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FURTHER EXPERIMENTAL EVIDENCE AGAINST "NEUROTROPISM" IN NERVE REGENERATION [1]

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FIFTEEN FIGURES

THE PROBLEM

Transection of a peripheral nerve leaves the two stumps in unequal condition. The proximal nerve fiber stumps, still connected with their central cell bodies, begin to grow out anew and represent the source of nerve regeneration. In the distal stump the axis cylinders and myelin sheaths degenerate, while the accompanying sheath cells hypertrophy and transform into the so-called cell cords of v. Büngner, which persist as such. In between the two stumps there remains a gap of varying extent which the regenerating fiber branches have to span if they are to reenter the cell cords of the distal stump which serve as conduits to the peripheral end organs. The fact that the stumps are in many cases found reconnected by fibers in much larger numbers than could be expected on sheer statistical grounds, has long been regarded as evidence that regenerative growth does not take place at random but that the distal stump exerts some influence on the direction of the outgrowing fibers. Systematic experiments to demonstrate this influence have been carried out by Forssman (1898, '00) and Cajal, '05; (reviewed in '28), and their interpretation of the observed facts has been widely accepted (Lugaro, '06; Tello, '23). Forssman called this influence "neurotropism," and Cajal, who had previously postulated similar influences in the embryonic development of a nerve fiber, interpreted them as of chemotactic nature. The assumption was that chemical agents emanating from the myelin residues or from the Schwann cells of the degenerating nerve stump could "attract" nerve sprouts emerging from the proximal stump. The existence of such distance action was questioned by Dustin ('10) and Ingebrigtsen ('16), apparently with little

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effect on the prevailing opinion, which remained that nerve fibers grow towards definite destinations, perceived through their chemical discharges. In fact, Cajal as well as Lugaro suggested that different types of nerve fibers might be guided by different chemicals selectively.

The alternative to "neurotropism" acting from a distance is the conduction of nerve fibers by contact with oriented substrata serving as pathway structures. In 1934, experimental evidence was presented which strongly supported this latter concept of "contact guidance" and excluded distance actions other than those operating indirectly by affecting the pathway structure (Weiss, '34). Later observations made it possible to reinterpret the facts described by Cajal in terms of the contact theory (Weiss, '41a). However, the conclusiveness of those earlier arguments against the concept of "neurotropism" could be questioned on the grounds that they were mostly based on experiments in tissue culture, under conditions radically differing from those of Cajal's and Forssman's experiments. The present report is intended to remove these objections.

In the literature on nerve regeneration the terms "neurotropism" and "neurotropic substances" have vicariously signified agents supposed to attract nerve fibers and agents merely beneficial to their growth, e. g., nutrients. Only in the former sense, the original one, will they be referred to in this paper. We shall likewise restrict our discusion to nerve regeneration, although the problem is basically the same in ontogeny.

For a concrete presentation of the problem, we turn to one of Cajal's basic experiments, illustrated in figure 1. It exemplifies nerve regeneration between two nerve stumps brought out of alignment, either side by side (a) or staggered (b). In either case a sizeable stream of nerve fibers can be seen to have bridged the gap between proximal and distal stump, giving the impression of an attraction exerted on regenerating fibers by degenerated nerve. While the fact that the presence of the distal stump has had some influence on the pattern of regeneration is evident, the nature of this influence cannot be immediately discerned from the results. One notes that only a certain proportion of the regenerated fibers lead into the distal stump, while many others have strayed about at random. Hence, the directive influence, whatever its nature, has had only limited success. Moreover, the existence of a connection between proximal and distal stump can be explained in several ways. (1) The peripheral stump may have had a directive effect on the growing fibers, either directly, as suggested in the theory of "neurotropism," or by calling forth an oriented path-

way structure in the sense of the contact theory. (2) The initial outgrowth of nerve fibers may have proceeded at random and in excess of needs, whereupon during a second, regressive, phase, those fibers which had accidentally found their way into the distal stump would have been preserved, while all others would have been resorbed according to a trial and error procedure. (3) Pioneering fibers might have grown out at random as in (2), but fibers accidentally connecting with the distal stump might thereby have acquired the ability to trap succeeding fibers on their surfaces and thus have guided them (selective fasiculation; Weiss, '41a). Possibly even a combination of these factors may be in operation. In short, the observations of Cajal and

a b

Fig. 1 Regeneration of nerve fibers into dislocated distal stumps. From Cajal (figs 124 and 139). Left: Regeneration from A into neighboring nerve whose distal portion B has been caused to degenerate by a proximal ligature. Right: Regeneration from A through a nerve fragment C into a distal stump D.

Forssman could be explained without recourse to the assumption of "neurotropism." However, it seems unprofitable to continue the discussion on an abstract plane, since concrete facts to guide our judgment are available.

It has been shown (Weiss, '34) that a piece of degenerated peripheral nerve placed in the vicinity of growing nerve tissue in tissue culture has no perceptible effect on the growing fibers. However, those experiments are open to the objection that the fibrin clot might have sealed off the end of the nerve fragment sufficiently to prevent effective diffusion into the cultures. This objection has been met by experiments of the kind illustrated in figure 2. A spinal ganglion with its attached nerve stump is explanted. The stump is then severed, leaving the cut surfaces in fairly close apposition. After 7 days, nerve fibers have

grown from the cut in all directions, and while spatial proximity has, of course, brought many into the distal fragment, whose preformed pathways then forced them into conspicuous alignment, one recognizes another equally extensive plexus of fibers which are wholly disoriented and unrelated to the distal stump. As nerve fibers have pervaded the peripheral fragment with great ease, diffusion of substances from that piece must likewise be assumed to have been unimpeded; yet, the course taken by the regenerating branches does not attest to any "neurotropic" potency of such diffusion.

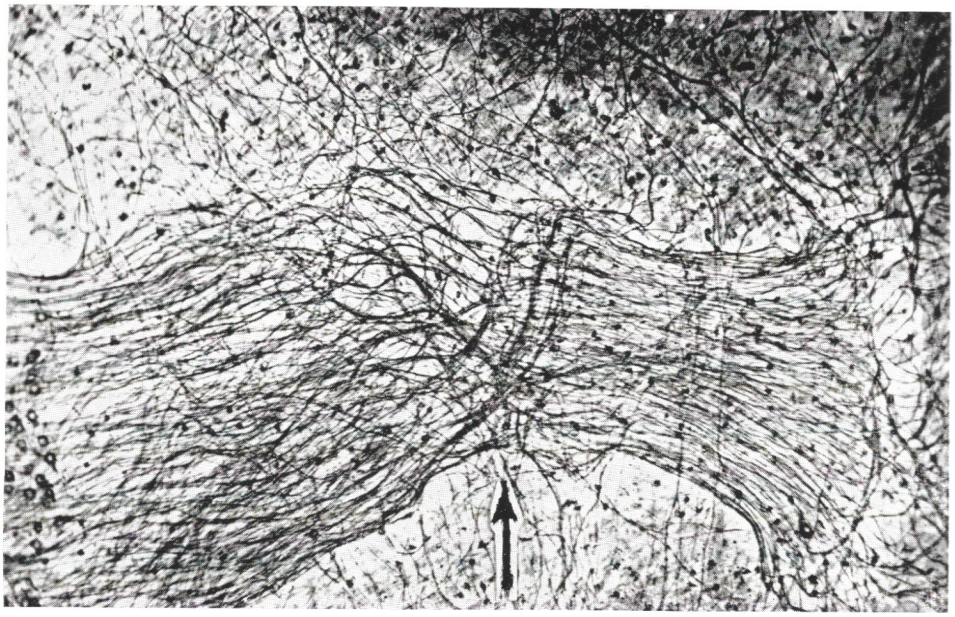

Fig. 2 Regeneration in vitro. Spinal ganglion, 11-day chick embryo, cultivated in plasma clot for 7 days. The attached nerve had been severed at the level of the arrow. Ganglion cells visible at far left. Bodian impregnation, total mount. × 94.

As these cultures were also set up in blood plasma clots, one could still contend that diffusion might have been too slow to maintain steady concentration gradients. As this objection does not apply to so-called liquid medium cultures, numerous cultures of this kind were made, in which an embryonic chick spinal ganglion and a pre-degenerated nerve fragment (embryonic or postnatal) were placed within close range in a drop of non-clotting medium consisting of Tyrode solution and blood serum in a ratio of 5:1. This permits free diffusion to occur between the degenerated nerve fragment and the spinal ganglion as source of regenerating fibers. Yet, in no case did the out-

growing nerve fibers converge upon the nerve fragment or show greater density of growth on the side facing that fragment or reveal any other effects than those attributable to the presence of the fragment as a mechanical obstruction. Figure 3 illustrates a typical case. Considering, however, that sheath cell growth in degenerating nerve in vitro is practically absent during the early days of cultivation (Ingebrigtsen, '16; Abercrombie and Johnson, '42), and that Cajal ('05) had regarded the sheath cells as the "neurotropic" source, even these experiments lacked full conclusiveness.

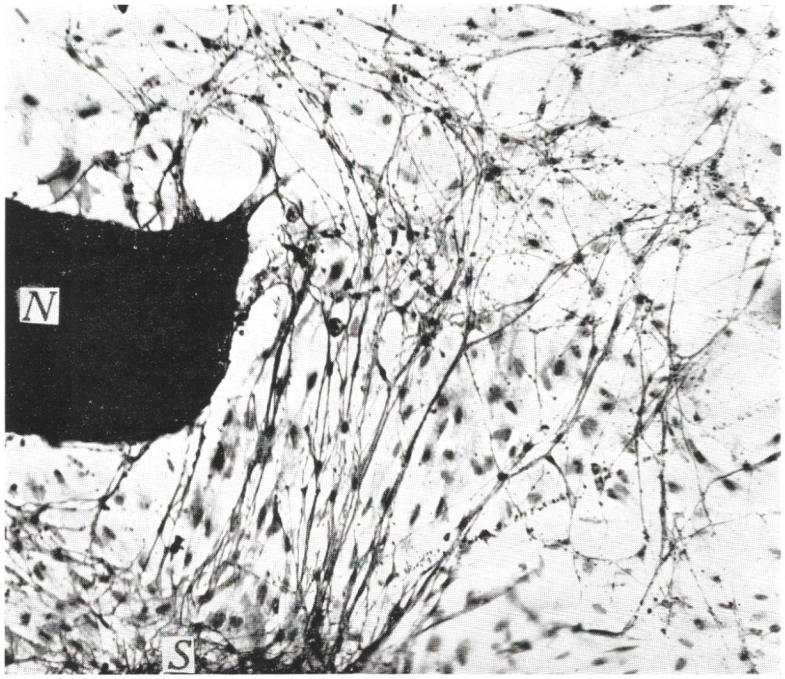

Fig. 3. Nerve fiber plexus grown from an embryonic chick spinal ganglion (S) in liquid medium in close vicinity of the end of a degenerated nerve fragment (N). Bodian impregnation. Total mount. × 160.

We, therefore, reverted to the living animal, devising experimental conditions more nearly comparable to those of Cajal's and Forssman's experiments, but, at the same time, more rigorously circumscribed and controlled. The main shortcoming of earlier work on "neurotropism" has been that it disregarded or underestimated the role of non-nervous tissues, particularly the connective tissue bed, in nerve regeneration. Perhaps because of the preferential use of selective nerve fiber stains, which relegated the tissue matrix to the background, nerve regeneration

has often been dealt with as if it occurred in a vacuum. To correct this situation, we let nerve regeneration proceed in a bed the constitution of which was well known from previous experience, namely, an arterial tube.

An account of the use of sleeves of artery for the reunion of severed nerves (Weiss, '43a), as well as a detailed histological analysis of the part played in the subsequent regeneration process by blood, connective tissue, macrophages, sheath cells and neurites, has been published previously (Weiss and Taylor, '43). Nerve regeneration under these conditions is so excellent, with fibers in large numbers passing straight from the proximal stump through the gap to the distal stump, that advocates of "neurotropism" might acclaim the result as a splendid manifestation of this principle, with the arterial tube serving as pipe line for the "neurotropic" substances. Even though sheath cells and axons have actually been proved to follow an oriented pathway structure through the gap, the postulated "neurotropic" diffusion gradient runs, after all, in the same direction. Certain incidents make it clear, however, that what counts, is the local contact structure and not any orienting action from a distance, "neurotropic" or otherwise. A striking example is furnished by the intersecting courses of regenerating nerve branches near the wall of the sleeve. While the great mass of nerve fibers in the interior travel longitudinally, some branches which happen to adhere to the artery course in circular direction, retracing the pattern of the elastic rings of the wall. A tangential section (fig. 4) reveals both fiber systems, crossing each other at right angles — a sort of Perroncito spiral on a gigantic scale.

An even more compelling test of neurotropism could be expected from experiments fashioned after the "multiple choice" experiments of the psychologists. Nerve fibers were to be given a choice between presumed "neurotropic" goals and other destinations or even blind alleys. For this purpose, bifurcating arteries were used: the proximal nerve stump as fiber source was inserted into the common stem, and the fork provided alternative channels for fiber growth. The ends of the two branches were variously left open or ligated or charged with a distal nerve stump as "neurotropic" lure or plugged with a piece of tendon. The possibility of selective orientation during the growth phase was studied in short term experiments, while long term tests were intended to reveal secondary effects of selective resorption.

MATERIAL AND METHODS

White rats of 120–180 gm. body weight were used in the experiments. The abdominal aorta with its terminal Y-shaped bifurcation into the

Fig. 4 Tangential section
of an arterial sleeve. The
nerve portion running
fiber branches circling

two common iliac arteries (Greene, '35) furnished a suitable preparation for our purpose. Two smaller branches, the inferior mesenteric and middle caudal, were tied off at their exit. Of the three channels thus left, the one receiving the proximal nerve source will be designated as "inlet channel," the other two as "test channels." When the aortic stump was to be used as inlet channel, it was taken mostly from donor animals of the same or smaller size than the recipients. When one of the iliac branches was to serve as inlet, larger donors

Fig. 4 Tangential section through the former gap region of a cut nerve reunited by means of an arterial sleeve. The center of the picture shows the marginal fibers of the regenerated nerve portion running proximo-distally (from bottom to top), while the background shows fiber branches circling around the inner wall of the arterial tube. × 230.

were chosen. The two iliac arteries are of unequal diameter and only the larger one could be used as inlet channel. The total length of the preparations varied between 12 and 16 mm.

For nerve fiber source, we used the sciatic nerve or its tibial branch severed in the upper thigh. The distal stumps of the severed nerves, unless intended for use in the experiments as explained below, were extirpated down to the knee. The proximal nerve stump was then introduced into the inlet channel by standard technique (Weiss, '41b, '43a). The stump was inserted only up to a distance of ca. 5 mm. from

the bifurcation point leaving that much space for the regenerating fibers to span before meeting the two test channels.

The condition of the test channels is illustrated in the diagram, figure 5. In experiment A, both test channels were left open; in B, one end was tied off with fine silk, the other left open; in C and D, a degenerating nerve fragment was inserted into one of the distal openings, the other one being either left open (C) or tied (D); in E, one test channel received a degenerating nerve piece, while the other received a graft of tendon of corresponding diameter. In series F to I, the larger one of the iliac branches received the nerve source, while the other iliac and the aortic stump formed test channels running in opposite directions. For the rest, the diagram is self-explanatory.

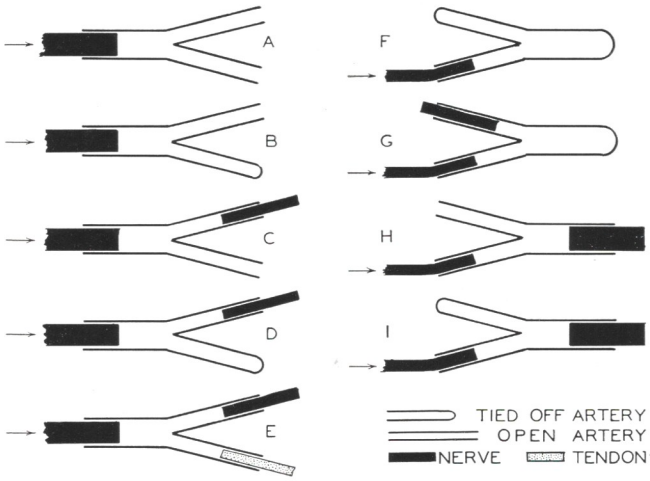

Fig. 5 Types of operations performed.

Those parts of the channels which were not occupied by nerve stumps or grafts were allowed to fill with blood. Blood also helped to keep channels distended. The fate of hemorrhages in nerve gaps has been described previously (Weiss and Taylor, '43). Breakdown of the erythrocytes and the appearance at the scene of potent fibrinolytic agents dissolve the clot within a few days into a liquid menstruum pervaded by sparse strands of fibrin. This medium retains its high liquid content into advanced phases of regeneration and, therefore, offers less resistance to the diffusion of substances, neurotropic or otherwise, than does the condensing scar tissue which ordinarily forms around nerve stumps left unprotected as in the experiments of Forssman and Cajal. The "lures" of degenerating nerve inserted into some of the test channels consisted either of isolated fragments or of the

distal nerve stump as such, the difference being that in the latter case the regenerating fibers had a chance to become eventually reconnected with their old periphery. These nerve ends were sometimes pushed far into the test channel close to the fork; however, in most cases they ended a few millimeters distal to the fork.

All operations were done in nembutal anaesthesia with aseptic precautions. No special means of attachment were used to hold the arteries in place. After closing the muscle wounds, sulfadiazine powder was applied, the skin was sutured and covered with a coat of synthetic resin plastic for protection. Of a total of twenty-four animals operated on, twenty-one came up for terminal study after periods of from 4 to 20 weeks. The experimental nerves were then recovered, fixed under moderate stretch in Bouin's solution, embedded in paraffin, sectioned serially in longitudinal direction at 10 micra, and silver-impregnated according to Bodian's Protargol method; some received an additional stain of Mallory's Triple Azan.

RESULTS

As in our earlier experiments with arterial tubulation, nerve regeneration was abundant. Ligated channels had remained well sealed, and no escape of nerve fibers from them could be detected either by functional tests or histological study. In all cases, a large volume of regenerating nerve fibers and accompanying sheath cells had grown down the lumen of the inlet channel and on into the test channels. Sleeves fitting too tightly again produced the two phenomena regularly associated with this condition, i. e., endoneurial edema and damming of axoplasm in front of the constriction (Weiss, '43b, Weiss and Davis, '43). A detailed description of the results follows.

Experiment A (three cases)

In this series there was free communication between the test channels and their surroundings through the distal openings. Any substances normally present in a nerve bed could at first penetrate freely. However, the channels did not long remain open. Connective tissue invaded them from their open ends. Only a small proportion of this invading tissue became fibrous; the main part turned into fat (fig. 6). Thus the open channels became gradually plugged. Biologically, they are to be rated not as "blind," but as terminating in fat tissue, which may or may not "attract" nerve fibers.

The regenerating fibers followed a straight longitudinal course down the inlet channel. Upon arriving at the fork, they separated into two

streams, dividing themselves nearly evenly between the two channels. Figure 7 shows the region at the fork in a specimen 12 weeks after the operation. This sample is representative of all three cases of this group, the others having been kept for 4 and 5 weeks, respectively.

The outstanding features of these cases are the following. The test channels have become filled to capacity by regenerating nerve fibers. The direction of these fibers is essentially longitudinal. They are grouped into distinct bundles, evidently as a result of "fasciculation." To what extent this results from (a) a tendency of fibers growing out later to cling to the surface of earlier pioneering fibers (Weiss, '41a), (b) a similar association among the Schwann cell cords on which the

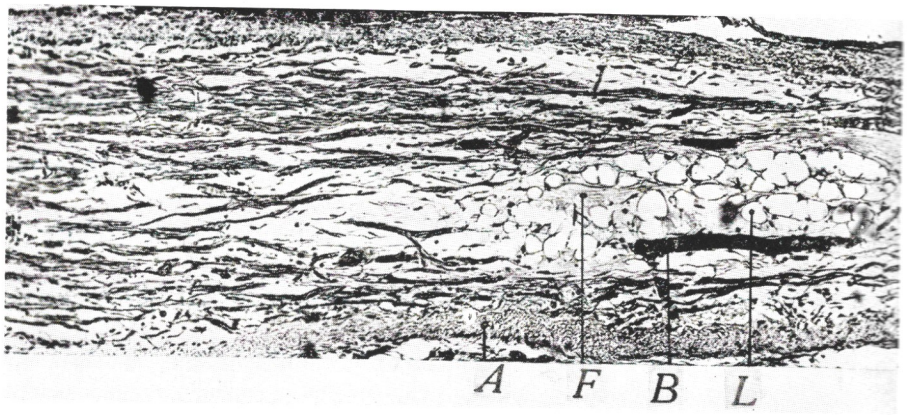

Fig. 6 Distal region of open test channel, 5 weeks after the operation. Fiber masses advancing proximo-distally (from left to right) are arrested by fibrous (F) and fat (L) tissue which has invaded the artery (A). Blood vessels (B) are nerve free. × 82.

axons travel, and (c) the development of islands of endoneurial connective tissue, remains to be determined. The fact that the individual bundles vary slightly in their courses, gives rise to a braiding effect (see also fig. 12). At the crotch many fibers cross and arch from one side to the other, depending on their chance positions. In some places, numerous branches have penetrated into the wall of the artery, but these seem to be collateral rather than terminal branches, and they have definitely not drained the fiber source in the manner observed in arteries with larger leaks (Weiss, '43a; Weiss and Campbell, '44).

Distally, the fiber masses have come to a rather abrupt stop at the border of the fatty invasion tissue. Figure 6 illustrates this zone, and other specimens show an even sharper boundary. As regeneration rate in the rat is of the same order as has been described for the rabbit by Gutmann ('42), namely, 2–3 mm. per day, most fibers must have

arrived at the distal plug (about 10 mm. from the proximal stump) within 2 weeks. As they have not advanced beyond this level after from 4 to 12 weeks, their arrest can be considered as permanent. Some loose connective tissue strands pervading the fat tissue or interposed between it and the wall of the artery may contain a few nerve fibers, but their number is negligible. The several large blood vessels running lengthwise through the fat have remained remarkably free of nerve fibers. As these vessels pass straight from the nervous into the fatty part, they could have served as entering wedges for fibers clinging

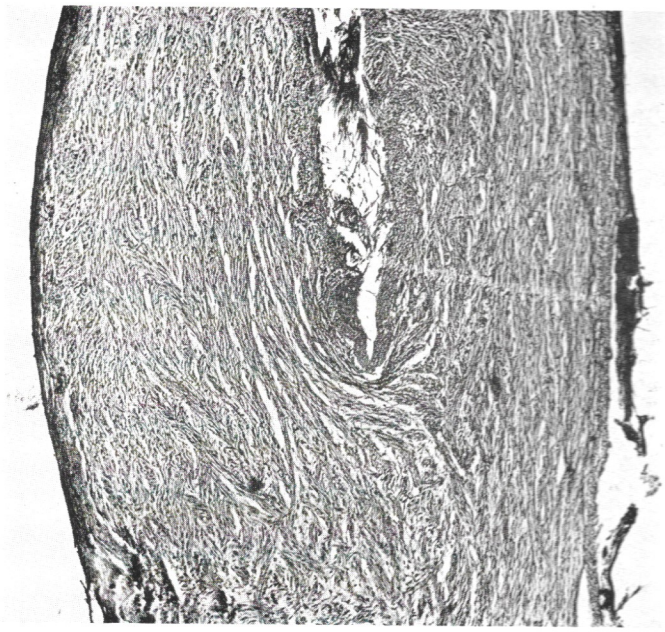

Fig. 7 Regenerated nerve at the transition from the inlet channel into blind test channels. × 72.

to their surfaces if there were any preferential association between nerve fibers and blood vessels. Occasionally this has occurred, but no more often than fiber penetration along narrow connective tissue tongues without accompanying blood vessels. These cases, therefore, furnish some of the most striking demonstrations of the indifference between nerve fibers and blood vessels in nerve regeneration.

The mode of termination of the arrested fibers will be described in the next section. Practically none of them have become reconnected with peripheral nerve, muscle or sense organs, or anything that could be considered a normal nerve destination. In spite of their functional

uselessness, there was no evidence, even after 12 weeks, of any resorption or atrophy (fig. 7).

Experiment B (two cases)

In this series, one of the test channels was distally ligated. The other, unligated, channel became again invaded by fibrous and fat tissue, just as in series A. As a result, one test channel ended blind, the other in a plug of fat tissue. The former has had no communication with the outside from the start, the latter admitted diffusion at least during the early phase of regeneration. However, these differences did not become manifest in the results. Preparations examined 5 and 12 weeks after the operation, show the stream of regenerated fibers evenly divided over both channels.

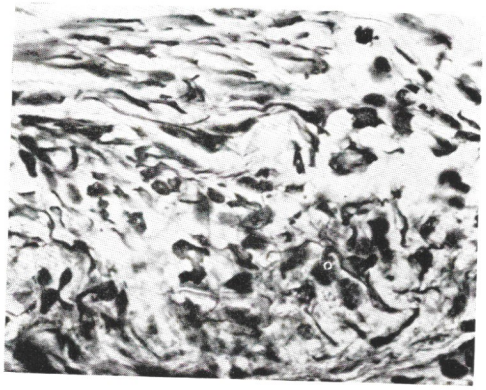

Fig. 8 Irregular terminal plaques of nerve fibers along the wall of the artery. × 500.

A histologic study of the ligated end has revealed the disposition of arrested fibers. While the impregnation used is not adequate for the resolution of finer histological details, the general character of the terminations can be recognized, as follows. The fiber tip widens to an irregularly shaped end lobe which, depending on the surroundings, may be either flattened or more bulbous. The neurofibrillar apparatus is somewhat loosened, but no end loops or other characters reminiscent of somatic nerve endings have ever been observed. Some of the fibers end directly on the inner surface of the artery, others penetrate into the wall. Figure 8 shows a group of them. They differ both in appearance and size from the large sphaerical or teardrop-shaped bulbs often seen in regenerated fibers in front of obstacles (Nageotte, '22; Dustin, '17; Cajal, '28).

Series A and B combined prove that regenerating nerve fibers may grow in saturation density over considerable distances as if toward a definite "neurotropic" goal, where plainly there is none. Evidently they simply follow a pathway structure to a dead end.

Experiments C and D (six cases)

In these experiments, one test channel was as in the preceding series, either ligated or left to be plugged by invading tissue, while the other channel contained a degenerating nerve stump. Although the two channels, one with the "lure," the other empty, thus competed for the oncoming fiber population, the volume, orientation, and distribution of the fibers were essentially alike in both.

Figure 9 shows an example after 5 weeks. The branch on the left is the one which contained the distal nerve fragment. In this picture,

Fig. 9 Forked nerve regeneration (15 weeks) into test channels, one containing nerve graft (left), the other empty (right). × 72.

more bundles converge upon the left than upon the right branch. This is true of this level only and is due to a slightly twisted position of the preparation relative to the plane of sectioning. Sections at deeper levels show the reverse. Even so, in this specimen, the total volume of nerve fibers was actually somewhat larger in the channel with the nerve graft than in the blind channel. However, this difference is purely accidental, as one may see from a comparison of the remaining five cases of this series. Discounting one case in which the blind channel had collapsed, two (after 5 and 20 weeks, respectively) showed equal nerve filling in both channels, one (5 weeks) showed a balance in favor of the channel with the graft, and one (5 weeks) in favor of the blind channel.

Evidently, therefore, the regenerating nerve fibers, when arriving at the fork, continue in both directions without discrimination. In none of these cases did it matter, whether the "lure" consisted merely of an isolated nerve graft or of the whole distal nerve stump continuous with its old periphery so that the regenerating fibers could grow through and reestablish functional connections with muscles and skin. The channel containing degenerated nerve has neither "attracted" nor "stimulated" nor oriented the outgrowth of nerve fibers any more than has the control channel containing no nerve.

This still leaves the question open as to whether fibers coming to a dead end might not later be resorbed, as suggested above in alternative explanation of Cajal's results. An animal kept for 20 weeks after the operation has provided the answer. One test channel had been ligated, while the other contained the distal stump of the tibial nerve. The tibial nerve and its muscles had become reinnervated and been in functional activity for some months when the animal was sacrificed. Histological study confirmed that the end of the blind channel was tightly sealed and that no nerve fibers had escaped. Both channels were found to have been filled about equally with fibers. Figure 10 shows the region of the fork, revealing no difference in the orientation or density of the fibers between the two channels. The one to the left is the blind one. From this we learn that fibers which have been stopped and permanently prevented from attaining functional connections have persisted in this condition without signs of reduction or resorption for at least 5 months, which is a sizable portion of a rat's life span.

In contrast to its mere persistence, the further elaboration of the character of the nerve fiber, referred to as "maturation" and recently strongly emphasized by Young ('42) and his coworkers, seems to be influenced by whether or not terminal connection is effected. The term

maturation covers all changes, visible and invisible, taking place after a fiber has become joined to its end-organ, including growth of caliber, myelinization, straightening of course, possible resorption of collaterals, "modulation" of central relations (Weiss, '36), etc. Our preparations are unsuitable for the study of myelinization, as no specific myelin stain was used. Fiber size, on the other hand, could be roughly

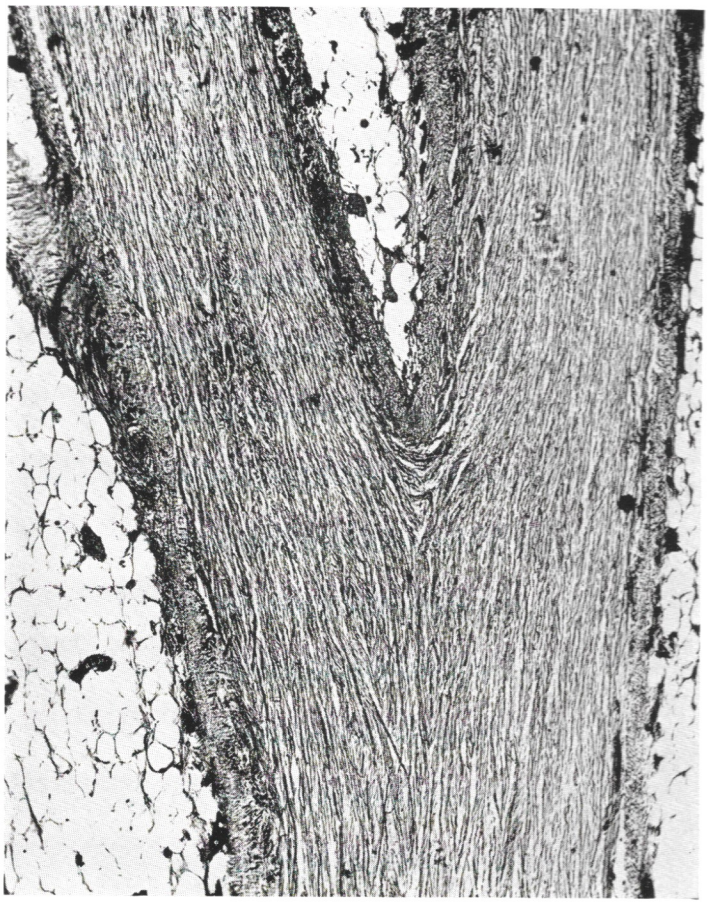

Fig. 10 Forked nerve regeneration (20 weeks) into a blind (left) and a nerve-connected (right) test channel. × 72.

assessed because the protargol treatment in many instances shows up the whole width of the axis cylinder, neuroplasm as well as fibrils. One fact becomes immediately evident from our preparations: even blind channels contain a considerable number of large nerve fibers, indicating that functional termination is not indispensable for recovery of a large diameter. Nor do nerves after 5 weeks of regeneration show per-

ceptible differences in the fiber size spectrum between blind and connected ends. Yet, the sample studied after 20 weeks reveals a remarkable excess of larger fibers in the half which had become functionally reconnected as compared with the blind half. Figure 11 shows the two fiber streams just proximal to the fork (blind channel to the left); the striking contrast in average fiber size between the two halves can be readily recognized. This would suggest that caliber growth is definitely enhanced after functional connection has been accomplished. Yet, not too much weight should be placed on a single case, and a further investigation of the problem is needed.

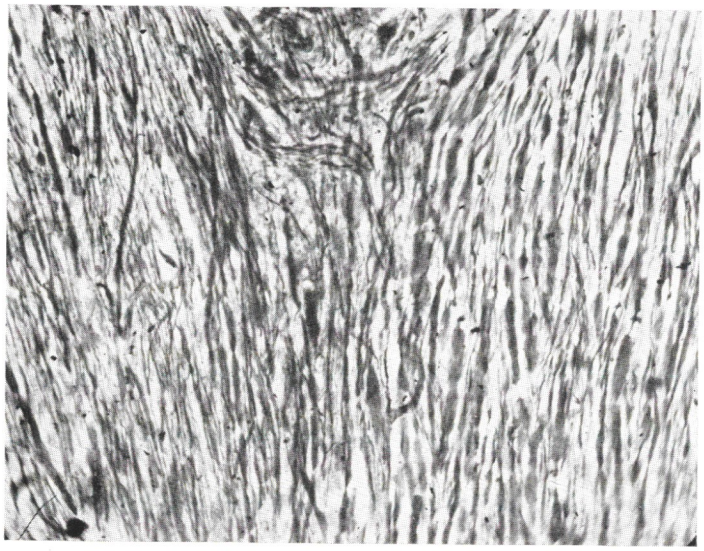

Fig. 11 Detail of the dividing fiber stream at the fork of the nerve shown in figure 10. Note difference of fiber sizes in left and right branch. × 230.

Any directive "neurotropic" effect of a nerve stump should, of course, increase in intensity as the fibers approach it. Quite to the contrary, however, the orientation of the fibers may become increasingly confused as they arrive at the distal stump. This fact, irreconcilable with a "neurotropic" concept, is fully in accord with the mechanism of "contact guidance." Figure 12, for example, shows the distal portion of a test channel with nerve graft after 11 weeks of regeneration. The graft (G) was of smaller diameter than the artery, thus leaving a space between its surface and the channel wall. At its base the regenerating nerve fibers, arriving in predominantly longitudinal orientation, can be seen to have suffered a marked deflection, with a majority

diverging into the space around the graft, rather than converging upon the graft. This effect is of mechanical origin. As explained in an earlier paper (Weiss and Taylor, '43), it results from pressure of the graft against the original blood clot in the gap. It can be avoided by moderate stretch, keeping the graft from pressing inward. In the present context, however, it serves as a striking addition to the evidence against the "neurotropic" potency of degenerated nerve.

Fig. 12 Proximal end of nerve graft (G) in test channel. Most oncoming fibers weave around the graft and proceed into the space between graft (G) and artery (A). × 105.

Experiment E (two cases)

In this experiment a nerve graft at the end of one channel was placed in competition with a tendon graft at the end of the other. As figure 13 shows, regenerating fibers have again moved into both channels in about

equal volume. The nerve graft has become fully pervaded, while only few fibers penetrated into the compact tendon plug. The chiasmatic crossing of fibers at the crotch seen in the picture is a common occurrence and attributable to the mechanical peculiarites of this region.

Fig. 13 Forked nerve regeneration (12 weeks) in test channels containing nerve (right) and tendon (left) grafts. × 72.

Experiment F (two cases)

In experiments F to I, the arterial Y was used in reversed orientation, inserting the proximal nerve stump into one of the iliac branches. The fact that thus only one of the test channels continued in the general direction of the inlet channel, while the other ran backwards under an acute angle, had a profound effect on the results. In all cases in which this recurrent branch had been left empty, whether open or ligated, it collapsed, its walls folding accordion-fashion, its lumen partly

shrinking to a narrow strand of vascularized fat and fibrous tissue, partly disappearing altogether. The reasons for this different behavior of the tubes, depending on whether they continue in the direction of the nerve or in the opposite direction, are a matter of conjecture. For the time being, no explanation is offered.

Only a small bundle of regenerating nerve fibers is found in the recurrent channel. These fibers have arched off the main stream at the crotch. The rest, constituting the vast majority, have continued straight ahead into the blind aortic end. The contrast between these results and those of series A and B is marked and underlines the importance of the mechanical configuration of the nerve bed in guiding the stream of regenerating fibers.

Experiment G (three cases)

This experiment differs from the preceding one in that a nerve graft had been placed into the recurrent test channel. Channels thus distended have not collapsed and they became well filled with regenerating fibers. As in series A-E, the inlet fiber stream was divided: one part continued straight into the blind end, the other turned into the side branch where it grew backwards into and through the graft. The course the latter group took after passing through the graft varied with the surroundings. In one case, the graft had become only moderately well filled. In another case, in which the branch containing the graft adhered to the adjacent proximal nerve stump, the fibers emerging from the graft continued to grow backwards along the surface of the nerve trunk for a considerable distance. This observation is valuable as evidence against the hypothesis of a polarizing force, e. g., electrical. Evidently, fibers grow with equal ease in proximo-distal and in the reverse direction. Similar observations have been made in series C-E, where many fibers, after leaving the distal end of the graft in the test channels grew backwards along the outer surface of graft and artery, fountain-like.

In the third case, the far end of the graft was surrounded by loose connective tissue in which the emerging fibers had formed an extensive messy neuroma. Apparently, many of them had then reentered the graft and grown back into the recurrent test channel, which finally brought them into the forward test channel, where they joined the straight bundles that had grown this way directly. The diagram, figure 14, illustrates these fiber courses, and figure 15 shows the actual fiber arrangement at the crotch. This case is particularly instructive not only in that it confirms the lack of polarity in nerve regeneration —

the fibers in the left branch have twice reversed their direction—, but also in that it shows how fibers which had already entered a supposedly "neurotropic" nerve fragment may leave it again for a blind channel.

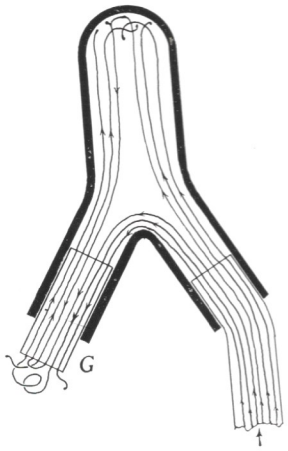

Fig. 14 Fiber arrangement in a specimen containing a nerve graft (G) in the recurrent channel. Explanation in text.

Fig. 15 Fiber pattern at the fork of the specimen outlined in figure 14. Inlet channel on right, test channel with graft on left, empty test channel in upper center. × 110.

Experiments H and I (three cases)

This series differs from experiment F in that the aortal end contains a nerve graft instead of being tied off. The recurrent test branch was again collapsed and, accordingly, had admitted only a small number of fibers. These came off the main stream in a bend around the crotch. The main stream continued straight into and through the graft.

DISCUSSION

In the reported experiments regenerating nerve fibers have been confronted with crucial test situations designed to reveal any possible attraction by, and deflection toward, degenerated nerve fragments as supposed sources of "neurotropic" substances. The results have proved that degenerating nerve exerts no such "neurotropic" action. Although, for the sake of simplicity, our description has focussed on the axons, it is clear that the evidence applies equally to the outgrowing sheath cell cords which in most cases precede the axons (Kirk and Lewis, '17; Nageotte, '22; Dustin, '17; Holmes and Young, '42; Weiss and Taylor, '43). Previous arguments against a chemotactic theory of nerve orientation (Dustin, '10; Ingebrigtsen, '16; Harrison, '35; Weiss, '41), have thus been given final experimental support. A galvanotactic theory, never seriously advocated for nerve regeneration, is equally untenable in view of the common occurrence of fiber growth in opposite directions, examples of which have been reported above.

This leaves the theory of "contact guidance" in control of the field. How this mechanism operates in organizing nerve regeneration in arterial sleeves, has been revealed previously (Weiss and Taylor, '43). Without repeating details, it may be recalled that one of the essentials enforcing longitudinal orientation among the regenerating nerve fibers was longitudinal stress transmitted to the blood clot inside the artery. While in our earlier experiments, the blood gaps had been relatively small — only a few millimeters at the most —, the present series shows that fibers regenerating in sleeves many times as long may maintain excellent longitudinal orientation, although not always quite as strict as has been observed in smaller gaps (Weiss, '43a). The longitudinal pathway structure must be ascribed to the combined action of (a) longitudinal stress imposed on the matrix in the test channels, such stress arising from the shrinkage of distal adhesions between the end of the artery and its surroundings (in the case of blind channels) or from retraction of the inserted distal nerve stump; (b) the syneretic shrinkage of the clot; (c) compression by the walls; (d) perhaps some

5. not seen often

elastic self-straightening of the fiber matrix. The observed disorientation (fiber crossing) in front of the crotch is to be expected, inasmuch as this triangular area lies in a sort of "stress vacuum."

The failure of fibers to fill recurrent channels is likewise explicable in terms of the mechanics of the situation. In order to enter these tubes, fibers have to turn off their straight path under a sharp angle or even double back. As long as there are alternative courses open, fibers seem to tend to follow the one most nearly in line with their immediately preceding orientation, conforming to what might be called a principle of conservation of direction. If nerve growth is supported by pressure from behind (Held's "vis a tergo") — and we have recently obtained additional experimental evidence that it is —, this would furnish an adequate physical basis for this principle. Even in tissue culture, fibers coming across an oblique pathway continue predominantly in the direction of the obtuse rather than the acute angle (Weiss, '34). The probability that a major proportion of the fibers may become deflected into a recurrent channel is, therefore, slim. In what measure the collapse of these channels has aggravated the situation, and the lack of fiber filling, in turn, has contributed to the collapse of the tube, is impossible to decide.

The presence of a nerve graft in a recurrent branch changes the picture. In that case a considerable mass of fibers enter. But whether this is merely due to the fact that the graft has held the channel in distension, thus greatly increasing its capacity, or whether a principle of "selective fasciculation" (Weiss, '41a) is involved in the sense that nerve fibers which have found easy travelling in a nerve graft become pathways of preferential application for follower fibers, remains to be determined. That the case cannot be claimed for the cause of "neurotropism," has been explained above. One must never lose sight of the fact that blind channels leading nowhere have, after all, become completely filled with fibers.

While "fasciculation," that is, the building up of fiber bundles around common pioneer fibers, is clearly expressed in the "braiding" pattern of many of our preparations (compare figs. 6, 7, 12, 15), we know neither the circumstances nor the mechanism involved. Previous experiments had led to the assumption that fasciculation would occur around fibers which have attained terminal connections, while fibers with free, roaming tips would remain solitary (Weiss, '41a). Applied to the present case, this would mean that the terminal networks of fibers arrested on the wall of the artery (fig. 8) would have to be rated as "terminals" in the indicated sense. Perhaps the critical

fact is merely that the fiber comes to a rest and can consolidate its surface configuration. However, speculation had better be deferred until more analytical experimental data have been obtained. In the case of nerve regeneration, especially, more attention will have to be paid to the role of the Schwann cell cords in the process.

Our ignorance of what constitutes an effective "terminal" — effective in that it insures the preservation and maturation of the nerve fiber concerned —, likewise affects the interpretation of the observed persistence of fibers in blind channels. In none of our cases was there any sign, even after 20 weeks, that fibers barred from reaching peripheral destinations would atrophy and be resorbed. It may be that still longer periods would have shown some reduction, but 20 weeks is a long time in terms of rat chronology. In discussing secondary resorption, we are not referring to the early withdrawal and autotomy of young branches sprouting from the tip of an advancing nerve fiber. Reduction of this kind is a regular feature of nerve regeneration (Cajal, '28; Speidel, '33). Rather are we referring to the late retrogression of already established fibers sometimes postulated to result from lack of functional utility (e. g., Young, '42; Weiss and Campbell, '44). In regard to this matter, it can only be stated that our knowledge is wholly inadequate to decide the question. While slow resorption of fiber branches in human amputation neuromas is said to continue for many years, the fact remains that the majority of the fibers is still in the field after all those years (Dustin, '17). We just do not know when and why fibers or branches of fibers persist or vanish. Our present results add, at least, the information that lack of connection with what could be considered a normal periphery is no cause for resorption.

Young and this figure Weiss

Our observation that fibers allowed to regain functional connections acquire larger diameters than fibers not so connected, opens interesting perspectives. But being based on a single case, the fact does not deserve more comment until further substantiated. In the present case, one could not even decide whether the small diameter of the fibers in the blind branch was due to their being "functionally unconnected" or to their being connected to a tissue — artery — whose innervation is typically of small caliber.

There being evidence of neither selective attraction nor selective resorption of nerve fibers in regeneration, it seems that the explanation of Cajal's and Forssman's results will have to be sought in the structural configuration of the bed in which their nerves were allowed to regenerate. The manner in which the stumps were displaced and attached; tensions set up during the operation or by subsequent retrac-

"fasciculation effect" still very tentative here

tion of the stumps and of the adhering connective tissue; the shrinkage of the hemorrhage in the wound bed so instrumental in the orientation of the substituting scar tissue (Weiss and Taylor, '43); the disposition of liquids in the wound; and possibly a "fasciculation" effect; all these may have contributed to the results which those authors were led to ascribe to "neurotropism," and which, as we now realize, could not possibly have been anything of the kind. Cajal says ('28, p. 195): "As for the theory of neurotropism, far from being for me a dogma, it is simply a working hypothesis which I am willing to correct or even abandon in the presence of better explanations." We feel the time has come to abandon it. "Contact guidance" affords a better explanation.

SUMMARY

The contention that regenerating nerve fibers are attracted by degenerating nerve (theory of "neurotropism") is contradicted by the results of the reported experiments.

1. No deflection of nerve fibers toward degenerating nerve has been observed in tissue culture, either in blood plasma or liquid medium.

2. Nerves in the rat were allowed to regenerate in forked arteries. The branches of the fork confronted the outgrowing fibers with alternative routes. These led either into blind channels or into channels containing degenerated nerve, tendon, or fat tissue.

   a. Nerve fibers grew into blind channels with the same density and orientation as into channels containing degenerated nerve.

   b. Nerve fibers were never deflected from their course toward channels containing supposedly "neurotropic" agents.

   c. Nerve fibers approaching the entrance of a degenerated nerve have shown no tendency to converge upon it.

3. Nerve fibers arrested in a blind channel form irregular terminal plaques. In spite of their lack of functional connection with appropriate end-organs, they have persisted in this condition for at least 20 weeks without signs of resorption.

4. An indication has been obtained that fibers connected with a functional periphery may grow to larger size (diameter) than fibers not so connected.

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