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The purpose of studying repair of nerves in experimental animals, such as the rat, is to perform model experiments in which the phenomena inaccessible to large scale investigation in human subjects may be analyzed under controlled conditions. It is neither intended nor to be expected that the results obtained in such model experiments will become immediately applicable for clinical use. What is to be expected, however, is that the lessons learned from those experiments, when properly interpreted in terms of the conditions prevailing in the human body, may furnish directives for clinical research and possibly clinical practice. In a preceding article [1] a method of reuniting severed nerves by means of arterial cuffs was outlined, and the superior results of nerve regeneration following "sleeve splicing" were described. Since that publication, the method has proved its value on several hundred experimental animals, including rats, chickens, rabbits, cats and monkeys. We have gained from these studies much information about the prerequisites of nerve regeneration; above all, insight into the reasons for the success of the sleeve-splicing method. While it would be idle to predict whether or not the arterial sleeve will make a suitable link in human nerves, the lessons which we have learned from analyzing the mechanism of its action in animals are of such a general and fundamental nature that they may well be heeded in whatever method one may elect to follow in surgical practice. These lessons pertain particularly to the early stages of regeneration. As will be shown in this paper, the whole course of nerve regeneration is essentially decided within a matter of days or, at best, weeks after the nerve union. Most of the processes thereafter are determined by the conditions laid down during the initial phase of regeneration, and the prospects of eventual nerve restoration will benefit or suffer, depending on the success or the failure of the early union.

It is with these facts in mind that we present in this article an analysis of the events following sleeve splicing and an evaluation of their significance for successful nerve regeneration. Some of these phenomena are common to all kinds of nerve reunion, and others are peculiar to the sleeve-splicing technic. Some of the latter

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1. Weiss, P.: Nerve Regeneration in Rat Following Tubular Splicing of Severed Nerves, Arch. Surg. **46**:525-547 (April) 1943.

are shared by other tubulation methods. In fact, Kirk and Lewis [2] in their excellent histologic study of nerve regeneration after fascial tubulization have noticed some of the phenomena emphasized by our studies. In the preceding study, the multiple functions of the arterial sleeve in facilitating nerve regeneration have been listed. These functions include insurance of straight and unbranched nerve fiber outgrowth, and prevention of escape of fibers, of invasion of connective tissue and of formation of fibrotic and growth-obstructing suture lines. The present study will furnish an account of how these functions are being exercised.

EXPERIMENTAL STUDY

MATERIAL AND METHOD

Our results are based on a study of 39 rat nerves, either tibial or peroneal, which were transected, reunited by means of an arterial sleeve and then studied between one and fifteen days after the operation. In general, the procedure described in the previous article [1] was followed. The arterial sleeves were so chosen as to fit comfortably over the nerve ends without producing constrictions. The ends of the nerves were not closely apposed but were left separated by a gap varying in extent from a fraction of to several times the diameter of the nerve, i. e., from about five-tenths to several millimeters. In some cases the gap became wider than was intended, owing to the partial retraction of one or both nerve stumps from the sleeve. In contrast to our earlier procedure, excess blood was not blotted from the interior of the gap but was left in place. This blood played an integral part in the healing process not formerly realized in its full meaning. As anesthesia induced with soluble pentobarbital U. S. P. was maintained for several hours, the seal between the nerve ends had become sufficiently firm by the time the animals resumed movement.

In order to investigate the phenomena taking place at the distal suture line of a graft in the absence of nerve fibers, splices between two peripheral nerve segments were effected. For this purpose, a piece several millimeters in length was excised from the proximal sciatic nerve. Two weeks was then allowed for the degeneration of the peripheral stump. After this period either the tibial or the peroneal nerve or both were transected and reunited by arterial sleeves. In this case neither stump contained intact nerve fibers, and such nerves will be referred to henceforth as "aneuritic." In 8 animals, simple biopsies of the union were made within two weeks after the operation, with special attention to the medium filling the sleeves between the nerve ends. The biopsy specimens from the remaining 31 nerves were straightened on cardboard, fixed in Bouin's solution, embedded in paraffin, sectioned serially at 10 microns and impregnated with silver according to Bodian, with an additional Mallory triple azan stain for the differentiation of connective tissue superimposed on the silver stain in sections selected at regular intervals. In all illustrations the proximodistal direction of the nerve is from bottom to top.

GENERAL RESULTS

Immediately after the operation, the artery is attached to the nerve ends by clotted blood and lymph. The gap between the nerve ends is filled with a dark purple blood clot visible through the translucent walls of the artery. This filling and the moderate tension along the nerve keep the arterial tubes from collapsing. Within four to five days after the operation the blood clot undergoes profound physical and chemical transformations. It is through these transformations that the severed texture of the nerve becomes rewoven in such a manner as to prepare the unimpeded transit of sheath cells and regenerating axons from one stump to the other. These changes are diagrammatically summarized in figure 1, representing the situation on the first, third and fifth days. Resolved into component steps, the reweaving process occurs as follows:

Phase 1.—Immediately after the operation, a firm connection between the nerve ends is established by the fibrin reticulum of the clotting blood in the gap, the fibrin threads inserting directly on the cut surfaces. The erythrocytes lie in clusters embedded in the fibrin meshes (fig. 1 *A*).

2. Kirk, E. G., and Lewis, D. D.: Regeneration in Peripheral Nerves, Bull. Johns Hopkins Hosp. **28**:71-80, 1917.

Phase 2.—Within twenty-four hours most of the erythrocytes disintegrate, while the fibrin framework persists. Later proteolysis along the inner wall of the artery breaks the connections between the clot and the sleeve. Thus detached all around its circumference, while firmly cemented to the two nerve stumps, the cylindric clot becomes subjected to longitudinal tension.

Phase 3.—The fibrin net assumes preponderantly longitudinal orientation corresponding to the lines of tension (fig. 1 *B*).

Phase 4.—By the third day, fibrinolytic liquefaction has set in within the clot. Its action is differential in that it dissolves mostly the transverse threads of the fibrin meshes while sparing the longitudinal ones (fig. 1 *B*). Somehow tension has rendered the longitudinally oriented fibrin fibers more resistant to proteolytic destruction.

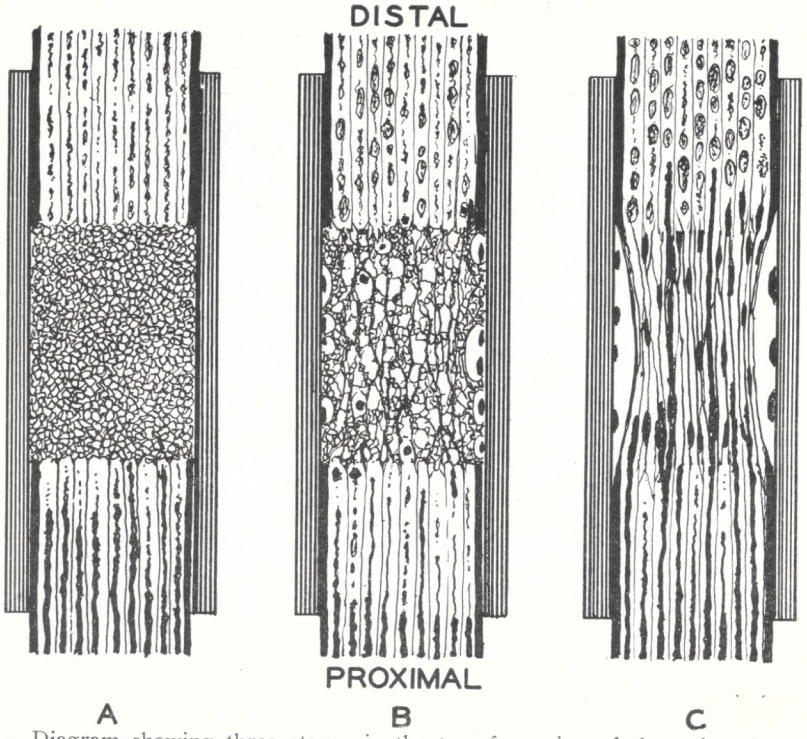

Fig. 1.—Diagram showing three stages in the transformation of the union tissue between sleeve-spliced nerve stumps, one (*A*), three (*B*) and five (*C*) days after the operation.

Phase 5.—By this time migration of cells into the region of the former gap has begun. Sheath cells and nerve fibers move out along the solid strands of fibrin and hence are likewise oriented in the longitudinal direction of the nerve. Simultaneously, macrophages move in and continue to clear the spaces between the fibers.

Phase 6.—By the fifth day, a continuous, straight cell bridge, followed by regenerating axons, extends across the former gap (fig. 1 *C*). The Schwann cells of this bridge stimulate the deposition of collagen along their surfaces, and a system of collagen fibers, oriented longitudinally, is laid down as the endoneurium of the nerve segment.

Phase 7.—When continuity between the nerve stumps is completely restored, channels are opened for the passage of endoneurial fluid, which may serve to keep the interior of the nerve in the required state of fluidity.

Comment.—This survey shows that the interweaving of severed nerve stumps occurs essentially by three successive processes: (1) the formation of an oriented scaffolding of fibrin fibers; (2) an oriented migration of living cells and nerve fibers, retracing and populating the fibrin matrix, and (3) a collagenization under the influence of these cells of the intercellular spaces in continuity with the stumps. By contrast, the scar forming between two nerve ends not protected by a sleeve fails to undergo longitudinal orientation, becomes invaded by all kinds of surrounding connective tissue cells and establishes a sort of foreign block in the continuity of the nerve. The two stumps, instead of being rewoven into an integral fabric over which sheath cells, nerve fibers, blood vessels and capillary liquid may pass freely and easily, are merely patched up by what amounts to a mechanical cementing tissue of low permeability.

These differences will become even more evident in the following day by day account of the healing progress. This account is based on the rate of progress as observed in the average case. While the timing varies slightly, depending on

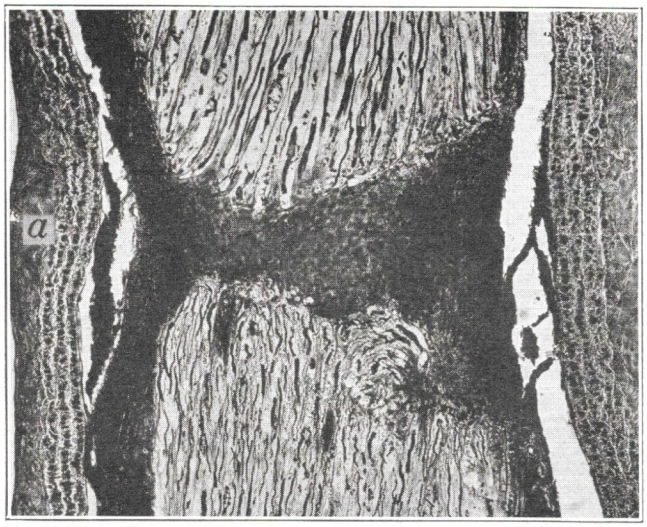

Fig. 2.—Union by blood clot of sleeve-spliced nerve stumps, one day after the operation; *a*, wall of arterial sleeve (× 120).

the age of the animal, the size of the nerves and the length of the gap, the fluctuations affect only the onset and duration, but not the order of succession, of the listed steps. For convenience, we shall designate the tissue filling the gap and reuniting the nerve stumps as the "union tissue," the term to be applied through all stages of transformation, beginning with the early blood clot and terminating with the restoration of the new nerve segment.

ONE DAY

Figure 2 shows the condition of the union after twenty-four hours. Degeneration of the axons in the peripheral stump has set in and some ovoids can be seen near the cut. Most axons are still intact, as it has been generally observed that wallerian degeneration within the sleeve is somewhat retarded, presumably owing to slight compression.[3] One notices in the picture that the two stumps are cemented

3. Weiss, P., and Davis, H.: Pressure Block in Nerves Provided with Arterial Sleeves. J. Neurophysiol. **8**:269-286, 1943.

by a blood clot firmly adherent to the cut surfaces. In contrast, there is a gap between the nerve and the arterial wall. This gap is an artefact resulting from the shrinkage of the preparation during fixation. However, the fact that this detachment has occurred along the arterial wall indicates that the clot does not adhere firmly to the artery, although its link with the two nerves is solid. The lack of firm ties between nerve and sleeve reduces, of course, the holding strength of the latter. On the other hand, we have regularly observed that the slipping out of the nerve ends from the sleeve is counteracted by the suction which such withdrawal would necessarily exert. With the sleeve gripping the surface of the nerve closely, the nerves act like the plungers in the barrel of a syringe; i. e., they could not be separated without creating a vacuum in between, and the suction of this vacuum helps to keep them in place as long as the pull remains moderate.

After the blood between the stumps has become clotted, it strengthens the formerly tenuous union between the nerve stumps. Blood in an otherwise empty artery, as one knows, would long remain liquid. In our arterial cuffs, tissue juice and exudates of the nerve ends obviously inhibit the antithrombic action of the artery. The

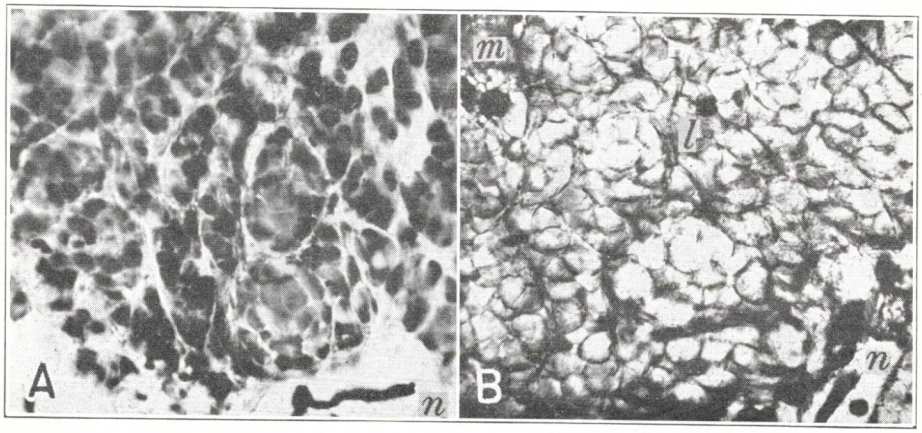

Fig. 3.—*A*, part of the union tissue of figure 2 under high magnification (× 630); erythrocytes dark, fibrin framework light; *n*, cut end of an axon. *B*, union tissue early on the second day (× 630); *n*, end of a cut nerve fiber; *m*, macrophage; *l*, leukocyte.

firmest attachment between artery and nerve is effected at the two ends of the artery where epineurial connective tissue grows over the sleeve, incorporating it in the continuity of the nerve. Under high magnification, the clot can be seen to be composed of small groups of erythrocytes, densely packed, lying in the meshes of a fibrin network (fig. 3 *A*).

TWO DAYS

Biopsy on the second day already reveals the presence of a liquid menstruum inside of the sleeve composed, evidently, in part of serum oozing from the clot owing to syneresis, and in part of liquids released by the progressive destruction of the red cells and the liquefaction of fibrin. The fluid is still dark colored. More details can be seen in the histologic pictures. As in the one day preparations, the solid parts of the matrix are firmly cemented to the nerve ends but adhere only lightly to the wall of the artery. The red cells have mostly disintegrated, although their ghosts are still intact. Inspection under high magnification (fig. 3 *B*) reveals the presence of a fine honeycomb of fibrin, the meshes of which are the spaces formerly occupied by the red blood corpuscles. The disappearance of the red cells

enables the white blood cells to stand out in the pictures. They consist of granular leukocytes, some lymphocytes and only an occasional macrophage. A sample count indicated about 3,000 of the larger leukocytes per cubic millimeter within the clot. There is a denser accumulation of these cells along the arterial wall, where many of them can be seen to liquefy the surrounding clot. Thus vacuoles appear in the clot all along its surface, each vacuole the product of a single cell. On becoming confluent, these vacuoles form a liquid layer, separating the clot from the artery. Figure 4 shows this process well advanced on the third day. Thus, while the clot from the beginning has failed to become sealed to the artery, it is now actively detached from it by this surface liquefaction.

This has a decided influence on the tensional pattern within the union tissue. The origin of these tensions is twofold. If the nerve ends tend to retract, they

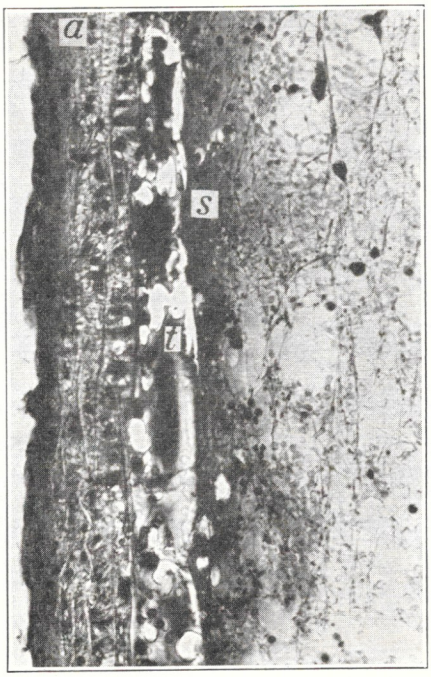

Fig. 4.—Proteolytic detachment of the union tissue from the arterial wall; note confluent cavities around liquefying cells; *a*, sleeve wall; *s*, condensed surface of clot; *t*, liquefied space between *a* and *s* (× 240).

put the intervening clot under elastic strain oriented longitudinally along the axis of the nerve. However, even in the absence of such extraneous pull, tensions arise within the clot owing to its syneresis. This syneresis, which is due to the progressive segregation of the solid from the liquid phases of the colloids of the clot, will contract the clot in those directions in which shrinkage is possible, i. e., where the surface of the clot is free. However, where it is attached internal tensions will arise between the points or surfaces of attachment. These elastic tensions will equal the force that would have to be exerted if the clot had first been allowed to shrink unimpeded and then been returned to the former length by actual stretching. Since the clot in all our cases is essentially a cylinder, attached at its base and top to the nerve stumps while free along its mantle, syneresis will lead to actual shrinkage

in the radial directions but to the setting up of elastic tensions in the longitudinal direction along which yielding is precluded by the resistance of the nerve ends.

In accordance with this tensional pattern, the fibrin framework, beginning with the second day, assumes a striking longitudinal organization (fig. 5 *A*), which, in view of the known orienting action of tension on the arrangement of fibrin fibrils, can be directly ascribed to the mechanical stresses in the clot. Concurrently with the increasing prominence of the longitudinal fibrin aggregates, a dissolution of the fibrin braces oriented otherwise than longitudinally takes place. Thus, the longitudinal parts of the fibrin network are strengthened while the nonlongitudinal ones are resorbed by proteolysis. Though this liquefaction is often more marked at first near the ends of the nerves, it likewise arises in many places in the interior of the clot more or less simultaneously. It results in a gradual increase in the size

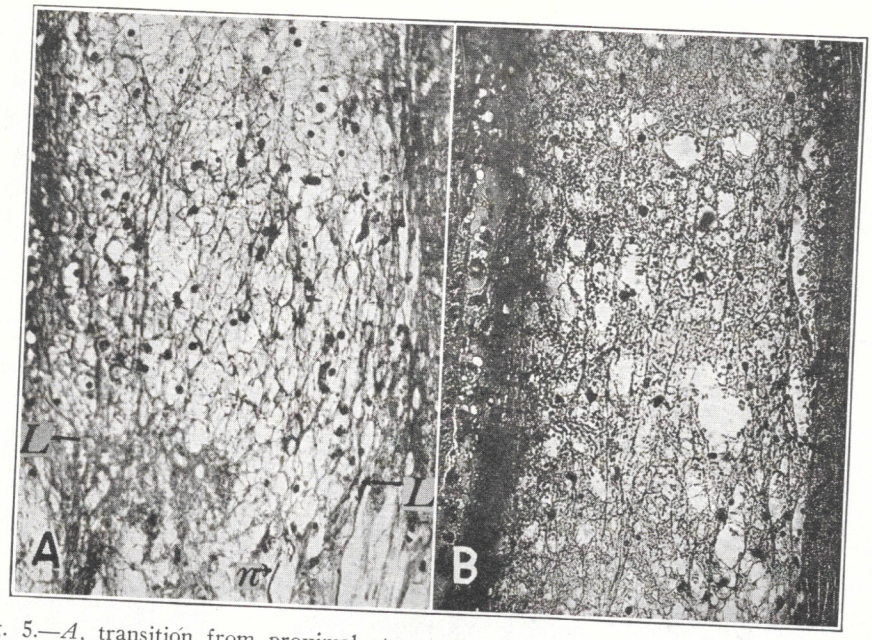

Fig. 5.—*A*, transition from proximal stump to union tissue late on second day. Heavy fibrin fibers begin to appear in the longitudinal direction (lines of tension). Note scattered leukocytes and macrophages and liquefying activity of the latter (cavities) in the denser outer parts of the clot. *L-L*, level of transection, containing the ends of cut nerve fibers (*n*) (× 230). *B*, union tissue on the third day. Note the destruction of the fibrin net, exempting the longitudinal strands (× 120).

of the individual meshes, more and more of the meshes of the original honeycomb becoming confluent as the partitions are dissolved.

THREE DAYS

By the third day, the condition illustrated in figure 5 *B* is reached. Only the gap region, measuring several millimeters, is shown in the picture. One can see the lumen of the sleeve filled with a spongy mass consisting, according to its staining reaction, of fibrin. These are the ruins, as it were, of the original compact clot. Liquefaction along the wall of the sleeve has become very extensive, and so has the consequent detachment of the union tissue from the arterial wall. Large liquid spaces pervade the clot. The longitudinal fibrin strands have become even heavier

and straighter, and many extend throughout the clot. Viewed at higher magnification, the details of the transformation of the clot become evident (fig. 6*A*). In some places, the outline of the original honeycomb of fibrin around red cell ghosts can still be seen, but most of the meshes have widened considerably, with marked elongation in the direction of the nerve. The heavier, longitudinal fibrin strands are clearly visible (compare also fig. 4), but they are still interconnected by cross links. As one can see from the picture, cells are still very scarce in the central portions of the union tissue. Near the nerve ends, however, cell migration has set in, as will be discussed presently. The longitudinal fibrin fibers are from their very first appearance anchored in the two cut surfaces of the nerve. This is already clear on the second day (fig. 5*A*) but is even more marked on the third day. As the nerve fiber substance near the wound is at this stage liquid, the solid frame of the cut surface of the nerve consists of the rims of the neurilemmal tubes and

Fig. 6.—*A*, central portion of the union tissue on the third day, showing the advanced transformation of the fibrin network into longitudinal strands (× 630). *B*, sheath cells (spindle-shaped) on the third day, climbing along the fibrin network; *a*, sleeve wall (× 630).

the endoneural collagen. It is only along these solid lines that fibrin fibers can insert. Therefore, the fibrin framework forms in direct continuation of the architecture of the nerve stumps.

The degree of cell invasion observed at the end of the third day varies greatly among different cases. While in some the union tissue may not contain more than a few isolated Schwann cells, in others some strands of such cells may have moved clear across and reached the opposite stump. In practically all cases, however, the invasion from the cut surfaces has started on a broad front. The outgrowing sheath cells (fig. 6*B*) move definitely as single, slender, spindle-shaped cells with far drawn out ends,[2] staining heavily with silver.[4] Several sheath cells may follow

4. Holmes, W., and Young, J. Z.: Nerve Regeneration After Immediate and Delayed Suture, J. Anat. **77**:63-96, 1942.

each other in tandem arrangement, but they do not, at this stage, offer the appearance of those smoothly contoured Schwann bands seen during later phases. The sheath cells can be seen to move without exception along the heavy fibrin fibers of the union tissue. While there are still some transverse fibrin connections, sheath cells may follow them to cross from one strand to another, as seen in figure 6*B*. However, such deviations are temporary and cease with the gradual breakdown of the fibrin cross links. Sheath cell migration proceeds from both nerve stumps. The presence of a noncellular "no man's land" between the stumps in our cases has permitted us to establish this point with certainty.

Along with the sheath cells, macrophages appear in the region near the wound. Since these macrophages have not previously been present in the interior of the clot in such numbers, it is evident that most of them have come from the nerve. They may be mobilized cells of the endoneurial tissue. Theoretically, there is a possibility that some of them may be transformed sheath cells. One of us (P. W.) has recently observed the transformation of sheath cells into cells of the macrophage type in tissue cultures of embryonic chick ganglions. However, whether this can also occur in the body remains to be demonstrated, and nothing we have thus far seen suggests this possibility. Macrophages seen after nerve section are usually described as engaged in a one way traffic into the nerve fibers. Yet our present observations prove that numbers of them move in the opposite direction, i. e., from the nerve into the union tissue. Their relatively late appearance on the scene disqualifies them as the primary agents of proteolysis. Liquefaction is well under way before they arrive in larger numbers. On the other hand, once present, they participate in the proteolytic activity and dissolve the remaining fibrin threads in their way. Again, as in the preceding phase, the longitudinally oriented fibrin fibers resist this dissolution.

FOUR DAYS

The fourth day brings merely an elaboration of the phenomena observed on the third day, with both the straightening of the union tissue and the immigration of cells into it progressing rapidly. Moreover, formation of new blood vessels and the outgrowth of regenerating axons are becoming more conspicuous. Well formed blood vessels can be seen to traverse the scar as early as on the third day, but more frequently on the fourth day. Significantly, these vessels arise as direct continuations of the vessels of the proximal and distal stumps and course in the longitudinal direction without much branching. This contrasts with the vascularization of an ordinary suture scar, into which the blood vessels penetrate from the epineurial and extraneural spaces (fig. 11*A*). Apparently the regenerating blood vessels, like the other components of the regenerating nerve, are guided by the fibrin architecture of the union tissue. A well oriented union tissue thus insures straight vascular reconnection between proximal and peripheral stumps.

Most of the axons of the peripheral stump have, in the meantime, disintegrated. In the proximal stump, the initial retrograde changes near the wound have been superseded by axon regeneration. Many of the new sprouts have arrived at the old level of the cross section and have proceeded into the union tissue. In doing so, they follow the same longitudinal guide lines as the sheath cells. The relative simplicity and clarity of the conditions in the clot make these cases favorable objects for the study of the relation between regenerating sprouts and sheath cells. As a general result of many observations on such preparations, we have become convinced that the vast majority of newly sprouting axons is closely associated with sheath cells. Contact need not exist over the full length of the axon, at least not during the earlier

phases. Some stretches may be bare, much as they are in early development.[5] This fact makes it difficult to decide whether there are any sprouts that travel in complete independence of sheath cells. That they can do so for a certain distance is evident in our preparations. It is equally clear, however, that such bareness is the exception rather than the rule.[6]

Some branching of the outgrowing sprouts does occur at nodal points of the fibrin network. However, such branching remains on a minor scale. Also most of the side branches are abortive, particularly those that do not run longitudinally. Presumably as the cross links among the longitudinal fibrin threads are progressively resorbed, nerve fibers which happen to have started to grow out along them are being deprived of their support and are then likewise reduced. This process soon leaves only those nerve fibers in the field which have grown out straight and essentially unbranched.

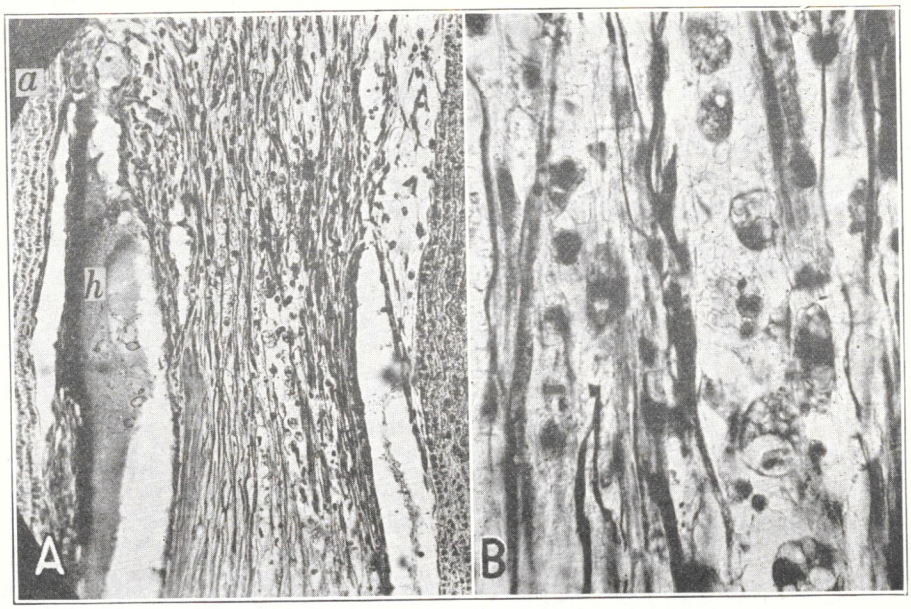

Fig. 7.—*A,* restored union between proximal and distal stump on the fifth day; *a,* sleeve wall; *h,* fresh hemorrhage (× 120). *B,* strands of sheath cells, axons and macrophages in the union tissue of the same case as shown in figure 7 *A* (× 630).

FIVE DAYS

By the fifth day, the orderliness of the reconnection between the proximal and the distal stumps has become fully apparent. By now, most of the union tissue has been pervaded by sheath cells and regenerating nerve sprouts, and the former clot has become fully converted into a new integral segment of nerve. As one can see from figure 7 *A,* the new nerve segment has cleanly retracted from the arterial wall, the space in between being taken up by a liquid residue of the pre-

5. Speidel, C. C.: (*a*) Studies of Living Nerves: I. The Movements of Individual Sheath Cells and Nerve Sprouts Correlated with the Process of Myelin-Sheath Formation in Amphibian Larvae, J. Exper. Zool. **61**:279-331, 1932; (*b*) II. Activities of Ameboid Growth Cones, Sheath Cells, and Myelin Segments, as Revealed by Prolonged Observation of Individual Nerve Fibers in Frog Tadpoles, Am. J. Anat. **52**:1-79, 1933.

6. Nageotte, J.: L'organisation de la matière dans ses rapports avec la vie, Paris, Félix Alcan, 1922. Kirk and Lewis.[2] Holmes and Young.[4]

ceding liquefaction. The streams of sheath cells, which have emigrated from the two nerve stumps, have met and merged into a continuous cell bridge. Restoration of their continuity obviously terminates their further migration and proliferation, for these sheath cell streams never show any tendency to continue to grow into local gliomas. Macrophages are now present in large numbers and by their liquefying power keep the channels between the outgrowing cell strands clear (fig. 7 *B*). Some scraps of the old fibrin network are still present in spots but are apparently being removed by the macrophages. At the same time, collagen begins to appear along the surface of the sheath cells, and the formation of neurilemmal sheaths and true endoneural connective tissue is thus initiated. Axons have continued to grow along the longitudinal pathways without further attempts at branching, and many of the most advanced sprouts have already penetrated into the distal stump, even in cases in which the original gap between the nerve ends measured as much as 5 mm. The original network of the clot has by now become completely resolved into a system of independent longitudinal strands.

SEVEN DAYS

By the end of the first week, the healing process is essentially accomplished. At this time, the former scar region can no longer be distinguished as such, and most traces of the former cut surfaces have become abolished, with the exception of the old margin of the perineurium, which has remained sharply outlined.[1] The nerve fibers extend now in strictly oriented straight and parallel strands from the proximal into the peripheral stump (fig. 8). They are still separated by larger liquid spaces than would be found in the normal nerve. These spaces, however, do not represent edema of the kind described in an earlier paper [7] but are residues of the local liquefaction which has been maintained throughout the healing process. Just when movement of endoneurial fluid along these spaces will be resumed, enabling such fluid to pass freely from the proximal to the distal stump, cannot be determined without special experiments. It is probable, however, that the endoneurial humors take over where the local liquefying activities of the healing process leave off in providing for fluidity of the nerve spaces.

LATER STAGES

A comparison of a splice after two weeks with that after one week reveals no new developments except an increase in the number of regenerated fibers. The new fibers tend to grow out along the surfaces of the more advanced ones, and thus small bundles are built up by a principle which has been described in an earlier paper [8] as "fasciculation." From then on, further improvement in the condition of the newly formed region will consist essentially of increase in the diameter of the nerve fibers, deposition of myelin and collagenization of the endoneurial tissue. Secondary resorption of excess collaterals, assumed to follow excessive branching and abortive sprouting in ordinary suture lines, is not encountered in our preparations, as branching has remained negligible.

As for the nerve sheath, the arterial sleeve may permanently serve as perineurium.[1] In many cases, however, enough epineurial tissue had been tucked into the sleeve during the operation to introduce a small source of epineurial proliferation into the lumen. However, since its growth is forced into the same longitudinal orientation that characterizes the rest of the union tissue, it merely forms a thin

7. Weiss, P.: Endoneurial Edema in Constricted Nerve, Anat. Rec. **86**:491-522, 1943.
8. Weiss, P.: Nerve Patterns: The Mechanics of Nerve Growth, Growth **5**:163-203, 1941.

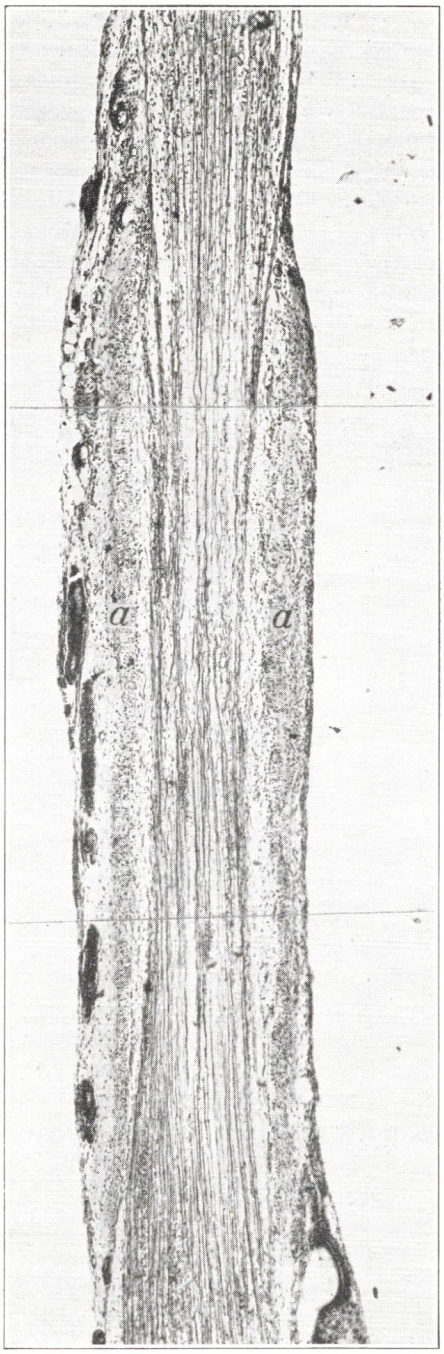

Fig. 8.—Completed union on the eighth day; *a*, sleeve (× 48).

cylindric layer separating the nerve proper from the inner arterial wall. In no case have we seen an infiltration of this tissue into the interior of the nerve.

THE UNION OF ANEURITIC NERVE STUMPS

In the case of nerve grafts, the connection between the proximal nerve stump, as source of innervation, and the graft is effected in the manner described in the preceding pages. The union of the peripheral end of a graft with the distal nerve stump, however, is somewhat different, in that it involves the union of degenerated "aneuritic" nerve fibers, none of which contain axons. Since difficulties encountered by nerve fibers when they later reach the distal end of the graft have often been blamed for the lack of success of grafting in general,[9] we made a special investigation of the healing process at the "distal suture line." As was to be expected, the events there were in all essential points identical with those observed at the proximal suture line; i. e., the arterial sleeve had the same organizing effect on the union tissue and, in further consequence, on the reconnection of the two severed ends. There were some minor differences, however, attributable to the different constitution and consistency of the aneuritic fibers in this region.

As was outlined on page 420, the nerves of this series were allowed two weeks of predegeneration prior to the splicing operation. They were therefore in a different condition from nerves in primary suture. The nerve fibers had become converted into true Buengner cords; axons and myelin had disappeared, and sheath cell hypertrophy and hyperplasia had already led to the formation of relatively solid bands of protoplasm inside the neurilemmal tubes, in accordance with the classic descriptions by Ramon y Cajal, Boeke, Nageotte and others. In brief, the irritative and regressive changes following primary nerve section had subsided, and the cells were already in proliferation. Consequently, it was not surprising to find that the reconnection between such aneuritic nerve stumps after sleeve splicing occurred even more promptly than that between stumps after primary transection. While the general organization of the union tissue two days after the operation is much the same as previously described for that stage, sheath cell migration is already as extensive as it would ordinarily be about the fifth day. This difference is readily explained by the fact that after primary nerve section the sheath cells are not immediately in a condition to migrate and will not acquire this faculty until after the breakdown of the neurite, while in the predegenerated nerve they had already passed through this preparatory phase.[10]

Sheath cell growth occurs again along a foundation of oriented fibrin, which arises in the same manner as in primary sutures. Figure 9 shows the borderline between one nerve stump and the union tissue on the third day after splicing. It clearly illustrates how the fibrin network is connected with the old collagen system of the nerve. This favorable picture has been obtained because sheath cell emigration in this case had, for some unknown cause, been delayed. One recognizes the anchoring of the fibrin network all along the old surface of the nerve, the longitudinal fiber strands becoming heavy and straightened, while the transverse connections are being dissolved. A moderate number of leukocytes and a few macrophages can also be seen. The membrane-like border between the old nerve

9. Davis, L., and Cleveland, D. A.: Experimental Studies in Nerve Transplants, Ann. Surg. **99**:271, 1934.

10. Ingebrigtsen, R.: A Contribution to the Biology of Peripheral Nerves in Transplantation: II. Life of Peripheral Nerves of Mammals in Plasma, J. Exper. Med. **23**:251-264, 1916. Abercrombie, M., and Johnson, M. L.: The Outwandering of Cells in Tissue Cultures of Nerves Undergoing Wallerian Degeneration, J. Exper. Biol. **19**:266-283, 1942.

fibers and the fibrin lattice consists of collagen. It can be attributed to the fact that the cross section of aneuritic nerves contains a solid mass of cells and intercellular matrix, while the cross section of freshly transected nerves is riddled with the lumens of nerve fibers in initial degeneration. It is unlikely that this fine collagenous lamella could present a permanent barrier to nerve regeneration, since macrophages coming up against it from the old nerve tubes seem to puncture it promptly.

At the end of seven days, the continuity between the spliced nerve stumps is fully restored by cellular strands in straight parallel alinement. As seen in figure 10 *A*, the nerve again assumes the center of the sleeve lumen, while the space between nerve and wall is filled with residual liquid from the liquefaction of the

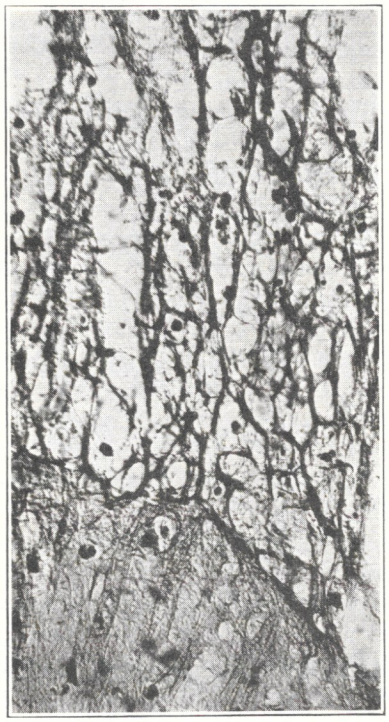

Fig. 9.—Transition from an aneuritic nerve stump (below) to the union tissue (above) on the third day (× 240).

clot. Blood vessels again pass straight from one stump to the other (fig. 10 *B*), following the general orientation of the union tissue. Figure 10 *B* gives a detailed view at higher magnification of the union tissue of the nerve shown in figure 10 *A*. One recognizes the long slender parallel cylindric bands of sheath cells, and a longitudinal blood vessel (*b*).

Preparations of this kind are particularly favorable for the study of the problem of collagen formation in nerves. This problem has aroused some interest in connection with the peripheral nerve tumors. Without entering into the neuropathologic aspect, we can state that our preparations make it absolutely clear that collagen is formed along the surface of the sheath cell. The longitudinal bands of sheath cells, being enclosed in a tubular neurilemmal sheath, are so characteristic in appearance that it would be impossible to confound them with the scattered elements of

the endoneurial connective tissue. In their outgrowth, the Schwann cell cords retain their characteristic tandem arrangement. While in an ordinary nerve scar these strands are intermingled with cellular elements of the scar region, the condition of the arterial splice lets them push forth into a cell-free medium, almost like tissue culture. We see these bands then glide along the fibrin strands into the former gap, often unaccompanied and undisturbed by any other types of cell. They represent, in a sense, pure cultures of sheath cells, and it is along their surface that the Mallory triple azan stain reveals the first identifiable deposit of collagen in distinct blue. Only later, finer collagen fibers appear in the spaces between individual cell bands, and even this may occur some time before endoneurial fibroblasts

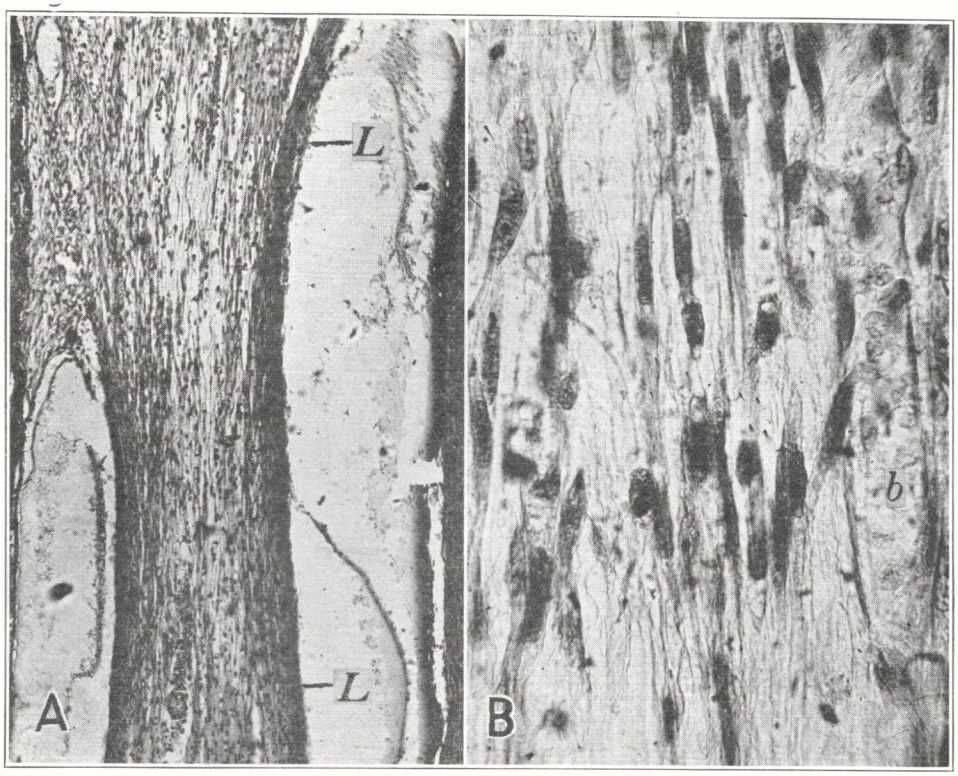

Fig. 10.—*A,* completed union between two aneuritic stumps on the seventh day. *L,* levels of the old cut surfaces (× 76). *B,* detail of the union illustrated in figure 10 *A; b,* blood vessel (× 630).

have moved in. No similar collagen formations were ever observed around other sorts of cells in the early union tissue. These observations, therefore, fully confirm the view of Nageotte [11] and Masson [12] that collagen, and particularly the collagenous sheath of the individual nerve fiber, is a joint product of the sheath cell and its surrounding matrix, with the surface of the sheath cell as the seat of the primary action. The question of collagen formation in nerve is of considerable importance in connection with the practical problem of nerve fibrosis. It seems that serious

11. Nageotte, J.: Substance collagène et névroglie dans la cicatrisation des nerfs, Compt. rend. Soc. de biol. **79**:322-327, 1916.

12. Masson, P.: Experimental and Spontaneous Schwannomas (Peripheral Gliomas), Am. J. Path. **8**:367-416, 1932. Murray, M. R., and Stout, A. P.: Schwann Cell Versus Fibroblast as the Origin of the Specific Nerve Sheath Tumor, ibid. **16**:41-60, 1940.

attention should be given to the sheath cells in this respect, for they represent a potential intrinsic source of fibrosis.

NERVE REUNION WITHOUT SPLICING

As a background with which to contrast the effects of arterial splicing, we have made a number of control experiments in which a nerve was merely transected and the two ends then left in close apposition without further intervention. If the apposition was good, mechanical reunion between the ends took place within a surprisingly short time. After five days, the two ends were found to be firmly cemented by intervening scar tissue (fig. 11 *A*). However, the constitution of the union tissue was fundamentally different from that observed in sleeve-spliced nerves. The main points of distinction are the following: The liquefaction within the clot has failed to occur. Instead, dense fibrous connective tissue from the surfaces

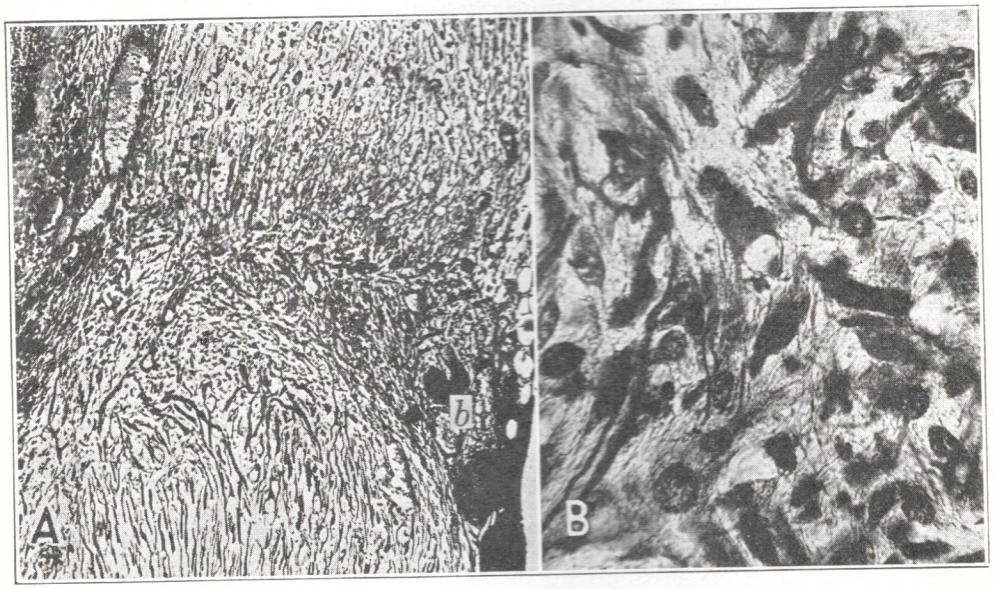

Fig. 11.—*A*, union between unspliced tibial nerve stumps on the fifth day. Note condensation and disorientation of scar tissue; *b*, epineurial blood vessels penetrating into the scar. The confused scar of this figure is to be compared with the well oriented union shown in figure 7 *A*, illustrating the sleeve-spliced peroneal nerve of the same animal; both nerves had been transected at the same level (× 63). *B*, enlarged view of the scar of figure 11 *A* (× 550). This figure is to be contrasted with figure 7 *B*, taken from the sleeve-spliced fellow nerve of the same animal.

of the nerve has penetrated into the region between the stumps and has given rise to a chaotic tangle of cells and fibers. Heavy lymphocytic infiltration accompanies the radial ingrowth of blood vessels from the vicinity. Adhesions between this scar tissue and its surroundings, resulting from the extraneural origin of these cells, cause the establishment of a tensional pattern which, in the main, converges radially toward the lesion. This is not conducive to the establishment of straight connections between the severed stumps. The outgrowing sheath cells and nerve fibers take correspondingly confused courses (fig. 11 *B*). As has been described by earlier observers, the regenerating axons branch profusely in the scar, with branches of the same fiber becoming distributed over widely separated regions of the scar and, hence, the distal nerve stump.

In short, unattended nerve stumps, even with cut surfaces closely approximated, fail to provide the conditions which we have recognized as indispensable for optimal nerve regeneration and which are actually obtainable by arterial splicing. The main shortcoming lies in the lack of lateral isolation of the union tissue. Indiscriminately adhering to the surrounding tissues as well as to the nerve ends, the initial clot develops a tensional pattern radiating in all directions and, consequently, preparing inroads from all sides for fibrous connective tissue. Moreover, as there are no provisions to prevent the dissipation of the proteolyzing factors from the union tissue, the latter cannot maintain that looseness of texture which ostensibly is a prerequisite of adequate nerve regeneration. As a result, instead of weaving an integral link between the nerve stumps, the union tissue becomes a foreign block in the continuity of the nerve. The fact that even so, owing to the luxuriant axon branching in such scars, numerous nerve fibers finally get through does not reduce the contrast between such unaided unions and the straight reunion of sleeve-spliced stumps. If we add that stitching the nerve ends with foreign suture threads, particularly in small nerves, only aggravates the disorganization of the tensional pattern at the suture line, the potential merits of sleeve splicing become apparent.

COMMENT

The observations reported in the preceding pages add up to a consistent and coherent picture of the healing process by which nerve stumps in an arterial sleeve are reunited. They likewise reveal the essential mechanisms involved. While individual cases show a certain variability of timing, the sequence of events is the same in all cases. These events duplicate essentially the course of action by which tension affects tissue growth in general and which was originally demonstrated and analyzed in tissue culture.[13] According to those earlier experiments, the mechanism operates in two phases: First, tension forces the colloidal matrix of the tissue to assume orientation along the lines of stress, which in turn gives rise to fibrillar arrangements of corresponding orientation; second, cells of the spindle cell type, when growing into the matrix, advance and proliferate along the oriented fibrillar framework of the matrix; as a result, the orientation of the cells coincides with that of the original tensions.[14] The same mechanism was later shown to underlie the oriented growth of nerve fibers as well.[15] At the same time, further evidence was obtained to show the active participation of the colloidal matrix in creating those tensions to which it owes its eventual organization. A matrix consisting of a fibrin clot, for instance, tends to shrink owing to the progressive segregation of the solid and the liquid phases (syneresis, coacervation). This sets up contractile forces in the solid parts. Tensions thus generated within the clot combine with those imposed from the outside. All this has been known from the tissue culture experiments. But we readily recognize now that exactly the same mechanism operates in the reunion of sleeve-spliced nerve stumps. It is supplemented, however, by some additional features which had not been previously recognized, as, for instance, the differential liquefaction of the clot. The primary role of the artery is to give this tensional mechanism free play. How it performs this role is briefly discussed in the following paragraphs.

13. Weiss, P.: Erzwingung elementarer Strukturverschiedenheiten am in vitro wachsenden Gewebe: Die Wirkung mechanischer Spannung auf Richtung und Intensität des Gewebewachstums und ihre Analyse, Arch. f. Entwcklngsmechn. d. Organ. **116**:438-554, 1929.

14. Weiss, P.: Functional Adaptation and the Role of Ground Substances in Development, Am. Naturalist **67**:322-340, 1933.

15. Weiss, P.: In Vitro Experiments on the Factors Determining the Course of the Outgrowing Nerve Fiber, J. Exper. Zool. **68**:393-448, 1934.

GAP FILLING

Nerve section invariably produces a gap between the stumps. Even what macroscopically appears as closest apposition still is a gap when viewed from the microscopic dimensions in which the cells that are to repair the defect operate. Gaps in tissue can be closed and repaired only by migration and growth of cells. This migration and growth can proceed only if certain physical and chemical requirements are satisfied, and foremost among these requirements is the presence of a proper physical substratum along which the cells may grow. Neither tissue cells nor nerve fibers can push forth into a homogeneous space, either gaseous or liquid; they extend only along phase boundaries (interfaces). The adequacy of a growth medium for cells and nerve fibers is, therefore, in part determined by its capacity to provide interfaces of the proper constitution, orientation, dimensions and numbers. The medium present between two severed nerve stumps is therefore of prime importance in that it must lay the foundation for the subsequent growth of a cell bridge.

Our experiments indicate that whole blood serves this function adequately. The fibrin of the blood clot furnishes the fabric subsequently to be molded by tensions into a system of tracks for cell and nerve fiber growth while the spacious meshes temporarily occupied by erythrocytes constitute spare room to be taken up by the invading cells. One must never lose sight of the fact that cells, like any physical bodies, can move into a circumscribed area only if they can displace a corresponding volume of substance. Liquids are easy to displace. However, if the clot consisted predominantly of compact matter, such as, for instance, fibrin, the cells would have much harder going and could penetrate only in limited numbers. It is for this reason that any filling above a certain density, even though having good cementing power and proper orientation, will be detrimental to nerve regeneration. This seems to be particularly true of pure or fortified blood plasma. Lacking the red cells, which after disintegration will contribute to the liquid phase, such a clot contains an unduly high ratio of solid fibrin to liquid constituents and therefore presents a handicap to growth. We have made a number of comparative studies, using blood plasma as filling in sleeves, and have found this medium almost invariably too dense for good nerve growth. It produces fibrotic islands leading to small local neuromas of arrested and choked nerve fibers. While the blocking effects of pure blood plasma may be negligible in the case of close apposition of the nerve ends, e. g., after "plasma suture,"[16] they would become definitely objectionable in the presence of a gap of even a few millimeters. We are still investigating the optimal composition of the gap filling, and if a matrix superior to whole blood should be found, the results will be reported at a later date.

Our observations have brought no confirmation of the earlier contention[17] that endoneurial fluid may be an important factor in determining the properties of the union tissue during the early stages of healing. While such fluid is probably instrumental in keeping the nerve channels clear after the first healing period is over, it seems to play no part in the establishment of the channels. Some contribution of the nerve ends to the gap filling is essential, however, in form of clotting factors. Whole blood enclosed in an arterial sleeve would remain liquid, owing to the anticoagulant properties of the wall of the sleeve. However, with nerve ends inserted, it clots.

16. Young, J. Z., and Medawar, P. B.: Fibrin Suture of Peripheral Nerves, Lancet **2**: 126-128, 1940. Tarlov, I. M., and Benjamin, B.: Autologous Plasma Clot Suture of Nerves, Science **95**:258, 1942.

17. Weiss (footnotes 1 and 7).

TENSIONAL ORGANIZATION OF THE UNION TISSUE

The events of the second and third days are essentially concerned with transforming the original blood clot into a system of longitudinally oriented pathways for subsequent cell and fiber growth. This could be ascribed to some unknown "orienting factors" emanating from the two nerve ends, but both past experience and the evidence of the present experiments identify the operating factor as tension. These tensions arise partly outside, partly inside of the clot. Extraneous tension is due to whatever pull the nerve ends exert. There is a definite tendency of freshly cut nerves to retract beyond the initial distance during the days following transection. This gradual retraction works in the right direction, in that it places the union tissue under longitudinal strain. It is here that the elastic properties of the sleeve come into play. If the nerve ends are rigidly connected by sutures spanning the gap, all tensile stresses in the direction of the nerve will be taken up by the rigid suture threads, and the parts lying in between will sustain no stretch whatsoever. Only an elastic or plastic link permits the transmission of tensions to the substance filling the gap, where they may then exert their molding effects. As mentioned before, another effective source of tensions lies, however, in the shrinkage of the clot itself, and this type can of course operate even within rigid tubes or between nerve ends otherwise rigidly united, provided that the right amount of contractile material is present between the ends.

The configuration of the tensional pattern depends on how and where the clot is attached to its surroundings. No elastic tensions can develop in directions in which the material is free to contract. The points of attachment, therefore, determine the resultant stresses. An ideal pattern of longitudinally oriented stresses will arise in a clot attached at both ends to the nerve stumps but unattached over the rest of its circumference. The arterial sleeve is instrumental in bringing about this optimal tension pattern (fig. 12 *A*). It does so by preventing the clot from adhering along its sides. From its very beginning, the clot fails to bind intimately with the arterial wall. During the second and third days, this lack of adhesion is then further accentuated by active detachment from the sleeve. This is effected by migrating cells of the leukocyte type which spread rapidly along the wall of the artery and liquefy the adjoining surface layer of the clot (fig. 4). How many of these cells have emerged from the interior of the clot and how many have come from the nerve stumps, it has not been possible to determine. Some of them may be remnants of the arterial endothelium. However, most of the original lining is presumably scraped off during the splicing operation. Moreover, the lateral detachment has also been observed in sleeves made from frozen-dried arteries, in which the original endothelium had certainly perished. Whatever their origin, these cells produce a liquid layer which effectively separates the clot from contact with the sleeve. They might achieve this either by secreting a fluid or by secreting proteolytic enzymes which will digest the fibrin and thus liberate serum previously entrapped in the meshes of the coagulum. The presence of distinct erosion cavities around each cell (fig. 4) suggests proteolytic action.

Any residual local attachment between clot and arterial wall introduces distortion of the longitudinal tension pattern and entails a corresponding diversion of cell and fiber growth. The extreme of this condition is observed when severed nerve ends are left unsheathed (fig. 11 *A*). In these circumstances the clot is attached along its entire surface, which, moreover, owing to its continuity with the tissue spaces, is extremely irregular. Consequently, the pattern of tensions resulting from the gradual contraction of the clot is likewise irregular. Granting that blood seeps more readily into the extraneural spaces than into the rather tightly packed

nerve, it is obvious that the predominant orientation of the resulting fibrin framework will be in the direction of the tissue spaces, i. e., transversal with regard to the nerve. Radiating fibrous adhesions with ample pathways for the ingrowth of fibrous tissue and capillaries into the gap and outgrowth of nerve fibers and sheath cells from the gap would be the result. These hazards can be avoided only by preventing the clot from forming lasting lateral adhesions. It is in their effect on this early phase of the healing process that the various methods recommended in the past for wrapping or tubulating the suture line will have to be assessed. Their varying effectiveness in this regard may explain the great variability of results obtained by different authors. The arterial sleeve still appears to be the most natural preventive.

It is understandable now why even small breaks or pores in the arterial wall cause disturbances of nerve regeneration out of proportion to their actual size.

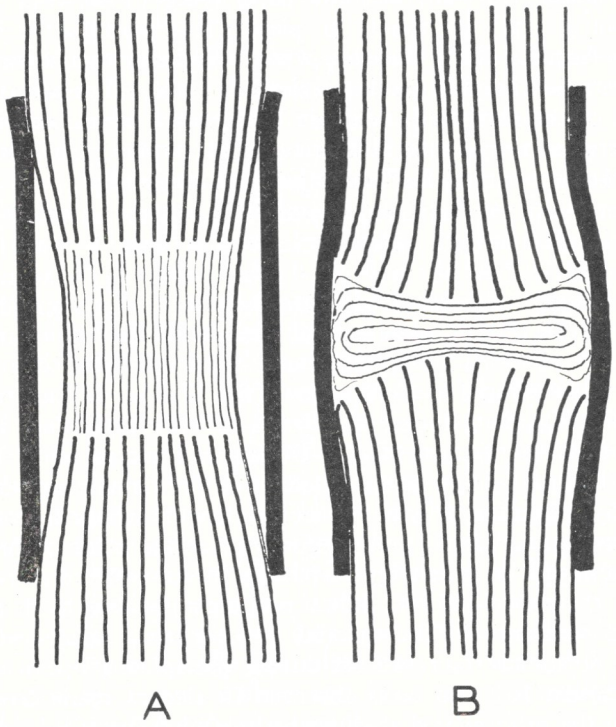

Fig. 12.—Diagram to illustrate the lines of stress in clots subjected to tension (*A*) or pressure (*B*) in the direction of the nerve.

They become centers of adhesion which deflect the whole tensional pattern in their direction (see fig. 5 of the earlier paper [1] on the subject). Similar difficulties have thus far militated against the use of heteroplastic arteries, both fresh and preserved. Large parts of the wall of a heteroplastic sleeve succumb to heavy lymphocytic attack in the foreign host body, and each focus of destruction becomes a potential source of adhesion and minor neuroma. If the host reaction could be delayed until there has been time enough for a well oriented union tissue to form, the sleeve would have served its main function, and the use of heteroplastic arteries might yet become feasible. On the other hand, there is no reason why other elastic tubes of nonirritant materials could not be substituted for artery, provided they

share the faculty of inducing fibrinolysis along their walls. Tubes fashioned from actively secreting epithelium or endothelium should serve this function adequately. We have found frozen-dried and rehydrated arteries fairly satisfactory, although occasional adhesions between union tissue and wall have been observed.

Aside from lateral adhesions, a major source of deflection of the tensional pattern is pressure of the two nerve ends against each other. Attempts at producing "close apposition" will have this effect. The nerve ends will become flanged and any blood clot forming in between, if it has no lateral outlet, will become subjected to compression in the direction of the nerve. The resulting tension pattern is shown in figure 12 *B*, and an actual example in a three day old splice is presented in figure 13. As subsequent growth of sheath cells and nerve fibers would retrace the fibrin pattern, the detrimental effects of this kind of union on nerve regeneration can easily be predicted from the pictures. By analogy we may assume that the presence between the nerve ends of air bubbles or liquid with excessive turgor will have similar effects.

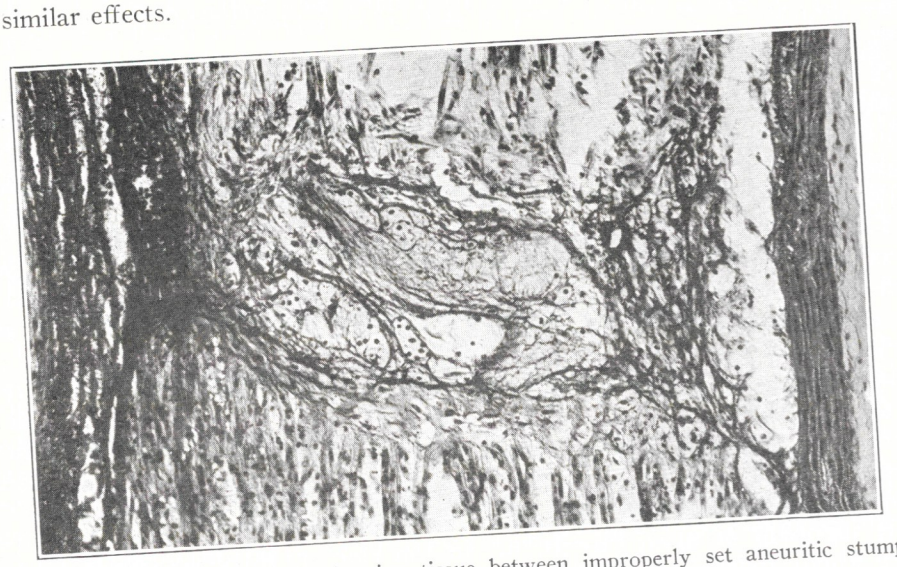

Fig. 13.—Transversally oriented union tissue between improperly set aneuritic stumps on the third day (× 120).

Once the correct tensional pattern has been established, the further course of events takes place almost automatically. The stress pattern first produces a corresponding orientation of the fibrin. The fact that oriented tension causes fibrin orientation in blood plasma clots has been a matter of record.[18] It is evident from our preparations that the fibrin fibers not only assume preferential lengthwise orientation but become heavier when oriented along the lines of stress. This increase may be ascribed to the greater ease with which smaller fibrillar units can aggregate into larger bundles when they are parallel. Eventually the lines of stress become thus embodied in a system of material lines of fibrin destined to form pathways for cells and fibers. This constructive process is soon joined by a destructive process in which the formation of oriented structures is further accentuated by the simultaneous destruction of unoriented ones.

18. Baitsell, G. A.: A Study of the Clotting of the Plasma of Frog's Blood and the Transformation of the Clot into a Fibrous Tissue, Am. J. Physiol. **44**:109-131, 1917. Nageotte.[6] Weiss.[13]

LIQUEFACTION

The dissolution of the interior of the clot begins about the second day (fig. 5 *A*) and from then on proceeds fairly rapidly. Biopsies after the second day usually show the sleeve filled with a pinkish fluid of low viscosity. This liquid menstruum is still rich in coagulable colloids, which when treated with the ordinary histologic fixatives produce a fine granulation. They give a strong Millon reaction for protein. Inasmuch as this liquefaction spares the heavier fibrin threads, which are oriented longitudinally, it does not lead to complete destruction of the clot but merely loosens its texture. In establishing longitudinal liquid channels, it prepares the ground for the later invasion by cells and fibers and, at the same time, counteracts any tendencies of the clot to become too compact and dense. In view of this fact, this phase of proteolysis seems almost an indispensable prerequisite for good nerve regeneration. The source of the liquefying agents becomes, therefore, a problem of considerable interest. While the problem has as yet been given only casual attention, the following facts have been gathered from our various observations.

Erythrocytes begin to disintegrate soon after the operation, and most of them have been destroyed by the second day. It is doubtful, however, that they release in their breakdown fibrinolytic enzymes, since it is unlikely that such enzymatic action would have remained undiscovered. A more familiar source of proteolysis is present in the blood clot in the form of granular leukocytes.[19] Studies on inflammation, as well as tissue culture observations, indicate that such white cells begin to disintegrate after about twenty-four hours, and, inasmuch as they are known to contain proteolytic enzymes, the liberation of such agents en masse at the end of the first day would readily explain the observed onset of liquefaction just about that time. A third possibility, namely, the exudation from the nerve itself of fluid rich in proteolytic enzymes,[7] cannot be wholly excluded, but our preparations reveal no fact that would encourage this view. If it were correct, liquefaction of the clot should proceed from the nerve ends toward the middle; yet no such systematic progress has been observed. On the contrary, centers of liquefaction are usually scattered more or less evenly throughout the clot (figs. 5 *B*, 6 *A* and 9). The fibrinolysis of the clot has no relation to blood vessels. It may appear prior to the ingrowth of blood vessels, or, in the case of early revascularization, it occurs wholly independently of the locations of the young vessels.

Proteolysis attacks first the fine tracery of fibrin outlining the spaces formerly occupied by the erythrocytes. Thus the meshes of the fibrin reticulum become larger and larger. It is only then that macrophages appear on the scene in larger numbers. A few scattered macrophages have been observed from the very first stages (figs. 5 *A* and 9). Wherever present, they liquefy the immediate surroundings. However, they are too scarce at first to be considered as the main agents of the primary liquefaction. After the third day, however, they become much more abundant, particularly in those parts of the clot adjoining the cut surfaces of the nerve (fig. 7 *B*), and from then on they seem to take a definite part in further fibrinolysis. Their accumulation around the nerve ends leaves no doubt that they have emigrated from the nerve stumps, for there is no other source that could have provided them. As they push toward the middle of the clot from both ends, they dissolve whatever fine interconnections between the longitudinal fibrin strands have survived the preceding stage of liquefaction. The

19. MacCallum, W. G.: A Text-Book of Pathology, ed. 6, Philadelphia, W. B. Saunders Company, 1936.

appearance of the macrophages, therefore, marks a secondary phase of intensified proteolysis. It is possible that this is followed by a tertiary phase during which the longitudinal channels are held open by endoneurial fluid seeping in the proximo-distal direction. This point, however, requires further examination. It is noteworthy that when a long tube of artery is attached to the end of a proximal nerve stump, with the free end ligated, it is found to be filled with fluid on the second day. When drained, the fluid is reformed. It has been observed as late as nineteen days after the operation.

The most remarkable feature of the liquefaction processes is that they spare that part of the fibrin network which has been oriented longitudinally under the influence of tension. Evidently this longitudinal fiber system is somehow protected against the proteolytic erosion. How this protection is achieved is still unknown. Two possibilities come to mind. The protection may be only apparent, in that the persistence of these fibers might merely be a result of their much larger size. In this case, all fibers would be attacked by the fibrinolytic enzymes indiscriminately, but the heavier ones would last longer, and the period of about one week, during which they serve as climbing ropes for the pioneering cells and fibers, would be insufficient for their complete dissolution. The other possibility is that the tensional orientation may have forced the surface molecules of the longitudinal fibrils into a more orderly and closer packing, which would provide fewer loopholes for the entry of proteolytic enzymes into the bodies of the fibers.

By the combined effects of tension and fibrinolysis, the framework of the nerve stumps has been rewoven throughout the former gap. The connecting fibrin threads now serve as guides for the outgrowing sheath cells and axons.

CELL INVASION

Ever since the classic experiments of Harrison [20] in tissue culture, the tendencies of cells to glide along fibrous structures have been known. The precise mechanism by which cells, and nerve fibers as well, are made to glide along surfaces is not known, but present evidence indicates that interfacial tensions are operative.[8] Our observations merely reaffirm earlier experiences. With the utmost clarity, the pioneering sheath cells can be seen to move from the nerve stumps into the union tissue along the fibrin fibers which they encounter at the very exits of the old nerve tubes. As we have previously said, these fibrin fibers are firmly anchored at the orifices of the neurilemmal tubes, and the emigrating sheath cells can therefore glide on into the scar along uninterrupted rails. Sheath cells emerging from the opposite nerve end behave similarly. As a result, the union tissue becomes populated with longitudinal, parallel, unbranched strands of sheath cells, with macrophages and occasional endoneurial cells interspersed (figs. 6*B*, 7*B* and 10*B*). Lack of branching of these cell strands may be ascribed to the same general principle established for nerve fibers, namely, that branching does not occur unless there is an incentive for it. Straight and unobstructed pathways offer no such incentive, and thus the sheath cells pass on straight from one stump to the other.

Evidently, sheath cells moving out from either end cease to migrate and proliferate as soon as they have made contact with similar cells coming from the opposite end. Whether it takes protoplasmic fusion or whether mere surface contact is sufficient to stop further growth seems irrelevant in the present connection. The main fact remains that the presence of straight guide lines between the two stumps insures that practically all sheath cells will be led head on against other sheath

20. Harrison, R. G.: The Reaction of Embryonic Cells to Solid Structures, J. Exper. Zool. **17**:521, 1914.

cells, thereby terminating growth in that particular line. In the end there will be just straight, simple bands of sheath cells connecting the two stumps (fig. 10*B*), and no trace of the extensive gliomas so commonly found around nerve stumps.[21] By the same token, the formation of neuromas by the regenerating axons of the proximal stump is averted. The very fact that the mechanism: tension → fibrin orientation → tissue growth, operates equally well in the presence or absence of neurites guarantees that sleeve-splicing between a graft and a peripheral nerve stump will leave no distal suture scar but will establish smooth and straight pathways for the later passage of nerve fibers from the graft into the periphery (fig. 10*A*).

AXON REGENERATION

By the time the axons of the proximal stump have recovered from their ascending traumatic changes and are ready to grow out anew, the main guiding features of the union tissue have already been laid down and the outgrowing fibers find a straight and direct pathway to the peripheral stump all prepared. This pathway is still composed in part of the old fibrin matrix, but in part it consists already of strands of sheath cells. As was mentioned previously, the new axons grow preferentially along the sheath cell strands, although free advance has been observed sporadically.

There is no indication that nerve fibers and sheath cells are guided toward each other by specific attractions, and in fact there is much evidence against such a view.[15] On the other hand, it is quite evident that as soon as axon and sheath cell are in contact, they tend to stick together. To quote a remote analogy, there is no force of attraction between a dry thread and a wet thread, but once both have come together, they stick. It seems that this is all that is needed to explain the association between nerve fiber and sheath cell if we keep in mind that the regenerating axons have never actually lost contact with sheath cells, as both are enclosed in a common neurilemmal tube. Therefore, if a regenerating sprout were to proceed into the scar independently, it would first have to detach itself from the sheath cell processes to which it adheres. As this detachment is apparently difficult to achieve, most axons stay in contact with sheath cells. It is possible, moreover, that specific mutual contact affinities [8] between sheath cell and axon make this adhesion firmer than would be that between an axon and another type of cell.

The regenerating axons seem to glide with great ease along the surface of the sheath cell strands, for club-shaped enlargements of the axon tips, indicating damming of axonal substance before an obstruction, are very rarely seen in the union tissue of a successful sleeve-splice. The frequency of terminal axon swellings is in direct proportion to the obstructiveness of the scar, and their lack indicates unimpeded outgrowth. Pioneering fibers have been found advanced as far as 8 mm. from the level of regeneration on the eighth day. Allowing four days for the early reconstruction phase, this would indicate a growth rate of 2 mm. per day, including growth through the "scar."

Branching, which is as much a function of obstacles across the path of growth as is the formation of terminal bulbs, has likewise remained at a minimum. Whatever little there is occurs during the earliest phase of outgrowth, when the longitudinal fibrin strands still carry side connections. Axons sometimes bifurcate at the crotches, with one branch following the main stem, while the other turns off along

21. Dustin, A. P.: Les lésions posttraumatiques des nerfs: Contribution à l'histopathologie du système nerveux périphérique chez l'homme, Ambulance de "l'Océan" **1**:71-161, 1917. Nageotte.[6]

the side branch. The fact, however, that little evidence of such axon branching is seen after the fifth day, indicates that the progressive resorption of the transverse fibrin threads, robbing nerve twigs attached to them of their support, must also have entailed the resorption of those axon collaterals. Only the straight fiber stem would thus be left to survive, or perhaps the vigor with which the nerve fiber advances in the main longitudinal direction drains growth requirements from the less vigorously growing collaterals and thus causes their atrophy. At any rate, the lack of branching can be definitely attributed to the orderliness of the preneural pathways. In contrast, branching in unspliced nerve unions or after faulty sleeve splicing is very profuse in accordance with the general disorganization of the scar.

The new nerve fiber tips of the proximal stump reach the level of the original cut surface usually several days later in sleeve-spliced stumps than in unspliced ones. This difference is explained by the fact that in the sleeve-spliced stumps the whole stretch of nerve inserted into the artery undergoes some traumatic degeneration. If this distance measures more than a few millimeters, it exceeds the normal range of ascending degeneration. The practical implications of this phenomenon would appear to be advantageous. It is a common experience that the segment lost by ascending degeneration is quickly repaired, inasmuch as the outgrowing fibers move unimpeded each in its own old tube. Consequently, their arrival at the nerve end would lag by no more than several days. On the other hand, their later arrival allows the union tissue more time to become organized and also gives the sheath cells, which move out from the level of section, a head start which they otherwise would not have. In fact, the more extensive ascending degeneration of the proximal stump may lead to the mobilization of a larger number of sheath cells than would normally be available at this end. It seems definitely more desirable to have the axons reach the gap only after the bed for their further growth has been properly prepared than to have them precipitated into the tangle of an early scar, which would leave marks of confusion in the restored nerve.

Our observations fully reaffirm the principle of contact guidance of the growing nerve fiber.[8] This principle states that the course of a nerve fiber is determined by the biophysical and biochemical organization of the surfaces along which the fiber moves, which implies that nerve fibers can move in no other way than by application to interfaces. On the negative side, the principle denies that agents not in immediate contact with the nerve fiber can affect its course, except indirectly by modifying the contact substrata. There is no "attraction" of nerve fibers toward distant sources of chemical emanations, such as are postulated in the theory of neurotropism.[22]

In spite of early opposition,[23] the theory of neurotropism has strongly influenced neurologic and surgical thought regarding nerve regeneration and nerve repair. The claim that degenerating peripheral nerve exerts a potent "attraction" on growing axons has been singularly intriguing. In view of the fact that surgical procedures based on this fallacious view are apt to be equally fallacious, it must be emphasized over and over again that this theory cannot stand up in the light of a critical evaluation of facts. To avoid repetition, we simply refer to previous

22. (*a*) Ramón y Cajal, S.: La rétine des vertébrés, Cellule **9**:119, 1893. (*b*) Forssman, J.: Zur Kenntnis des Neurotropismus, Beitr. z. path. Anat. u. z. allg. Path. **27**:407, 1900. (*c*) Tello, F.: Gegenwärtige Anschauungen über den Neurotropismus, Vortr. u. Aufs. ü. Entwcklngsmech. d. Organ., 1923, no. 33.

23. Dustin, A. P.: Le rôle des tropismes et de l'odogénèse dans la régénération du système nerveux, Arch. de biol. **25**:269, 1910. Ingebrigtsen, R.: Experimentelle Untersuchungen über freie Transplantation peripherer Nerven, Zentralbl. f. Chir. **43**:864, 1916.

presentations of the evidence by Weiss [24] and corroborative reviews by Harrison,[25] Detwiler [26] and Young.[27] The observations of Ramón y Cajal [22a] and Forssman [22b] that continuity is restored between severed nerve stumps even when the latter have been separated and brought out of line, are, of course, quite correct. However, this connection is effected not by "attraction" but by the influence of the stumps and their surroundings on the configuration of the union tissue, plus the fact that the earlier fibers making successful connections constitute a pathway of preferential application for fibers growing out later ("fasciculation").[8]

As a sample of additional evidence contradicting chemotropism in nerve growth, which will be reported more fully elsewhere, we cite the following experiment. A proximal nerve stump was inserted into the stem of a Y-shaped artery, with the fork offering alternative pathways to the regenerating fibers. A peripheral nerve stump was inserted into one of the open ends, while the other end was either left open or tied off or plugged with a piece of tendon. In no case were the regenerating nerve fibers "attracted" toward the peripheral nerve fragment, but the fiber stream divided itself more or less evenly and continued into both channels regardless of the kind of destination awaiting them.

Comparable observations were made in experiments mentioned previously, in which a proximal nerve stump was introduced into the end of a long arterial tube, the other end of which was ligated. This blind tube remained dilated first by blood, and subsequently by a liquid, composed presumably partly of fibrinolysate and partly of nerve exudate. There was a fibrin framework in the interior, much as in gaps between two nerve ends, and sheath cells and nerve fibers had grown in large numbers down the full length of the tube, only to be finally stopped at the blind end. They obviously grew toward no "destination," but simply along an established pathway with no exit. Growth in such arterial tubes is never as strictly oriented as it commonly is in sleeves between two stumps. Nevertheless, the main orientation is longitudinal. This may be attributed to the fact that adhesions of the ligated end of the arterial tube set the latter under longitudinal tension. Encouraged by these experiences, we are at present exploring the possibility of using stretched blood-filled sleeves in the role of grafts to bridge larger nerve gaps. This method would circumvent many of the shortcomings of the "tubulation" practices of the past.

FIBROSIS

Collagen formation in the union tissue sets in immediately after cell invasion. The role of the Schwann cell in this process has already been discussed. The earliest deposition of collagen in the otherwise fibrinous matrix appears along the surface of the Schwann cords. From there the process spreads into the interstices, establishing a new endoneurium. If it went no farther, collagenization would simply restore to the new nerve parts normal histologic features. However, in many instances it goes beyond limits compatible with the requirements of good nerve regeneration and nerve function. The result may be anything from moderate to serious fibrosis. The heavier the fibrosis, the denser the interstitial tissue will be; and the denser the tissue, the fewer nerve fibers will penetrate, the more will

24. Weiss (footnotes 8 and 15).

25. Harrison, R. G.: The Croonian Lecture on the Origin and Development of the Nervous System Studied by the Methods of Experimental Embryology, Proc. Roy. Soc., London, s.B **118**:155-196, 1935.

26. Detwiler, S. R.: Neuroembryology, New York, The Macmillan Company, 1936.

27. Young, J. Z.: The Functional Repair of Nervous Tissue, Physiol. Rev. **22**:318-374, 1942.

be prematurely arrested, the slower will be the advance of the successful ones, the less their ability to recover normal diameter and the greater their danger of becoming strangled and pressure blocked.

The arterial sleeve reduces the sources of fibrosis, but it does not altogether abolish them. It reduces fibrosis, first by shielding the interior from highly collagenous extraneural scar tissue, and second by turning whatever trace of fibroblastic tissue may have penetrated into the sleeve immediately into a longitudinal course parallel to the nerve, where it can do no harm. Naturally, these two protective features are predicated, the former on a hermetic seal between artery and nerve, and the latter on the presence of longitudinal stress. Accordingly, methods aimed at preventing fibrosis by tubulating or otherwise wrapping a nerve suture are apt to remain ineffective to the extent to which they fail to realize those two prerequisites. Yet, even with all extraneous sources of fibrosis excluded, there still remains the problem of intrinsic fibrous transformation, particularly of degenerated nerve. We have planned a special study of this problem and hope to be able to present tangible data at some later date. In the meantime, a few occasional observations deserve to be noted.

In a number of our cases, excess collagen was found in that portion of the distal nerve stump tucked into the arterial sleeve. The indications are that this represents a tissue reaction of the nerve to its compression by the sleeve. Compression is caused by the swelling of the peripheral stump during early degeneration (see figs. 1 and 2, Weiss,[7] 1943), which amounts to tightening of the arterial grip. In response, the nerve produces new collagen. When limited to a short segment, this may be innocuous. However, it seems that some such hypertrophy of collagen may occur throughout the distal stump, possibly as a direct reaction of sheath cells and endoneurium to the increasing turgor of the degenerating fibers. The excellent nerve fiber growth commonly observed in peripheral stumps indicates that hypercollagenization of moderate degree is no bar to successful regeneration. Evidently, later shrinkage of the degenerated fibers, with or without reinnervation, removes the internal pressure, which if it were kept up might easily lead to progressive fibrosis. Extraordinary circumstances, however, may lead to just that, and it is worth investigating whether some instances of intraneural fibrosis may not be blamed on irregularities of the degeneration process, such as, for instance, delay in the resorption of the "ovoids," congestion of the tubes with hyperplastic sheath cells, etc. Continued research into the causes of fibrosis, it is hoped, will produce measures of nerve repair which are not only "afibrotic"—keeping fibrosis out—but also "antifibrotic"—counteracting it where it has occurred. Preserving a certain fluidity of the nerve spaces seems to be a prime factor of success. The role of the endoneurial fluid in maintaining this fluidity has been suggested [7] but remains to be demonstrated.

Nerve grafts seem to be particularly susceptible to fibrosis, but without a detailed analysis it is impossible to give the reason for this change or to formulate possible means of averting it. Fibrosis of the densest sort is what makes alcohol-fixed nerve grafts unfit for use. While some nerve fibers may penetrate,[28] regeneration through the graft and its replacement tissue remains insignificant,[29] even though

28. Huber, G. C.: Operative Treatment of Peripheral Nerves After Severance, More Particularly After Loss of Substance: A Critical Review, J. Lab. & Clin. Mèd. **2**:837-848, 1916-1917. Nageotte.[6]

29. Sanders, F. K., and Young, J. Z.: The Degeneration and Reinnervation of Grafted Nerves, J. Anat. **76**:143-166, 1942.

the orientation of the tissue is of the required kind. We have convinced ourselves of this fact in experiments on the rat.

In conclusion, it can be stated that proper orientation of the matrix is not enough to insure optimal nerve regeneration; the matrix must also be of the right composition, and this includes a correct balance between collagenous and noncollagenous constituents.

CONCLUSIONS

In the light of our analysis, the arterial sleeve appears to fulfil a variety of functions.

1. It unites the nerve ends. However, the initial holding power of this link is not sufficient to withstand more than moderate tension. It could not maintain a forcible approximation of the nerve ends such as may be attempted for the reduction of a sizable nerve gap. In such cases, stay sutures at a safe distance from the nerve ends could be used to take up the main stress, leaving just enough stretch to keep the union taut. The presence of some blood-filled gap between the cut surfaces seems imperative.

2. The sleeve serves as container for the cementing blood clot.

3. By liquefaction along its inner walls, it prevents lateral adhesion of the union tissue. This throws all tensional stresses into a strictly longitudinal direction, thus bringing the fibrin matrix of the union tissue into alinement with the severed nerve fibers and creating a guiding pattern for the transit of cells and axons across the former gap.

4. The sleeve prevents the dissipation of the products of liquefaction, as well as of endoneurial fluid, and thus preserves a degree of fluidity optimal for nerve regeneration.

5. The sleeve prevents the ingrowth of extraneural connective tissue into the gap, as well as the escape of sheath cells and axons from the nerve. This it achieves not so much by grossly walling off the interior from the exterior, as by permitting a tissue matrix to form which will deflect all cell and fiber streams in the longitudinal direction.

6. This action likewise accounts for the lack of branching among the outgrowing axons and Schwann cords and for the suppression of the neuromas and gliomas which would otherwise develop.

7. The sleeve permits direct vascular reconnection between proximal and distal stumps through the gap, while excluding the intrusion of extraneural blood vessels. Whether there is any significance to this point remains to be determined.

These points constitute the crucial advantages of arterial sleeves. The list can serve a double purpose. Firstly, if the use of arteries should prove impracticable in man one could try to resort to composite procedures, i. e., substitute for the single action of the arterial sleeve a combination of measures, for instance, one providing for the sealing, another for the tension pattern, a third for liquefaction, etc. Secondly, the list establishes criteria by which the prospects of tubulation and wrapping procedures in nerve repair may be assessed. Unless such procedures score favorably when tested point for point down the list, they are not likely to measure up to the results hitherto obtained with arterial sleeve splicing. A survey of the literature on tubulation shows that many past procedures falling in that class bear only superficial resemblance to arterial sleeve splicing. None of them fulfils vigorously all requirements of our list. Some are excellent in one respect but fail in others. Practically all of them deprive the union tissue of the benefit of longitudinal tension. Others leave spaces between tube and nerve, perhaps small

in macroscopic terms but huge for cells which operate in microscopic dimensions. Still others introduce foreign substances, such as gelatin or agar-agar or air, into the gap, creating a situation diametrically opposed to what would aid the nerve. The idea that a medium will be favorable for nerve growth just because it is "soft" or "nutrient" or rich in vitamin B_1, etc., is based on a serious misconception of the mechanism of nerve growth. Such notions can usually be traced to the tempting analogy between a nerve sprout and a plant root. Yet nerve fibers decidedly do not grow after the fashion of plant roots. They grow by surface application, and the physical configuration of their matrix is as important as is the chemical composition of the medium.

To replace conjectures about the requisites of nerve regeneration by factual knowledge seems the only safe way toward the creation of a solid and rational basis for the improvement of nerve repair. The present article is presented as a contribution toward that goal.

SUMMARY

A study of the processes following the reunion of severed nerve ends by an arterial sleeve in the rat has revealed a typical sequence of events, as follows:

A blood clot cements the nerve ends.

Within a day the red cells break up, leaving the fibrin net of the plasma standing.

Owing to liquefaction along the arterial wall, the union clot becomes detached laterally, while remaining firmly fused to the nerve ends.

This results in longitudinal tensions transmitted from nerve to clot to nerve.

These tensions orient the fibrin fibers in longitudinal directions.

Proteolysis throughout the clot, beginning about the second day, gradually destroys all fibrin fibers except the ones oriented lengthwise. Thus a system of longitudinal fibrin strands bridging the gap from stump to stump is established.

Macrophages move into the gap, continuing the clearing of liquid channels. Sheath cells move out from both nerve stumps, glide along the fibrin rails and give rise to the deposition of endoneurial collagen on their surfaces.

Axons regenerate, mostly in application to sheath cells, along the same straight and direct guide lines. Lacking obstructions, they proceed essentially without branching.

The main framework of the nerve union is completed by the end of the first week, and subsequent developments concern mainly the utilization of the bridge by increasing numbers of nerve fibers and the maturation of the latter.

These experiments have established a number of conditions prerequisite for optimal nerve regeneration. They point the way toward the avoidance of confused suture lines, neuromas, gliomas and nerve fibrosis. Even if the arterial splice as such should not be applicable in clinical practice, the lessons learned from this analysis of its merits may guide the search for improved methods of nerve repair.

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