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Regeneration of Nerves.

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[PLATE 15.]

In 1901 two of us published a paper on Nerve Degeneration,* a subject which it is almost impossible to study without taking into account the closely-related subject of nerve regeneration. From the microscopic study of the distal portions of divided nerve trunks we arrived at the conclusion that the activity of the neurilemmal cells has some relation to the development of the new nerve-fibres. At an early stage in degeneration they multiply; later they participate with phagocytes in the removal of the broken-up myelin droplets; subsequently they elongate, and, becoming connected end to end, lead to the formation of what some term embryonic nerve-fibres. These three stages are illustrated by some of the microphotographs published in the paper alluded to. (See especially figs. 22, 25, and 27.)

We were, however, extremely doubtful whether this appearance really indicated the formation of real nerve-fibres capable of conducting impulses, and felt that such incomplete observations could not be considered as serious objections to the view of those who, from Waller onwards, have taught that the axis cylinder is the branch of a nerve-cell which grows towards the periphery.

In a preliminary communication† which we published two years ago we called special attention to the work of Howell and Huber‡ on the subject. These observers, who employed both histological and experimental methods of observation, and who noted, as all other writers before and since have done, the neurilemmal activity, arrived at the conclusion that although these peripheral structures are active in preparing the scaffolding, the axis cylinder, which is the essential portion of a nerve-fibre, has an exclusively central origin. We further stated that the more work we have done on the subject the more have we become convinced that this view is the correct one.

The purpose of this paper is to state more fully the evidence that has led us to this conclusion.

* "The Chemistry of Nerve-Degeneration," by F. W. Mott and W. D. Halliburton, 'Phil. Trans.,' B, vol. 194, pp. 437—466, 1901.

† 'Proceedings of the Physiol. Soc.,' March 19, 1904; 'Journal of Physiology,' vol. 31.

‡ 'Journ. of Physiology,' vol. 13, p. 333 1892); vol. 14, p. 183 (1893).

Since we commenced our work, the subject has risen into considerable prominence, and numerous papers have been published on the question. Some investigators, like Purves Stewart, and Ballance,* in this country, and Bethe† in Germany, have advanced the view that the new nerve-fibres have a peripheral origin, while others, like Langley and Anderson,‡ and S. Ramon Cajal,§ have defended the older Wallerian doctrine. In Cajal's paper a very complete bibliography of the large number of investigations devoted to the enquiry is also given.

We ventured to suggest in the preliminary notice of our work that the manifest activity of the neurilemmal cells is related in some degree, probably nutritionally, to the successful repair of a divided nerve, for in situations like the central nervous system, where the neurilemma does not exist, not only is the removal of degenerated myelin a very slow process, but, as is well known, regeneration does not occur. When the nerve is regenerated, and conducts impulses, the elongating strands of neurilemmal cells are seen to be situated outside the new axis cylinder, and in longitudinal view the latter is thus frequently concealed; but on transverse section the axis cylinder is seen to be quite distinct and separate within this sheath, although the neurilemmal nuclei retain an abnormal thickness for some time.‖

The idea that the sheath is nutritive has been advanced in a somewhat modified form more recently by Graham Kerr.¶ He points out that there are three main views regarding the development of motor nerves in vertebrata:—

1. Each nerve-fibre develops as an independent outgrowth from a ganglion cell, which gradually grows outwards and finally becomes united to its special muscle. This view is associated especially with the name of His, and is favoured by the majority of embryologists.

2. The nerve trunk is regarded as multicellular in origin, and consists at first of a chain of cells, in the substance of which the nerve-fibres are developed later as fine strands passing continuously from one cell body to

* 'The Healing of Nerves,' Macmillan and Co., 1901.

† 'Neurol. Centralbl.,' January, 1902, p. 60; 'Allgemeine Anatomie und Physiologie des Nervensystems,' Leipzig, 1903.

‡ 'Journ. of Physiology,' vol. 31, p. 418 (1904).

§ "Mecanismo de la regeneración de los Nerviós," 'Trabajos del Laboratio de investigaciones biologicas de la Universidad de Madrid,' vol. 4, pp. 119—210 (1905); "Mécanisme de la Régénération du nerf," 'Compt. rend. Soc. de Biol.,' vol. 59, p. 420; "Critique de la théorie de l'autorégénération," *ibid.*, p. 422.

‖ This is well illustrated in fig. 30, p. 464, of our 'Phil. Trans.' paper.

¶ "On some Points in the Early Development of Motor Nerve Trunks and Myotomes in Lepidosiren," by J. Graham Kerr, 'Roy. Soc. Edin. Trans.,' vol. 41, pp. 121—128 (1904).

another. The elements forming the original chain are regarded as ectodermal elements which have wandered out from the spinal cord rudiments. The sheath is derived from those parts of the original cell chains which retain their protoplasmic character.

3. The nerve trunk is not a secondarily formed bridge between the spinal cord and the peripheral organ, but exists from the first, and in subsequent development it merely undergoes elaboration and increase of length as the distance between the spinal cord and the periphery increases with the increasing size of the animal.

His study of the development of motor nerves in the fish Lepidosiren leads Kerr to adopt the third view as regards the nerve trunk itself, and the second view as regards its sheath. In early stages the motor trunk is naked, but later masses of mesenchymatous protoplasm, laden with yolk, become applied to the nerve trunk, at first over only a small portion of its length ; these masses gradually spread over the whole trunk, from which they are clearly distinguishable by staining reactions. As development goes on, the yolk in these masses is used up, their protoplasm becomes less and less conspicuous, and eventually is only to be detected in the immediate vicinity of the nuclei. At first the central protoplasmic strand in each fibre is simply granular, but later the passage of impulses finds expression in the marking out of definite fibrillar tracks, some undifferentiated protoplasm remaining as the inter-fibrillar substance.

It is pretty clear that the great function of the sheath is as a nutritive organ ; its protoplasm is at first laden with yolk, and this is gradually used up as the nerve trunk develops within it.

That the main function of nuclei, apart from reproduction, is to control cytoplasmic metabolism is well recognised, and the nuclei of the sheath are able to exercise this control over the active metabolism of the developing nerve trunk, which is destitute of nuclei of its own. Connected with this relation is, no doubt, the active multiplication of these nuclei observed in early stages of nerve regeneration. In such regeneration it may well be that the protoplasmic matrix of the nerve simply repeats the process of its original development, increasing in size and then developing nerve fibrils within itself.

On this view the process which takes place in the peripheral segment of a cut nerve would be somewhat as follows :—

The fibrils, no longer subject to the stimulus of passing nerve impulses, revert to their protoplasmic condition, and the sheath becomes highly active : it increases in thickness, and its nuclei multiply ; its protoplasm digests the remains of the medullary sheath. Supplied with nourishment by the activities of the surrounding sheath, the protoplasm behaves just as it does in ontogenetic development; it grows—probably slowly—and so gaps are bridged over; as soon as it becomes continuous, nerve impulses begin to play backwards and forwards in its substance and cause again a differentiation into fibrils. As part of the impulse tracks persist at the central stumps of the fibrils, the regenerated parts of the fibrils will naturally develop in exact continuity with these.

We have reproduced Professor Graham Kerr's views at some length because they support, in an entirely independent manner, our own views on the nutritive properties of the nerve sheath. Whether, however, such nutritive control is able to act so far as to produce actual fibrillæ we are extremely doubtful. Graham Kerr admits that in a degenerated and regenerating fibre the protoplasmic strand can only be demonstrated with extreme difficulty, and he further admits the necessity of union with the central stump to ensure functional regeneration. In our own experiments, as will be more fully described later, we have never seen anything of the nature of an axis cylinder or neuro-fibrillæ in the peripheral segment of a divided nerve, provided connection with the central end is successfully prevented.

Graham Kerr further emphasises the fact that his observations apply to one animal only, and that he does not wish to draw general conclusions from them as to what occurs in other vertebrates. He, however, points out that Bethe's observations on the chick, although apparently supporting the cell-chain view, are in no way irreconcilable with the observations he chronicles. With regard to the view he advances that the original nerve trunk exists from the first as a link which stretches in length with the growth of the animal, it appears to us that it is incapable of explaining how a gap an inch or so long can be bridged across when one excises, as we have done in some of our experiments, a portion of nerve of that length. The union that occurs, often very rapidly, even when the ends are not sutured together, must be due to growth of nerve fibres from the central to the peripheral stump or in the reverse direction.

In a paper published by the veteran histologist, von Kölliker,* shortly before his death, the theory that regeneration can occur in the peripheral end of a cut nerve without connection or control from the central end is very vigorously criticised. Kölliker himself was instrumental many years ago in putting forward the view that the nerve sheath, known as the neurilemma, is a secondary formation in the mesoblast into which the axons penetrate, and in his later years became an exponent of the neuron theory. It is mainly

* 'Anat. Anzeiger,' vol. 25, p. 1 (1904).

from these two points of view that he has attacked Bethe's position, and it certainly appears on *a priori* grounds exceedingly improbable that mesoblastic cells should be capable of giving rise to such a highly specialised structure as an axis cylinder, which so far as is known is exclusively epiblastic in origin.

We may, however, now pass from the region of speculation to consider briefly a few typical researches dealing with the facts of regeneration, before describing our own observations.

The propositions maintained by Waller,* namely, the integrity of the central end of a cut nerve, the degeneration of the peripheral segment, and its regeneration by fibres growing out from the central stump, were questioned a few years later by Vulpian and Philippeaux.† These observers cut nerves in various, but mainly, young animals, excising long portions to prevent reunion with the central end. Some months later they were surprised to find that the peripheral ends had regenerated and were excitable; to this phenomenon they gave the name of "autogenetic regeneration." These results were regarded with great scepticism by most writers,‡ until, in 1874, Vulpian§ repeated his experiments, and obtained the same results as before. This time, however, Vulpian put forward a new explanation and, in fact, accepted the Wallerian doctrine, because he found that connection with the central nervous system had been re-established by means of fibres growing into the peripheral stump from other nerves in the neighbourhood. He had also, with Philippeaux, observed regeneration in portions of nerve trunks transplanted under the skin of the abdomen, and explained this in 1874 in the same way.

In the revival of the controversy that has taken place within the last few years the position of the disputants has been almost exactly the same as that occupied by Waller and Vulpian more than half a century ago.

Ballance and Purves Stewart‖ hold the view that the new nerve-fibres have a peripheral origin, but rely exclusively on histological evidence; one method they employed was Golgi's, which can hardly be considered for this purpose a trustworthy one. It is well known that black streaks are produced by this method by structures which are not nervous at all. A

* "Nouvelle méthode anatomique pour l'investigation du système nerveux," 'Comptes Rendus,' 1852; "Expériences sur les sections des nerfs," 'Gazette Médicale,' 1856.

† "Note sur les expériences démontrant que les nerfs séparés des centres nerveux . . .," etc., 'Comptes Rendus,' 1859.

‡ See, for instance, Ranvier, 'Leçons sur l'histologie du Système Nerveux,' 1873, pp. 158, 186, and 190.

§ 'Archives de Physiologie,' 1874, p. 704.

‖ *Loc. cit.*

strand that looks like a nerve-fibre is not really such unless it can be experimentally shown to be both excitable and capable of conducting nerve impulses. In the absence of any such proof we regard it as probable that these observers have mistaken for nerve-fibres the chains of neurilemmal cells which undoubtedly form in the peripheral portions of cut nerves.

Bethe* is at present the most prominent supporter of the autogenetic theory, and his observations on the return of histological structure have been coupled with experimental testing. He finds that the peripheral ends of cut nerves are in young animals excitable without union with the central end. He does not, however, exclude the fallacy which underlay the old experiments of Vulpian and Philippeaux, and which has more recently been pointed out by Langley and Anderson.† These observers showed that, in spite of any obvious connecting strand with the central end, new nerve-fibres find their way often by devious channels into the peripheral stump from nerves in skin and muscle cut through in the operation. Bethe, in fact, by burying the peripheral end of a nerve in the neighbouring musculature in order to prevent reunion with the central stump, provides an excellent means for the muscular nerves to carry out that union with the central nervous system which it was his object to avoid.‡

At the commencement of their work Langley and Anderson thought they had obtained evidence of purely peripheral regeneration, and it was not until they carried out careful dissections that they convinced themselves that union with the central nervous system had occurred in the manner just mentioned. They, therefore, once more cut the central end of the nerve, and of any other nerves which might possibly have established a connection with the peripheral segment of the nerve they had under observation. The second operation would cause degeneration of the fibres which had established a central connection and leave intact any fibres which had regenerated autogenetically. Even this was not always sufficient to prevent reunion once again of the peripheral end with the central nervous system. But in cases of success no autogenetically formed nerve-fibres were found; eight to twelve days after the second operation, no sound medullated fibres were found in the peripheral end, and the nerve was quite inexcitable. These experiments, like Bethe's, were mostly performed upon young animals (kittens and rabbits), and in some, after a period of nearly two years, no trace of autogenetically-formed new nerve-fibres could be detected.

* *Loc. cit.*

† *Loc. cit.*

‡ This view is also taken by Münzer, 'Neurol. Centralbl.,' December 1, 1902 (p. 1090), and January, 1903 (p. 63).

All the medullated nerve-fibres found in the peripheral end of a cut nerve, which had been sutured to the central end, degenerated when the central end was again cut nearer the spinal cord; in other words, all the nerve-fibres were fibres which were connected with the central nervous system. If the regeneration had been autogenetic, this would mean that every one of them had become united with the end of a nerve-fibre in the central stump; on the autogenetic theory this is highly unlikely, whereas it is quite simply accounted for on Wallerian lines. If the number of medullated fibres in the peripheral end was small, then the connection with the central end was found to be slight. If no connection occurred, then medullated nerve-fibres were entirely absent. Bethe admits a variability in the number of medullated fibres, and this again, though easily explicable on the view that such fibres come from the central end, is not accounted for at all by the autogenetic theory.

Langley and Anderson further point out that Bethe admits age to be an important factor in the case; in adult animals he has little or no proof of autogenetic regeneration. This is a very weak point, for surely the difference is due to the greater ease with which reunion with central nerves occurs in young and growing animals.

Lugano* objects to Bethe's views on much the same lines as Langley and Anderson.

Cajal,† from the histological point of view, has made a very complete rejoinder to Bethe's work. He has also controlled his results by noting the effects of stimulating the nerves. We are unfortunately not able to read the full account of his work in the original Spanish, but the numerous illustrations he gives remind us very forcibly of what we have seen in our own microscopical work. We have, therefore, to rely on the brief summary he has published in French. By the help of his new silver method he comes to the conclusion that the new formation of nerve-fibres in the peripheral stump is exclusively due to growth from the central end. He describes the long and often contorted course of these growing fibres in the swelling at the cut central end, and shows that they ultimately reach their goal, the peripheral stump, in time and in spite of all hindrances. The greater the obstacles interposed, the later does the union and consequent regeneration in the peripheral end occur. He also calls attention to the olive-shaped swelling at the free end of each growing axis cylinder. These are also figured by Marinesco and Minea.‡

* 'Riv. di Patologia nervosa e mentale,' November, 1904, p. 550.

† *Loc. cit.*

‡ "La loi de Waller, et la régénérescence autogène," by M. G. Marinesco and J. Minea, 'Revista Stiintelor Medicale,' No. 5, September, 1905, Bucharest.

The view taken by the last named observers seems, like Graham Kerr's, to be somewhat of the nature of a compromise.

They point out that all authors are agreed as to the activity and multiplication of the neurilemmal cells in the peripheral segment of a cut nerve, and that these unite together into long strands, but if the central end is compared with the peripheral the activity in the former is much greater and the formation of nerve-fibres is there rapid; these grow in length, assume a contorted course, become myelinated, and an axis cylinder is well defined. In the peripheral end the process is much slower, excitability never returns if union with the central end is prevented, and no real differentiation of axis and sheaths takes place. If the ends are united, then restoration of the peripheral end is more rapid.

They point out that this is due to the fibres at the central end remaining in communication with their cells of origin and so are under their influence. The cutting of the nerve has suppressed in the peripheral end all functional acts, and in consequence all nutritive acts depending on the nervous centres cease also. In the central end, on the other hand, the internal work elaborated by the cells still reacts on the fibres and favours their nutrition and growth.

By the help of Cajal's silver method they describe and figure rows of granules formed in the peripheral segment, especially in the neighbourhood of the neurilemmal nuclei and independently of central union, and believe that these unite to form neuro-fibrillæ which, however, do not become functional until activated by union with the central fibres.* Cajal, however, criticises this part of their observations and conclusions, and does not believe that the striations described are real neuro-fibrils.

In a sense, therefore, they believe in autogenetic regeneration; in fact they regard it as the only form of regeneration, and that the difference between what is seen at the central and peripheral ends is one of degree only, being more active and efficient at the former situation.

They, however, part company from Bethe, for they regard the functional activity of the central nervous system as being absolutely indispensable for the return of function in the nerve-fibres and also for their complete histological restoration. They consider that the nerve cell and its axis cylinder constitute a functional unit, and regard the mere fact that the latter undergoes degeneration when cut off from its cell of origin as sufficient evidence of that.

Perhaps the most steadfast upholders of the theory that peripheral nerves

* Similar appearances were also described by Büngner, 'Ziegler's Beiträge,' vol. 10, p. 321 (1891).

regenerate independently of the central nervous system have been certain surgeons.* The statements made by clinical observers that in man (including adults, in spite of Bethe) sensation rapidly returns after freshening up and suturing together the ends of a nerve which has been divided a long time previously would be very valuable evidence in favour of the "peripheral theory" if it was entirely trustworthy. Since commencing this enquiry, one such case recently in King's College Hospital was carefully observed by one of us (A. E.) and it throws useful light on the subject. The case was not one of actual division, but compression, which comes to much the same thing. Two thick silver wires put on to secure the ends of a fractured humerus had included the musculo-spiral nerve. The operation consisted in removing the wires and freeing the nerve from the scar. A short time afterwards the man stated he was again able to feel, but these sensations rapidly subsided and sensation did not really return until months later. The preliminary sensation was doubtless subjective; the irritation of the nerve-fibres in the scar lasted some hours, and the sensations so produced were referred by the patient's mind to the original terminals of the fibres.

At the meeting of the British Medical Association in Oxford in 1904, Dr. Kennedy,† of Glasgow, brought forward a number of cases in support of the autogenetic theory. Here again, the genuineness of the early recovery is doubtful; in spite of sensitiveness to such tests as needle pricks there is usually absolute anæsthesia to the far more delicate test of stroking the hairs over the affected region until quite late dates. Head and Sherren‡ have also recorded a number of surgical cases, but they found no evidence of early recovery; they showed by very careful work that there is considerable difficulty in localising the stimulus so that it should not affect the hyperæsthetic marginal zone of the anæsthetic region, and also that the deep sensibility of the subjacent parts, such as would be excited by needle pricks, is entirely independent of cutaneous sensation. The fibres subserving this form of sensation run mainly with the motor nerves and are not destroyed by division of all the nerves to the skin. Neglect to recognise these facts will no doubt explain the results recorded by Kennedy and those who have published similar cases.

The difficulty of obtaining absolutely trustworthy evidence from patients themselves induced Head§ to combine with his experiments on animals and

* See, for instance, Bowlby, 'Lancet,' 1902, vol. 2, pp. 129, 197.

† 'Brit. Med. Journ.,' 1904, vol. 2, p. 729; see also R. Kennedy, 'Phil. Trans.,' B, pp. 188, 257 (1897).

‡ 'Brain,' vol. 28, p. 117 (1905).

§ Head, Rivers, and Sherren, "The Afferent Nervous System from a New Aspect," Brain,' vol. 28, p. 99 (1905).

his observations on patients an experiment on one of the sensory nerves of his own arm. This experiment gave an opportunity for the study of the phenomena by a trained observer upon himself. He certainly experienced no early return of function, and the date at which sensation did come back coincides closely with the dates obtained in animals by physiologists for the reappearance of new fibres. He has further made the interesting suggestion that the first kind of sensation to return and which is of a vague nature (termed *protopathic*) is associated with the activity of the fine medullated nerve-fibres which replace the degenerated ones at an early stage. Return of protopathic sensibility begins about the eightieth day. The more elaborate sensations and power to accurately localise them return at a much later date, and this *epicritic sensibility*, as Head terms it, is usually not perfect until many months, or even a few years, after the regeneration started. By this time, as was shown by experiments on animals, the fine nerve-fibres which subserve protopathic sensation are largely admixed with a later growth of larger nerve-fibres, and he believes epicritic sensation is subserved by these. He also postulates that the three kinds of sensation (deep, protopathic, and epicritic) are related to different kinds of end organs in the peripheral structures. It is not, however, necessary to enter more fully into these results, for we have made no special study of the varieties of sensation, nor have we in our experiments on animals kept them alive for a sufficiently long period to enable us to see the fibres formed at very late stages.

After this introduction we propose to pass now to the consideration of our own experiments. After the very conclusive researches of such investigators as Langley and Anderson and of Cajal, it may seem rather a work of supererogation to describe any more experiments which tell against the autogenetic theory. We will only plead that every piece of confirmatory evidence is useful if the upholders of that theory are to be convinced they are wrong, and, moreover, in some directions our experiments are new, and approach the problem in rather a different way from that followed by other workers.

We will describe them under the following heads:—

(1) Experiments in which union of central and peripheral ends was prevented.

(2) Experiments on transplanted pieces of nerve.

(3) Experiments on the degeneration of regenerated fibres.

(4) Experiments on the rate of medullation in regenerating nerves.

(5) Experiments on the influence of stimulus on regeneration.

In carrying out this work we should mention that the experiments on animals (monkeys and cats) have been carried out at King's College, London, by two of us (W. D. H. and A. E.), using anæsthetics (ether and chloroform) during all cutting or stimulating operations and with strict antiseptic precautions. The histological portion of the research has been carried out by the third (F. W. M.).

(1) *Experiments in which Union of Central and Peripheral Ends was Prevented.*

In some of our previous work we noticed that the excision of any inch or so of nerve was entirely inefficient to prevent union of the two ends together; we were later aware, from a preliminary communication made by Langley and Anderson* of their results, that the peripheral end is often invaded by new nerve-fibres from the nerves in skin and muscle divided in the operation.

We, therefore, made extremely small incisions, and the parts were separated from the nerve trunks by the handle of the scalpel, and with as little cutting as possible. In cats one incision over the buttock allowed us to divide the sciatic nerve high up; another in the ham enabled us to divide the two popliteal nerves. The intervening portion of the sciatic nerve, about 4 or 5 inches long, could then be easily pulled out. Additional security to prevent union with central fibres was in some cases obtained by enclosing the upper end of each popliteal nerve in closed caps made out of small sterilised drainage tubes about half an inch long. A period of 100 to 150 days was then allowed to elapse in order that if regeneration was going to occur in the peripheral segments of the nerve it might have an opportunity of doing so. At the end of this time the animal was again anæsthetised and the nerves tested by electrical stimulation. In all cases they were entirely inexcitable to strong faradic currents, and the wasted muscles supplied by these nerves had also largely lost their power of response to this form of stimulation. To the naked eye the nerves were pale. The animals were then killed and the nerves subjected to microscopical investigation, but they were found to show no trace of regeneration. The chains of neurilemmal cells, without a sign of either axis cylinder or medullary sheath, were all that could be seen, and in those cases of later date where the nerves had been placed in tubes it was very difficult to recognise any nervous structure whatever.

* 'Proc. Physiol. Soc.,' December 13, 1902; 'Journ. of Physiol.,' vol. 29, p. ii.

(2) *Experiments on Transplanted Pieces of Nerve.*

We have already seen that Vulpian and Philippeaux transplanted pieces of nerve under the skin of the abdomen, and observed in them what they at first thought was evidence of auto-regeneration, a conclusion subsequently withdrawn by Vulpian. Similar experiments, recently published by Kennedy,* are regarded by him as proving absolutely conclusively the autogenetic origin of nerve-fibres. He excised a portion of the sciatic nerve and transplanted it into the subcutaneous tissues of the same animal. Six months later he killed the animal and examined the nerve which had been transplanted. He found among a number of degenerated fibres some which were definitely formed with a medullary sheath showing distinct nodes of Ranvier. He concluded that these fibres had been formed by the tissues of the excised portion of nerve.

He conceives objectors to this deduction maintaining that these nerve-fibres have grown into the sheaths of the transplanted nerve from cutaneous nerves divided in the course of the operation, and meets this objection as follows :—

"Such an explanation is, I think, far-fetched, and assumes an extraordinary affinity between the supposed young nerve-fibres and the old nerve trunk, an affinity which, if established, would, I am sure, secure almost certain spontaneous union of nerve trunks after division, unless very extraordinary barriers were placed between the divided ends.

"The number of cases, however, in which secondary suture has to be performed shows that divided nerves cannot be confidently left to nature to repair, which I am sure might almost uniformly be the case did such strong affinity between young nerve-fibres and the distal degenerated segment exist."

It has, however, been shown that a large nerve trunk on division curls up at the end, forming a contorted leash of fibres. Further, a nerve trunk on division retracts, and a considerable amount of scar tissue is formed. This cicatricial tissue by the time the nerve has begun to regenerate has become dense fibrous tissue. In the case of nerve trunks, therefore, "extraordinary barriers" are the rule. Moreover, in spite of such barriers spontaneous union often occurs, and in animals when the cut is made more cleanly than occurs in an accident in man, and when antiseptics are used, the amount of cicatricial tissue formed is less, and union may be confidently anticipated without suture either immediate or secondary. In the case of small nerve trunks the case is also different; for example, after operations involving

* 'Brit. Med. Journ.,' 1904, vol. 2, p. 729.

extensive skin incisions, anæsthesia is always found on one side of the incision, varying in extent according to the direction of the incision and the number of cutaneous nerves divided. It is, however, the rule for these anæsthetic areas to completely recover.

Again, when a longitudinal incision is made through the rectus abdominis muscle it is very rare to find any permanent paralysis of portions of the muscle, although division of its nerves must be very common. Temporary paralysis, of course, must take place, but there is so little fear of ultimate paralysis and atrophy of the muscle that many surgeons prefer an incision through the rectus to any other method of performing laparotomy.

To further study this point we repeated Kennedy's experiment, with the variation that a portion of a nerve was not only inserted underneath the skin, but a similar portion was inserted into the peritoneal cavity. This was performed as follows: the peritoneal cavity of a cat was opened through an incision in the left hypochondrium, and the stomach drawn up into the wound. The portion of sciatic nerve which had been previously removed from the same animal was laid on the anterior stomach wall. A row of Lembert sutures of catgut were then passed so as to invaginate a groove of tissue around the nerve; the sutures were continued for a short distance at each end of the nerve so as to completely enclose it in a sheath of stomach wall lined by peritoneum. In this way there was no possibility of nerve-fibres growing in from the skin, and but little possibility of their coming from the stomach.

As a control experiment a wisp of catgut was enveloped in the stomach wall in a precisely similar manner.

After 150 days, the animal was killed and the nerves examined. In one case the whole of the nerve tissue had completely disappeared; apparently it had been entirely absorbed by leucocytes, and the suture line was the only sign of the operation.

But in other experiments, where the nutrition of the nerve had probably been more effectually maintained, the nerve could be readily seen both in the stomach wall and under the skin. It was thinner and more transparent than when it was inserted. It was examined in teased specimens and in sections. It can be readily teased out into its constituent fibres, and this method is perhaps the most valuable one of investigating its structure.

When thus prepared, each fibre looks like a long tube; they have no double contour and there is no trace of a medullary sheath. There are very abundant nuclei which take nuclear dyes rapidly and intensely. At the point where a nucleus is situated there is usually no increase in the diameter of the fibre, the nucleus being either central or projecting into a central lumen. In some fibres, on the other hand, there is a distinct swelling at the site of the nucleus, and here there is a considerable amount of protoplasm accumulated around the nucleus. There were no traces of nodes, and nothing resembling an axis cylinder or fibrillæ could be seen. The diameter of the fibres varied from 2·5 to 3 μ. These appearances are illustrated in figs. 1 and 2.

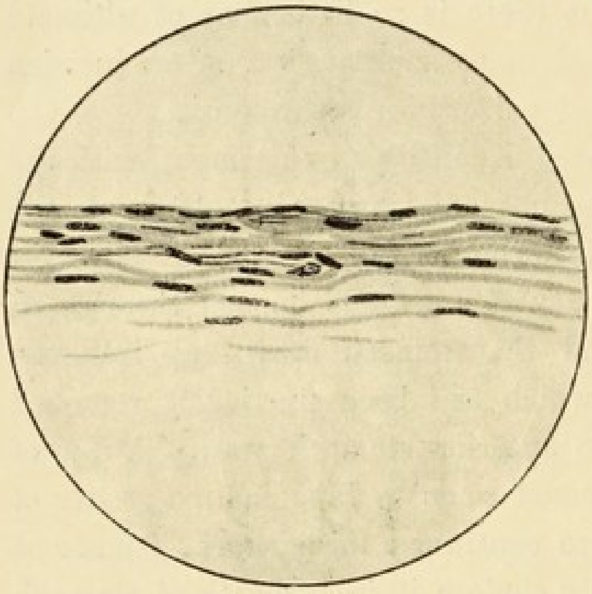

Fig. 1.

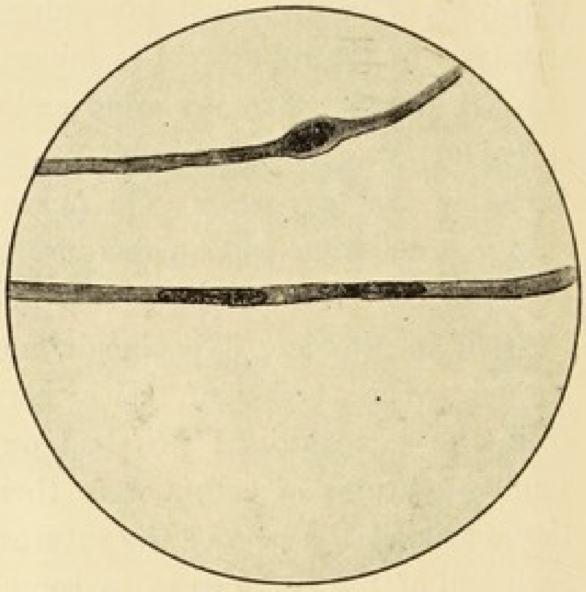

Fig. 2.

The sections (some of which were stained by Cajal's new silver method) showed merely the divided ends of these tubular structures cut across. It is true that the appearance of the long nuclei in transverse section was at first sight suggestive of axis cylinders, but their size, apart from the evidence of longitudinal sections and teased preparations, disproves this view.

The appearances, therefore, are merely those which have been so often described in the peripheral segment of a divided nerve in which regeneration has not occurred, and which all agree is due to neurilemmal activity.

The remains of the nerves which had been transplanted under the skin showed the same appearances. Here also a large uumber of well formed blood-vessels were found. These grow in not only at the ends, but actually break through the surrounding connective-tissue sheath into the interior. The accompanying drawing illustrates how a blood-vessel breaks through the sheath into the substance of a degenerated nerve bundle.

From this irruption of blood-vessels it is a very small step to the introduction of nerve-fibres into the interior of the transplanted nerve. It seems reasonable to suppose that where the one could penetrate the other would have no difficulty in doing likewise, and this would account for the presence of the medullated nerve-fibres which Kennedy discovered. In our own preparations of transplanted nerves under the skin we could by careful search discover a few medullated fibres, but this was not the case where the nerve trunk had been transplanted under the peritoneum, in which situation invasion by neighbouring nerves is nearly impossible.

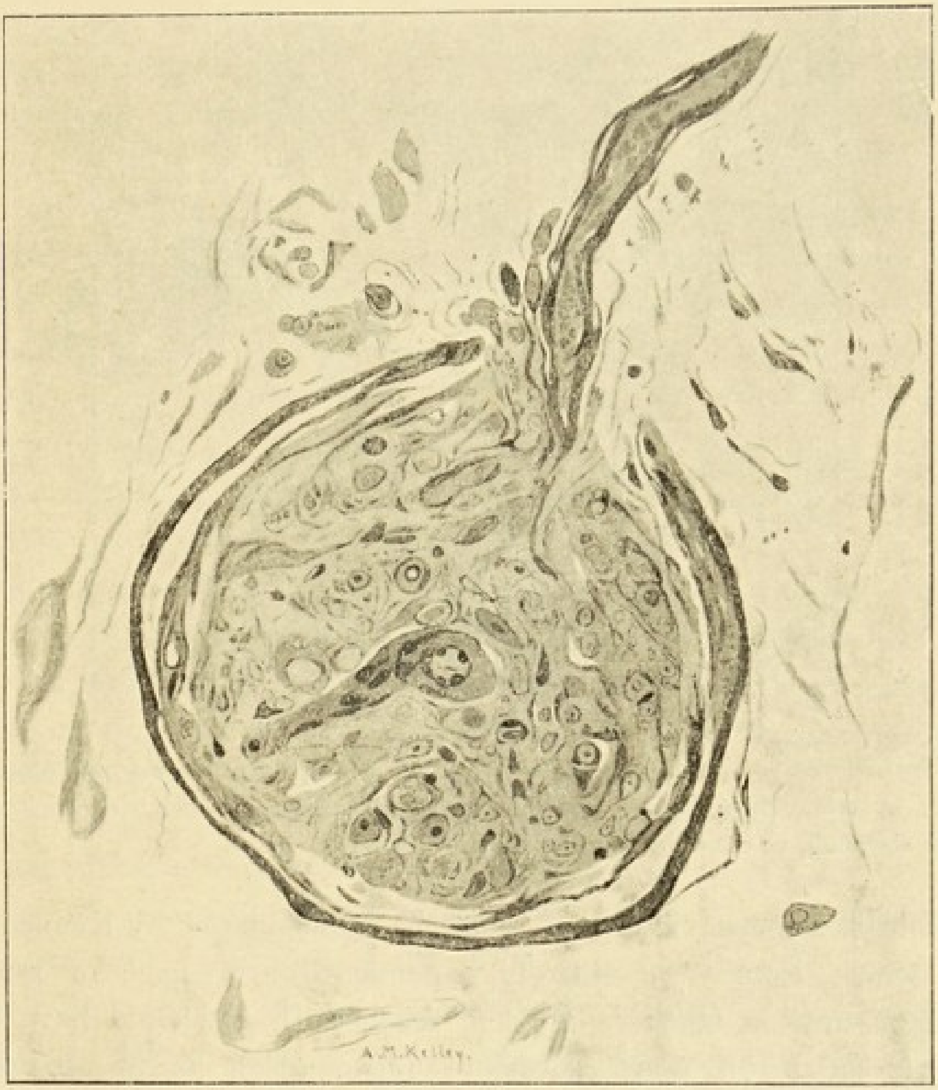

Fig. 3.—A small blood-vessel entering the sheath of a fasciculus of transplanted nerve, as described in the text. 280 diameters.

The connective tissue between and around the bundles of nerve-fibres retains its normal relationship. This is, however, not a property of nerve tissue in particular, for any structure of a similar shape would become enclosed in a sheath of fibrous tissue unless it were completely absorbed. This is well illustrated in fig. 4, which shows a transverse section of a strand of catgut which had been enveloped in the stomach wall.

The connective tissue has organised around the catgut threads, forming a framework which strongly suggests the sheath of nerve fasciculi.

We may sum up our results of these experiments by saying that we have found no evidence of the appearance of new nerve-fibres in nerves which have been transplanted into the peritoneal cavity. Such nerves may either undergo absorption, or be replaced by that variety of tissue which is found replacing nervous tissue proper in the distal segment of a divided nerve.

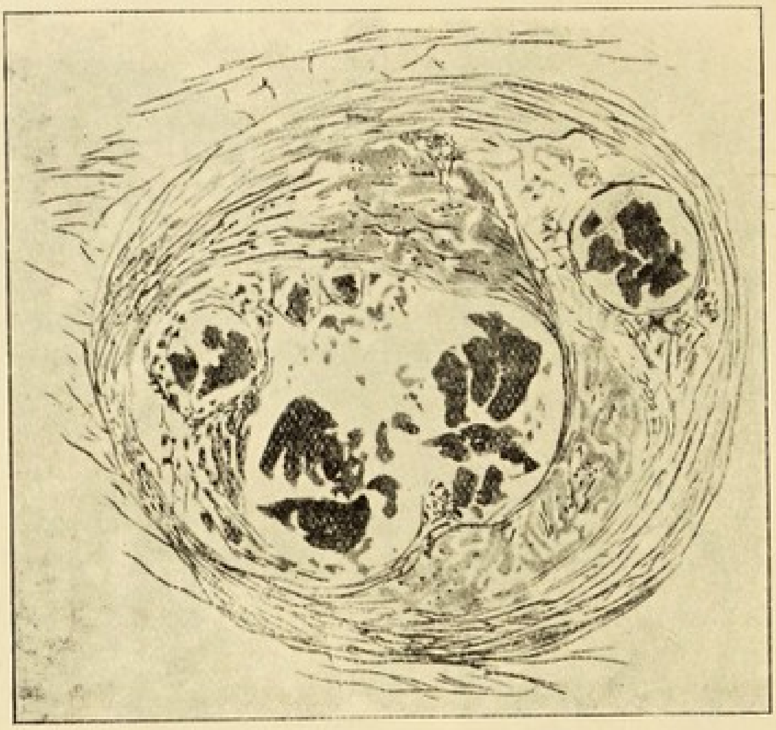

Fig. 4.—Transverse section of strand of catgut which has become surrounded by a sheath of connective tissue, after being transplanted beneath the peritoneal coat of the stomach, as described in the text.

It is at least remarkable that the nerves transplanted by Kennedy into a position where they were entirely dependent on soakage of lymph for nourishment until a blood-supply had reached them should have been so specially fortunate in their powers of regeneration as to produce well-developed medullary sheaths and nodes of Ranvier. It is difficult to understand on the autogenetic theory why so few of the degenerated fibres should regenerate in this way, for all were under precisely the same conditions. Such an occurrence is easy to account for, if our own view is adopted that the well-formed fibres seen had grown into the transplanted nerve from nerve-fibres cut through in the operation.

(3) *Experiments on the Degeneration of Regenerated Fibres.*

These experiments were suggested to us by Professor Gotch, and have been performed once on the monkey and twice on cats. A large nerve (the ulnar in the case of the monkey, the sciatic in the case of the cats) was divided and the ends sutured together with sterilised silk. After a sufficient length of time had elapsed, restoration of function led us to suppose that regeneration had occurred. A second operation was then performed in each case. The animal was anæsthetised, and the nerve was exposed; the union of the two ends was found to have been accomplished, and the nerve was excitable both above and below the junction. A piece of nerve about half an inch long was then excised, an inch or so below the junction. On histological examination of this all traces of degenerated products were found to have disappeared, and it was made up of fine nerve-fibres, many of which had acquired a delicate medullary sheath. After this second operation the wound was closed and the animal allowed to live 10 days longer; it was then killed and the nerve both below and above the second cut was examined. No degeneration was found in the nerve-fibres above the second lesion, but Wallerian degeneration was shown by the Marchi method to have occurred in the medullated fibres of the peripheral portion, which was quite inexcitable. The direction of degeneration is the direction of growth, so this experiment shows that the growth of the nerve-fibres had not started from the periphery centralwards, but in the reverse direction.

In the monkey in which this experiment was performed, the second operation took place 70 days after the first. In the case of the cats a longer interval was allowed to elapse, namely, 147 and 162 days respectively; by this time myelination of the nerve-fibres was more pronounced, and so degeneration was more readily seen by the method employed.

We give below (fig. 5) a drawing of the degeneration seen in a teased preparation of the peripheral segment after the second operation.

We noted an interesting point in these experiments which illustrates the great rate at which the regenerating fibres grow. Although only 10 days had elapsed between the second operation and the killing of the animal, and although quite half an inch of nerve had been excised and no efforts made to approximate the ends, yet there was a well-marked strand of union uniting the two segments, both in the experiment on the monkey, and in one of the two experiments on cats.

We think that these experiments, taken in conjunction with the somewhat similar experiment already alluded to in the work of Langley and Anderson, in which, after union of the two ends of a divided nerve, degeneration in the regenerated fibres set in after a second operation of dividing the central end of the nerve nearer the spinal cord, conclusively prove the regenerated fibres to be in anatomical continuity with the fibres in the central end, and so with the central nervous system. They thus serve to prove the doctrine that the regenerated fibres have grown from those in the central stump.

At the time that we undertook these experiments we were under the impression that they had never been previously performed. But on looking up the literature we find this is not the case. Like so much of the present work set going by the revival of the autogenetic theory, this experiment has also been performed by others. As long ago as 1859 Philippeaux and Vulpian did it, and obtained the same result as we have; we can hardly doubt that this formed one of the factors that later led Vulpian to abandon the autogenetic theory.

Fig. 5.—Wallerian degeneration of regenerated nerve-fibres. Cat. Drawn from a teased preparation with the camera lucida. Marchi method of staining. Magnified 820 diameters.

Bethe also has repeated the experiment; he states that a nerve which has regenerated autogenetically degenerates afresh when it is again cut; that this degeneration occurs only in the peripheral segment, and that the central end remains intact. According to him this experiment convinces him in a striking way that the integrity of the central end does not depend on its connection with the cells in the central nervous system. This is an excellent example of arguing in a circle, for his first assumption that autogenetic regeneration has occurred is entirely unwarranted.

(4) *Experiments on the Rate of Medullation in Regenerating Nerves.*

Another very important piece of evidence which supports our general views was obtained by examining regenerated nerve-fibres in various parts of their course. In the preliminary announcement of our results we stated:—

"We think in some cases that the more distant the situation from the original point of section, the less perfectly developed the nerve-fibres appear to be; myelination has progressed less in the distal portion of their course."

This conclusion was derived from the examination of a large number of specimens, but as no special measurements of the distance in each case from the point of section had been made, we thought it advisable to examine the matter more systematically.*

We accordingly divided the sciatic nerve of a cat high up in the thigh, and sutured the two ends together. In time the resulting paralysis had largely disappeared, and from this we judged that regeneration of the nerve had occurred, and 84 days after the operation the animal was anæsthetised, and the nerve was found to be excitable both above and below the junction to a weak faradic current, such a current as could just be felt by the tongue. The animal was then killed, and a piece of the sciatic nerve immediately below the junction was placed in Marchi's fluid for subsequent microscopical examination. A piece of the lower end of the posterior tibial at the level of the ankle was similarly treated. The histological study of longitudinal and transverse sections of the two portions of nerve showed a much greater degree of myelination at the upper than at the lower end.

In order to make the experiment still more exact, we proceeded in rather a different way in a second experiment. A possible objection to the first experiment would be that the posterior tibial is a motor nerve, and regeneration of motor fibres is as a rule slower than that of sensory fibres; the greater amount of medullation observed at the upper end of the sciatic might possibly have been due to the fact that there we were chiefly examining sensory fibres. This objection does not really hold, because all the bundles in the upper end of the sciatic were almost equally well myelinated. Still, it appeared wise to meet the objection by somewhat varying the manner of observation. The sciatic nerve of a cat was cut and sutured; 91 days later the animal was killed by chloroform, and during the anæsthesia that preceded death the nerve was found to be irritable to weak faradisation as before, both above and below the junction. In the dissection of the nerves, a large motor

* We find that this observation was also made by Langley and Anderson (*loc. cit.*, p. 425). They say, "In one or two of our cases we examined the whole length of the nerve and found that the medullated fibres decreased towards the periphery."

and a large sensory bundle in the leg were traced up into the main sciatic trunk, and by dividing the sheath they could be easily separated in the trunk for a considerable distance. A small piece of the upper and lower ends of both the motor and the sensory bundle were then removed, placed in Marchi's fluid, and examined in sections as before.

There was a noticeable difference between the amount of medullation in the motor and sensory nerves, that in the latter being, as is usually the case, more advanced. Both, however, showed at their upper ends numerous myelinated fibres; whereas at the lower end, which was 4 or 5 inches distant, myelination was much less distinct. We illustrate these appearances by the figures on the accompanying plate, which show high and low power views of transverse sections of the motor trunk at the two levels.

This second cat, although it was killed a week later than the first, showed less advanced myelination. We have called attention in our previous work to the difficulty of assigning any exact dates, even in the same species of animal, to any particular stage of the regeneration process. The dates vary within somewhat wide limits with the vital reaction of different animals. In the case of the two cats we are at present concerned with, the first animal was a young and lively one, whereas the second animal was large, lethargic, and older. It is therefore not surprising that regeneration should have been more rapid in the first than in the second case.

It can hardly be doubted that the medullary sheath is a developmental appendage of the axis cylinder; it appears in the fibres of the central nervous system where the neurilemma is absent; it degenerates with the axis cylinder when a nerve-fibre is cut, and completeness of function is associated with its appearance in development and with its reappearance in regeneration.

We therefore think we are justified in concluding that the late appearance of the medullary sheath in those portions of the regenerating fibres which are most distant from the place where the nerve was originally cut is a conclusive piece of evidence in favour of the view that the new nerve-fibres have grown from the central end in a peripheral direction.

Von Büngner,* von Notthaft,† Strœbe,‡ and Wieting§ all agree that the formation of nerve-fibres takes place more rapidly near the junction than at the periphery.

* 'Ziegler's Beiträge,' vol. 10, p. 321 (1891).
† 'Zeitsch. f. Wissen. Zool.,' vol. 55, p. 376 (1892).
‡ 'Centralbl. f. path. Anat.,' vol. 4, p. 49 (1893).
§ 'Ziegler's Beiträge,' vol. 23, p. 63 (1898).

(5) *Experiments on the Influence of Stimulus on Regeneration.*

These experiments were performed on Rhœsus monkeys. A monkey's arm was rendered immobile by the division of a number of the upper posterior roots on one side. In order to be perfectly certain, we usually divided from the second cervical to the second or third thoracic roots inclusive. The anterior cornual cells from which the corresponding motor fibres originate are thus not subjected to stimuli from the periphery and, as Mott and Sherrington were the first to show, the arm is as much paralysed as if the anterior roots had been cut. We have, however, again noticed in some of these animals under the influence of strong emotion (for instance, when the monkey is prevented from reaching with the sound hand a piece of apple) that some efforts were made to move the paralysed limb. These efforts are ineffectual and are limited to associated movements in the upper segment of the limb; fine movements were never noticed. When the animal is living in its cage under ordinary conditions it makes no effort to move the limb, which in successful experiments (*i.e.*, when a sufficient number of roots have been entirely cut through) hangs helpless like a flail.

In the case of one or two of the wilder animals we first operated on, the hanging limb was apt to get injured by their jumping about the cage. Wounds so produced heal with difficulty, and the presence of such wounds necessitated the sacrifice of the animals. Later we obviated such accidents by providing the animals with jackets, the sleeve containing the paralysed limb being well packed with cotton wool.

After the healing of the neck wound produced by the cutting of the posterior nerve roots was accomplished, a matter usually of one or two weeks, a large nerve in the arm (median or ulnar)* was then divided and the ends sutured together. The corresponding nerve was cut and sutured on the non-paralysed side as a control experiment. The animal was finally killed; the interval between the operation and death varied in different experiments, but the best time for making the observation we finally determined to be between 60 and 70 days after the nerves had been cut. This is a date when regeneration is perfectly evident, but not too far advanced; it, therefore, permits of comparative observations on the amount of regeneration.

* We found a considerable anastomosing branch passing from the median to the ulnar nerve in the lower part of the fore-arm; some branches which, still lower down, apparently come off from the ulnar, are really median in origin. A non-recognition of this fact is apt to cause confusion (as it did in some of our early experiments), for after section of the ulnar some of the branches to the last two fingers show no sign of degeneration.

Union of the divided nerves occurs on both sides of the body, and in our early experiments the nerve on the side corresponding to that on which the posterior nerve roots had been divided was found to be the less excitable to the faradic current. Histologically this nerve showed a looser texture, and new nerve-fibres, though present, were somewhat less numerous than on the control side. In these early experiments also we found that the posterior cornual cells in the cervical region were atrophied, and that there was a considerable overgrowth of neuroglia tissue in the posterior horn.

Further examination (by the methylene blue and erythrosin stain) of these spinal cords showed, however, that there had been a considerable number of small hæmorrhages in the cord, sufficient in some cases to cause degeneration in various descending tracts in the cord. It, therefore, became quite possible to explain the effects observed by this complication. We are inclined to think that the hæmorrhages are not due, or not chiefly due, to mechanical injury of the cord during the operation, but are to be explained by the loss of support in the cord tissue which follows degeneration of the entering posterior root fibres.

In several of the later experiments in which cord hæmorrhages did not occur to any great extent, we have been unable to detect any marked changes in the anterior or posterior cornual cells, or any difference to stimulation or in microscopic structure between the regenerated nerves of the two sides.

This conclusion fits in with some experiments of H. K. Anderson;* he showed that in developing animals, section of all the posterior roots connected with a limb exercised no retarding influence on the development of the corresponding anterior roots.

Warrington† has stated that when posterior nerve-roots are cut, the anterior nerve cells undergo the chromatolytic change associated with inactivity. We do not wish to dispute Warrington's observations which apparently were made at an early date after the division of the roots. If the change does occur, it can only be temporary, and in the animals which we have killed at the later dates mentioned, and also some killed at earlier dates (including one killed 17 days after the division of the roots), it was not possible with any certainty to tell by looking at the anterior nerve cells of the two sides which was the side on which the posterior roots had been divided.

In further experiments we sought to cut off the cerebral influence by removing the cortical arm area of the opposite side in addition to dividing posterior roots as before. In this case also the regenerated nerves of the two

* 'Journ. of Physiology,' vol. 28, p. 499 (1902).

† 'Journ. of Physiology,' vol. 23, p. 112 (1897—98); vol. 24, p. 464 (1899); vol. 25, p. 462 (1899—1900).

sides were equally responsive to stimulation, and histological evidence of any marked difference between them was also lacking.

Finally, we still further reduced the action of innervation currents on the anterior cornual neurons by cutting off the stimuli which enter by the posterior roots, as well as those which descend from the brain. The latter was accomplished by combining either a semisection or a complete transection of the cord in the mid-thoracic region with the division of the posterior nerve roots which correspond to the lower limb of one side.

The two sciatic nerves were then divided and sutured and, as before, an interval was allowed to elapse until these nerves regenerated. Here, again, the result was negative. Both nerves regenerated equally well, and both were equally responsive to excitation.

It is, therefore, quite evident that the paths which even under these circumstances remain open (commissural and association tracts) are sufficient to maintain the activity of the anterior cornual cells in the sprouting forth of new axons in a peripheral nerve, although they may be insufficient to induce those cells to send effective impulses along them.

It is also evident that in order to reduce the activity of the anterior nerve cells to a sufficient degree to prevent the regeneration of their axons, it would be necessary to insulate a group of them so as to prevent all impulses reaching them from every part of the nervous system. We did not see our way to accomplish such an operation without interfering with the blood supply of such an island of cord matter. The nearest approach we have obtained to such a condition of things is to be found in those early experiments in which hæmorrhages and neuroglial overgrowth occurred as a complication. Here certainly the diminution in the number of regenerated fibres points to a reduced activity or, it may be, destruction of some of the anterior cornual elements, but in either case these experiments, so far as they go, are in favour of the view that new nerve-fibres grow from central axons, and are not formed autogenetically.

General Conclusions.

We have put forward the foregoing five sets of experiments as a contribution to the discussion now in progress as to whether the regeneration of nerve-fibres is autogenetic or not. These experimental methods approach the subject in different ways, and in no one case was any evidence forthcoming of auto-regeneration.

The facts recorded, taken in conjunction with those published by such observers as Cajal and Langley and Anderson, form on the other hand strong pieces of evidence in favour of the Wallerian doctrine that new nerve-fibres are growths from the central ends of divided nerve trunks. The experimental facts recorded by those who, like Bethe and Kennedy, hold the opposite view are susceptible of easy explanation, mainly on the lines emphasised by Langley and Anderson of accidental and unnoticed connection of the peripheral segments with the central nervous system by means of other nerves cut through in the operation. If such connection is effectually prevented, real regeneration of structure and restoration of function never occurs.

Moreover, the regenerated fibres always degenerate in a peripheral direction, and in a peripheral direction only, when the link that binds them to the central nervous system is again severed. Perhaps the most striking of the facts brought out in our own work is in reference to the development of the medullary sheath; this appendage of the axis cylinder appears earliest at situations near the point where the ends of a nerve have been joined together, and reaches the distal portions later.

What takes place in the peripheral segment of a divided nerve is a multiplication, elongation and union into long chains of the neurilemmal cells. The same change is even more vigorous at the central termination of the cut nerve; and the view of the phagocytic and nutritive function which we attribute to this sheath has been supported independently by some striking observations of Graham Kerr which we have fully referred to. At the central end this nutritive function is effective and provides for the nourishment of the actively lengthening axis cylinders. At the peripheral end, unless the axons reach it, it is ineffective in so far as any real new formation of nerve-fibres is concerned. If, however, the axons reach the peripheral segment, the work of the neurilemmal cells has not been useless, for they provide the supporting and nutritive elements necessary for its continued and successful growth. The neurilemmal activity appears to be essential, for without it, as in the central nervous system, regeneration does not take place.

According to Graham Kerr the formation of neuro-fibrillæ may possibly take place in the protoplasmic residue of the degenerated axis cylinder; according to Marinesco this property is assigned to the neurilemmal elements themselves, a proposition we regard as extremely improbable, seeing that these elements are mesoblastic. In either case these two observers consider that the neuro-fibrillæ, however formed, are ineffective until they are activated by union with those of the central axons. Our own observations do not entirely exclude this view, but on the other hand (as we have never seen these fibrillæ in the peripheral segment unless union with the central end has occurred) they lend it no support. All our facts are readily explicable, however, on the theory that the nerve-fibres are growths from the central ends of divided nerves.

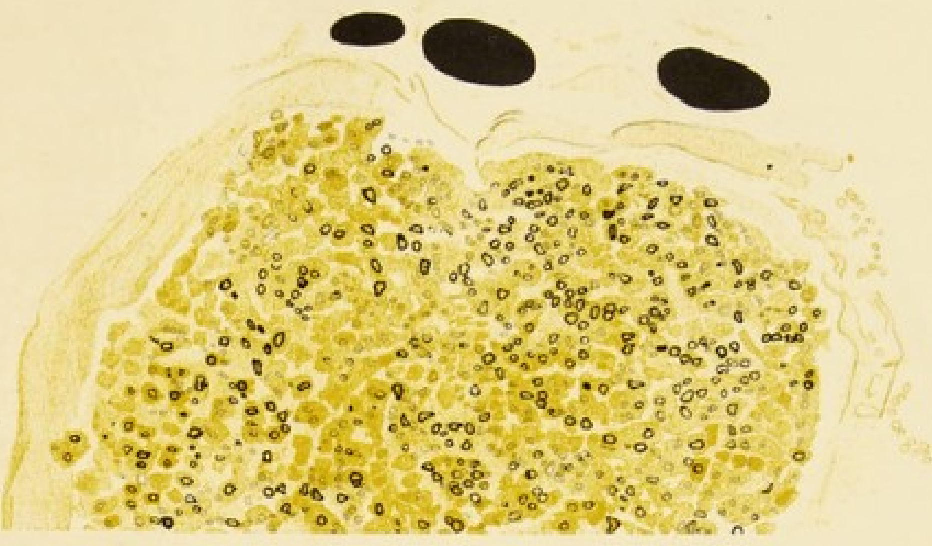

Fig. 6a.

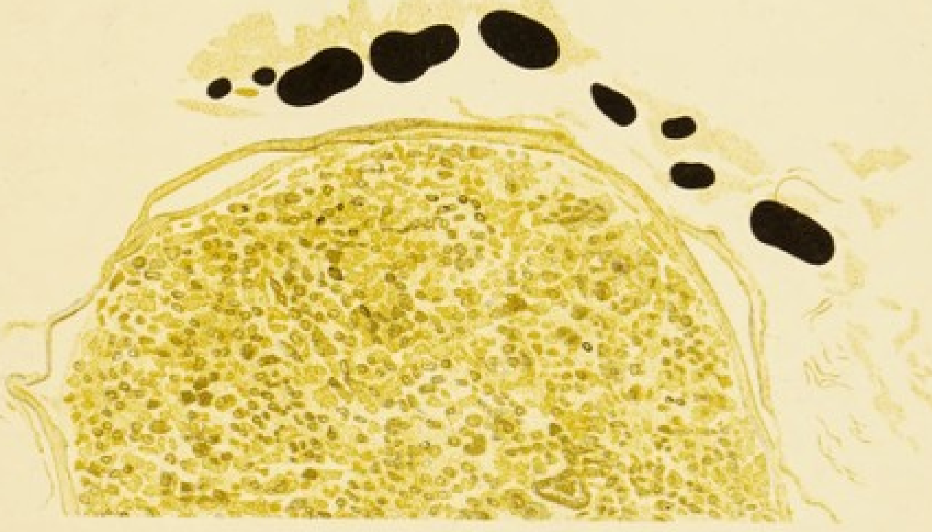

Fig. 7a.

Fig. 6b.

Fig. 7b.

Bale & Danielsson, Lith

Towards the expenses of this research we have received contributions from the Government Grant Committee of the Royal Society, and from the Scientific Grants Committee of the British Medical Association.

DESCRIPTION OF PLATE.

Fig. 6a.—Regenerating motor bundle of cat's sciatic nerve, 91 days after the nerve was divided and sutured. Marchi method of staining. The transverse section shows well-marked myelination in the majority of the fibres; it was taken from the upper end of the nerve. Magnified 350 diameters.

Fig. 6b.—A small portion of the same, magnified 820 diameters.

Fig. 7a.—The same bundle 4 or 5 inches lower down. Myelination much less advanced. Magnified 350 diameters.

Fig. 7b.—A small portion of the same, magnified 820 diameters.

All the drawings were made with the camera lucida.

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