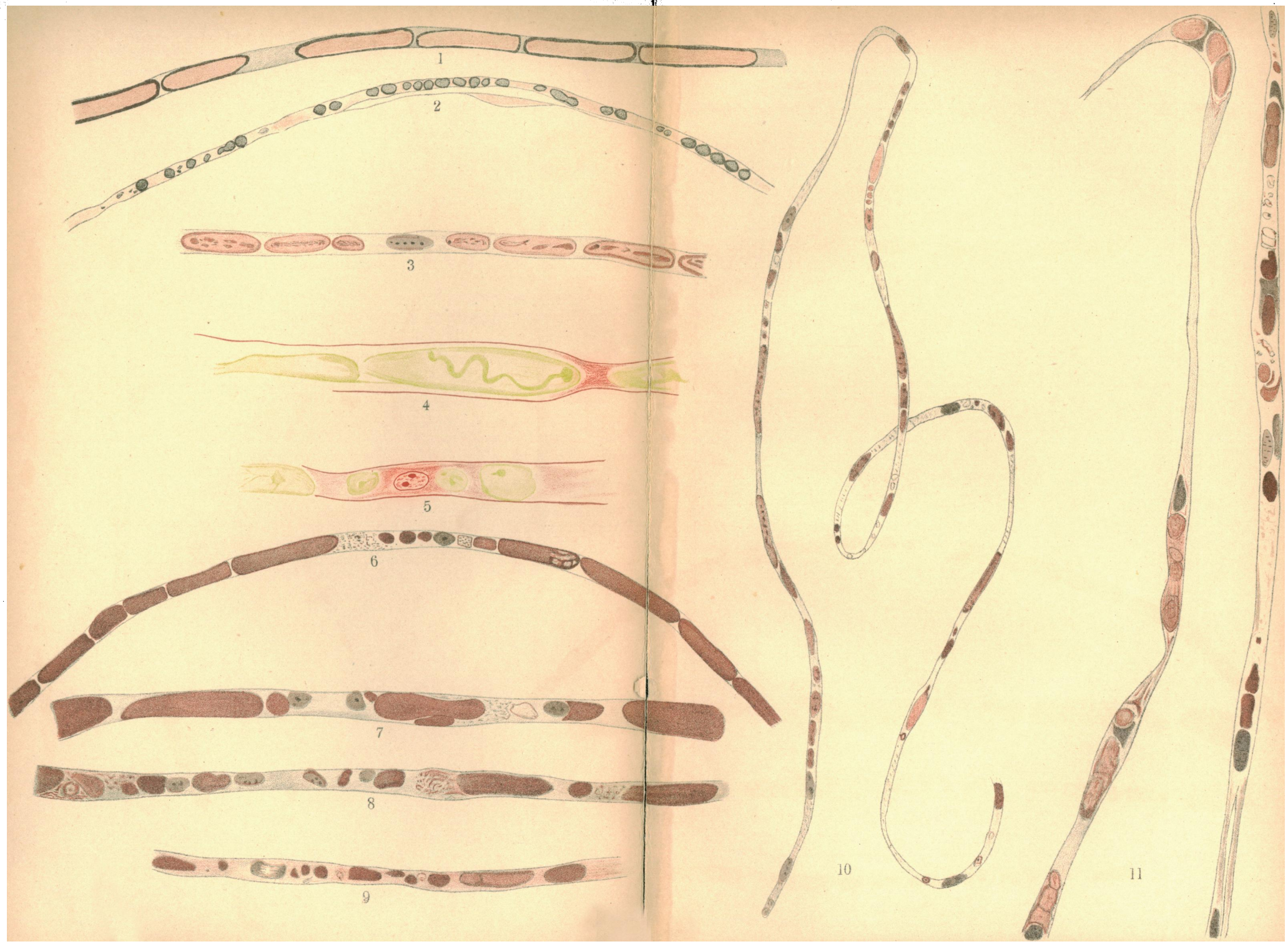
Figs. 70, 71, 72. To show the connection between the axis cylinder of the old and the new fibre. Compare 71 with figs. 64, 65. Exp. XXII. Central ulnar after 75 days, no union. Picric acid and haematoxylin.

Fig. 73. To show the formation of two embryonic fibres in a fibre of central end near the wound, connecting a distal piece which has not degenerated. Central end of human ulnar $6\frac{1}{2}$ months after injury. Gold and haematoxylin.

Fig. 74. To show intercalated pieces of newly formed fibres without degeneration of distal end. Exp. XVII. L. S. 4 weeks. Wound. Osmic acid and haematoxylin.

Fig. 75. The same, from central end of ulnar (Exp. XXII.) 75 days after section, shows the axis cylinder in the intercalated piece and its connection on each side with the intact axis of old fibre. Picric acid and haematoxylin.

Fig. 76. The same. Exp. XXII. Central end of ulnar after 75 days. Picric acid and haematoxylin.



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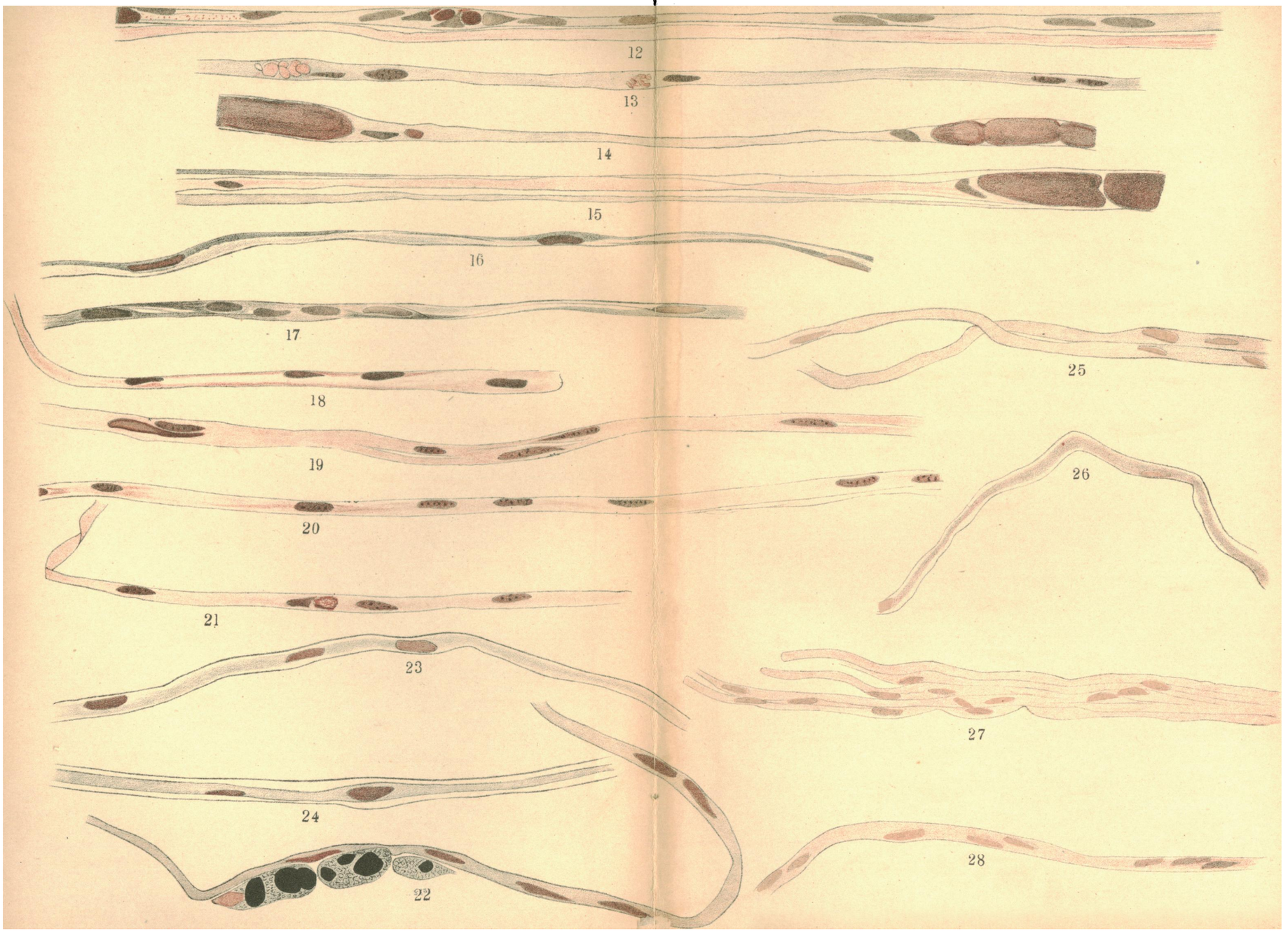

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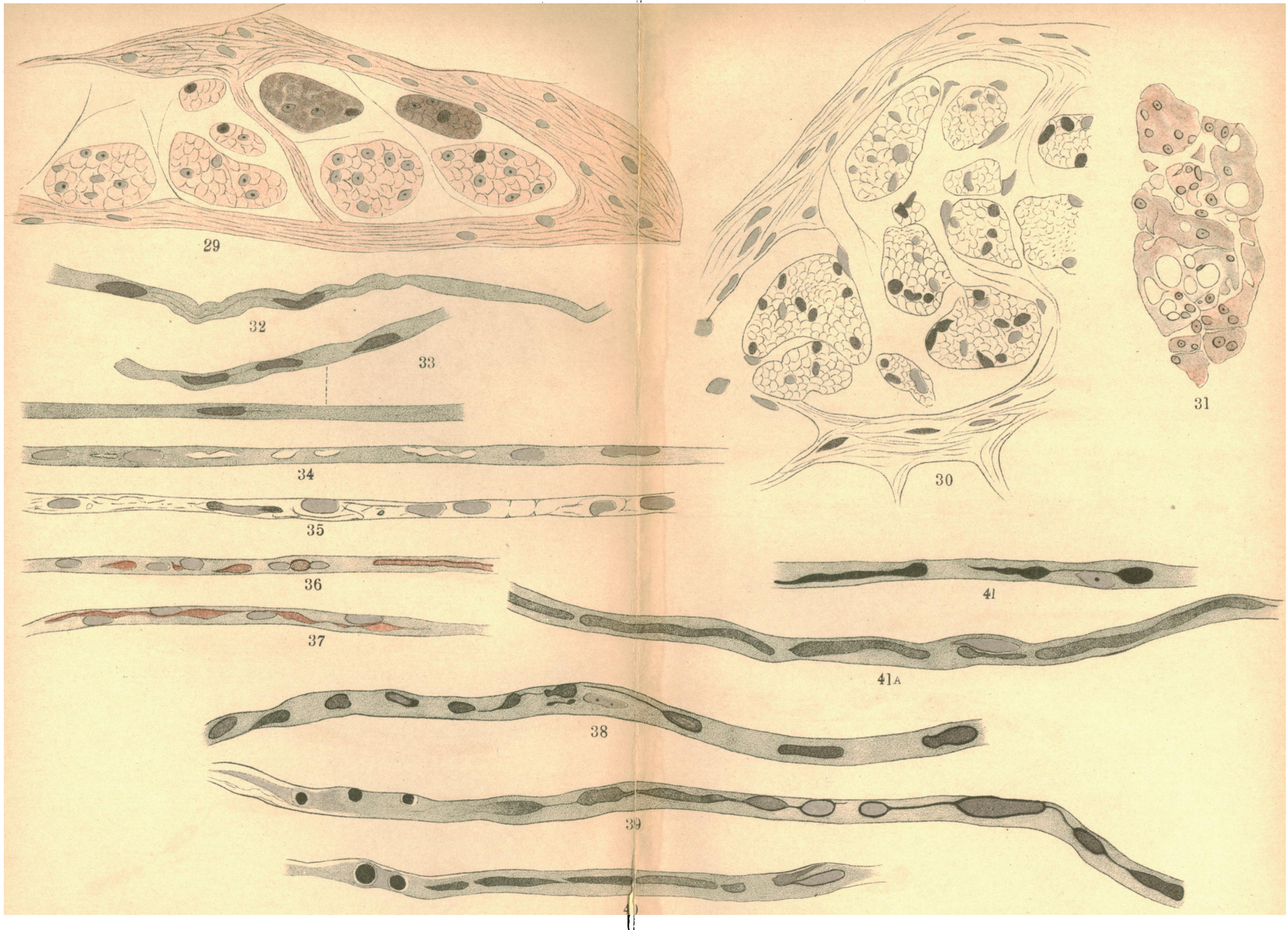

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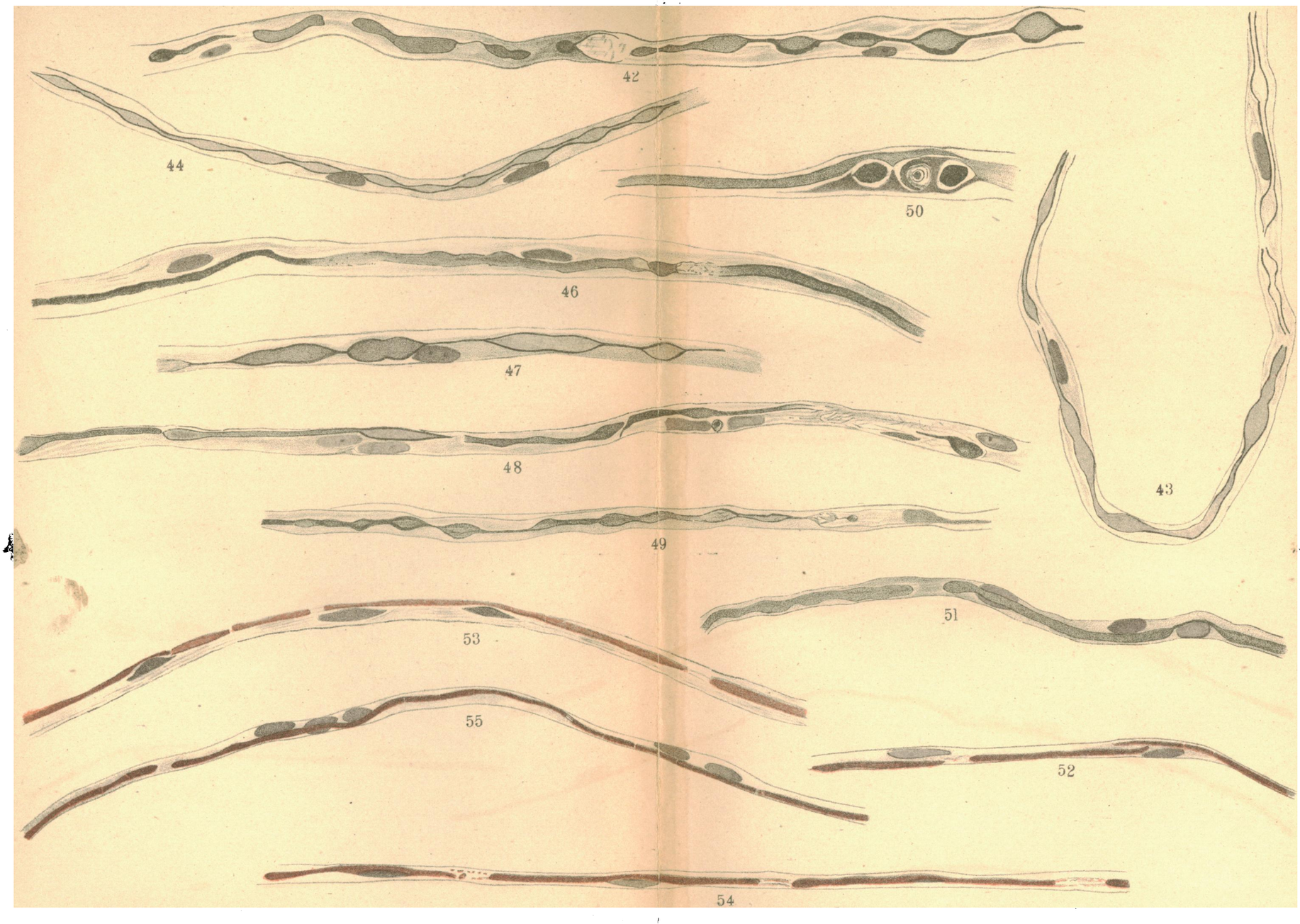

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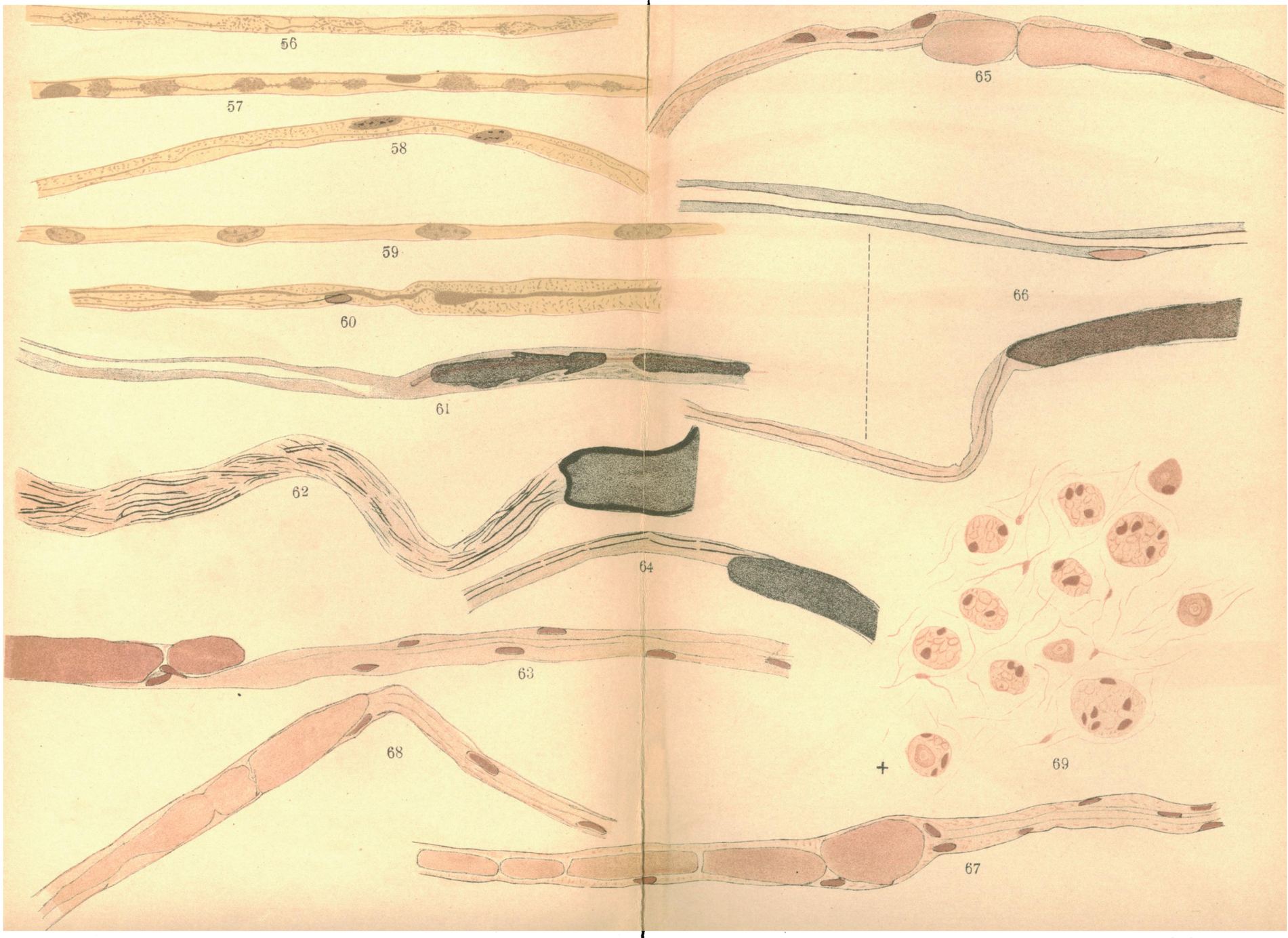

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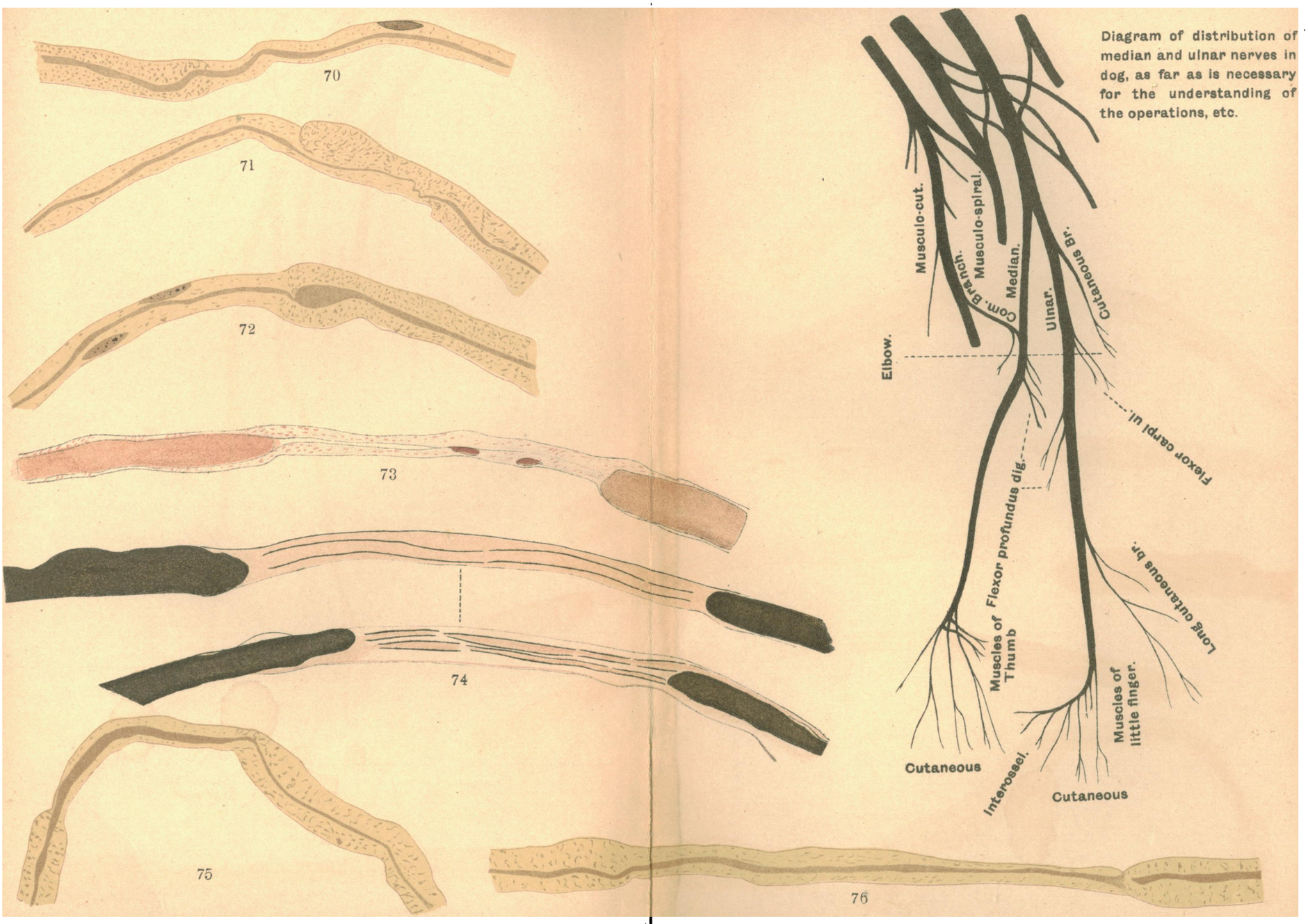

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