

ROGER W. SPERRY

THE TECHNOLOGY OF NERVE REGENERATION:
A REVIEW. SUTURELESS TUBULATION AND
RELATED METHODS OF NERVE REPAIR

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ideas re nerve outgrowth*

BY
PAUL WEISS

Reprinted from *Journal of Neurosurgery*
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THE TECHNOLOGY OF NERVE REGENERATION: A REVIEW. SUTURELESS TUBULATION AND RELATED METHODS OF NERVE REPAIR*

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INTRODUCTION

IN SEVERAL recent publications,^{183,186,188,189,195,200} a method of sutureless reunion of severed nerve stumps by means of cuffs of artery was described and, on the basis of its merits in the laboratory, was recommended for possible adaptation to clinical use. The conclusions were based on experiments with a total of over 700 nerve unions effected in rats, rabbits, chickens, cats and monkeys, followed in the majority of cases by functional and histological studies. These experiments, in conjunction with a broader study of the mechanism of nerve regeneration, have led to important insight into the prerequisites for optimum nerve restoration. Arterial sleeve splicing complies with more of these prerequisites than do other methods, and this has been the basis of its success. But further application of the lessons thus learned gives promise of even more substantial improvements, and sleeve splicing may eventually be superseded by some other, more meritorious procedure incorporating its experiences. The emphasis lies more on the principle than on the current form of its application. An unbiased survey of existing methods of nerve repair, including sutureless sleeve splicing, shows plainly that no one of them is sufficiently superior to the others to deserve a monopoly of attention. In times of urgency such as these, the weighing of one method against another had therefore better give way to a concerted effort to extract the best features from all available methods and combine them to the best practical advantage.

Sleeve splicing bears a certain, though only superficial, resemblance to earlier procedures of joining the stumps of a severed nerve inside a tube ("tubulization") and of wrapping suture lines, procedures which have been practised on and off for more than sixty years (see Table 1). It also shares certain features with the method of plasma suture.^{207,163}

None of the reported methods has been generally adopted, and there has been some controversy regarding their merits or demerits; many, in fact, have been thoroughly discredited.^{155,130} Nevertheless, trust in their potential usefulness keeps manifesting itself in persistently recurring attempts to reintroduce them into the practice of nerve repair in either the old or some new form.^{172,152} The very persistency of these attempts, aside from those simple

* Some of the experimental work referred to in this article was carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago; also aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

TABLE 1

Main publications on nerve sheathing and tubulation

No.	Author	Year	Material Used
1	Gluck ⁵⁸	1880	decalcified bone
2	Vanlair ¹⁷⁰	1882	decalcified bone
3	Kölliker ⁹⁸	1890	decalcified bone
4	v. Büngner ²³	1891	artery; vein
5	Huber ⁸⁵	1895	Cargile membrane
6	Payr ¹²⁴	1900	magnesium
7	Pomerancew ¹³¹	1900	decalcified bone
8	Lotheissen ¹⁰⁶	1901	gelatine
9	Foramitti ⁵¹	1904	artery; live or fixed
10	Craig & Ellis ³⁴	1905	Cargile membrane
11	Sherren ¹⁴²	1906	Cargile membrane
12	Treutlein ¹⁶⁹	1906	artery
13	v. Auffenberg ⁷	1907	decalcified bone
14	Hashimoto & Tokuoka ⁷⁵	1907	preserved arteries
15	Tilmanns ¹⁶⁸	1907	
16	Wrede ²⁰⁴	1909	vein
17	Röpke ¹³⁵	1910	
18	Perekropoff ¹²⁵	1913	artery, vein
19	Denk ³⁷	1914	fascia
20	Eden & Rehn ⁴⁴	1914	fat tissue
21	Hirschel ⁷⁹	1915	preserved arteries
22	Auerbach ⁵	1915	galalith
23	Fullerton ⁵⁷	1915	vein
24	Hans ⁷⁰	1915	epineurium
25	Heile & Hezel ⁷⁶	1915	unvulcanized rubber
26	Kirk & Lewis ⁹⁵	1915	fascia
27	Kredel ¹⁰⁰	1915	fascia and fat
28	Nageotte ¹¹⁷	1915	vein
29	Bethe ¹⁶	1916	artery
30	Edinger ⁴⁵	1916	agar tube
31	Mauclaire ¹¹⁰	1916	trachea
32	Meisel ¹¹¹	1916	fascia
33	Auerbach ⁶	1916	casein preparation
34	Stracker ¹⁵⁷	1916	veins; agar tube
35	Blencke ¹⁹	1917	agar tube; artery
36	Burk ²⁴	1917	agar tube
37	Dustin ⁴¹	1917	artery; vein; fascia
38	Eden ⁴³	1917	artery <i>in situ</i>
39	v. Enderlen & Lobenhoffer ⁴⁷	1917	artery; feather quill; agar tube
40	Hohmann & Spielmeier ⁵⁴	1917	agar tube; artery
41	Kirschner ⁹⁷	1917	agar tube; fascia and fat
42	Müller & Berblinger ¹¹⁵	1917	agar tube; artery
43	Perthes ¹²⁶	1917	fat tissue
44	Spitzzy ¹⁵¹	1917	agar tube
45	Steinthal ¹⁵⁴	1917	rubber
46	Bielschowsky & Unger ¹⁷	1918	preserved dura
47	Meuriot & Platon ¹¹²	1918	rubber
48	Platt ¹²⁹	1919	greased fascia; vein
49	Huber ⁸⁷	1920	artery, fascia, Cargile membrane
50	Stopford ¹⁵⁶	1920	fascia; vein
51	Kraus & Reisner ⁹⁹	1940	muscle, fat
52	Verne & Iselin ¹⁷²	1941	parchment
53	Spurling ¹⁵²	1943	tantalum

duplications of effort based on unfamiliarity with facts and literature, signifies that the belief in tubulating or wrapping suture lines stems either from a basic truth or a common delusion. The lack of unanimity in the matter can be ascribed to several reasons. One reason has been the fragmentary state of knowledge about nerve regeneration. Ignorance of the requirements of the different tissue components in nerve regeneration has turned some basically sound procedures into failures merely because some trivial fact, the importance of which had not been known or suspected, was neglected. Another difficulty has been the lack of objective and uniform standards in assessing and describing the experimental and clinical results. Moreover, there were many reports without adequate documentation and many claims unsupported by facts.

This state of affairs has made it desirable to reexamine the whole subject in the light of the increased knowledge and more critical standards of today. The following report undertakes to do this in the spirit of scientific analysis. Neither the biological process of nerve regeneration nor the various techniques to facilitate it for the sake of functional nerve restoration are indivisible wholes. All components of a nerve and of its surroundings—axons, sheath cells, fibroblasts, macrophages, erythrocytes, leucocytes, plasma, collagen, capillaries, etc.—all these contribute in some degree to the outcome of nerve regeneration, and their various peculiarities and requirements want to be taken into account. Every technique of nerve union, in turn, consists of a variety of steps and measures each of which is apt to affect differentially the various tissue components, some beneficially, and some adversely. The net balance decides the outcome for nerve regeneration as a whole, whether beneficial, harmful or irrelevant. There is no justification, therefore, for either accepting or condemning any one method *in toto*, since each one must contain elements worth preserving and exploiting, as well as elements patently detrimental, and elements that do neither good nor harm. By sorting out these elements, scientific analysis prepares the synthesis of improved methods, which will emerge from the combining of the positive features of the different techniques and the elimination of their recognized drawbacks. The results, subject to verification in laboratory experiments, may then be adopted as blueprints for clinical procedures.

THE MECHANISM OF NERVE REGENERATION

Due regard for the realities of nerve regeneration is essential both in judging existing methods of nerve repair and in devising new procedures. Any methodical approach to the problems of nerve repair must be based on strict observance of the growth requirements of regenerating fibers, which in turn presupposes intimate knowledge of nerve growth, and this means not merely descriptive knowledge of the phenomena as recorded in macroscopic and microscopic observation, but analytical understanding of their mechanics. Some valuable progress in this direction has been made during recent years, even though it is still small in the light of what remains to be done. As a di-

rective for future research, it must be stressed that progress in this field is not to be expected simply from continued search for more facts along the classical lines of nerve regeneration studies, but from a basic change of viewpoint. Problems of nerve regeneration will have to be taken out of the province of naturalistic description and transferred into that of physical and chemical reality, where nerves are treated as material systems with verifiable physical and chemical properties. A similar reorientation is noticeable in all current biological thinking, and the field of pathology has greatly benefited from it. Morphological features formerly described as such must be resolved into the dynamics operative in their formation.^{182,122} In the case of the nerve fiber, the mere listing of such phenomena as local swellings, terminal bulbs, spiraling, branching, etc., must give way to a dynamic concept of the mechanism of nerve growth so consistent that all those phenomena will appear as logical manifestations of that growth principle under the locally prevailing conditions.

There have been some good efforts to that end in the past, but most of them were either very abstract or based on inconclusive experimentation. The very phenomenon of "growth" of the nerve fiber has only occasionally been stated in sufficiently articulate form to invite analysis.^{185,205} If "growth" connotes augmentation by synthesis of the living substance, how much of the elongation of a regenerating axon is due to real growth, and how much to mere draining of preexisting substance from the cell body? And does what actual growth there is occur in the cell body, at the advancing tip, or over the whole length of the axon? And where are the ingredients for this synthesis taken in—centrally, at the tip, in the whole naked part, or at the nodes of the old stump? And does what is valid for the elongation of the fiber hold equally true for its continued growth in width? It is tangible questions like these that will have to guide research, rather than just general talk about "growth" in all its indefiniteness. What sense is there, for instance, in speculating on how essential are blood supply, vitamins, etc., to the young fiber sprouts if one does not even know to what extent the latter depend on local sources for their oxygen and nutrient supply?

Another example of the lack of concern for physical realities which characterizes much of the past work on nerve regeneration is the not uncommon tendency to view nerve fibers, at one time, as if they were growing in a vacuum, and at other times, as if they were not corporeal. One encounters descriptions (occasionally in connection with tubulization experiments) according to which the space into which nerve fibers grow is "empty."^{19,43,151,157} Now, physically speaking, there can be no empty space anywhere in an organism; there must be either solids, liquids, gases, or mixtures of them, and the behavior of the nerve fiber will vary significantly, depending on how the space is occupied. The fallacy of identifying lack of histological distinctiveness with physical homogeneity is obvious. On other occasions, the ability or inability of nerve fibers to "grow through" other tissues is discussed with insufficient regard for the physical mass of the fiber. This is very pertinent to

generation.^{31,119} Degeneration precedes and, in a sense, prepares the ground for regeneration. It involves regressive and progressive changes. Most prominent among the regressive changes of the distal stump is the breakdown of the myelin sheath and the axis cylinder, followed by the dissolution and resorption of the resulting debris (with formation of so-called digestive chambers or "ovoids"). Progressive changes include the appearance of large numbers of macrophages removing nerve debris, and the conversion and proliferation of formerly tubular Schwann cells into solid cell strands (Bünger's cords). In addition, common traumatic and inflammatory reactions may occur at the wound surfaces. In the proximal stump, degenerative changes similar to those of the peripheral stump remain confined to the vicinity of the wound, their extent varying with the nature of the trauma from a few millimeters to several centimeters. They are soon to be superseded by the regenerative processes in which the proximal stump takes the lead.

Save for a few resistant fibers, the regressive changes of the distal stump are nearly completed within a few days after transection. Whether or not degeneration proceeds in proximo-distal direction down the nerve, is still controversial.^{23, 83, 159, 123, 84, 136} The factors controlling Wallerian degeneration are, in spite of some analytical attempts,^{119, 90} still largely unknown. Its rate varies with the circumstances; it is markedly delayed by nerve compression.¹⁹⁴ Experiments to determine whether nerve fragments which have degenerated at different rates and under different conditions are all equally suitable for neurotization, are under way.

The neurilemmal tubes survive the lytic processes of early degeneration. They become distended by the accumulation of decomposition products and macrophages. This increase of turgor, causing swelling of the nerve as a whole (for actual measurements, see Weiss¹⁸⁷) and obliteration of the endoneurial spaces, may interfere with blood circulation and stimulate collagen deposition,¹⁰⁹ but no further information on these points is available. Variations of turgor may be the clue to the later differences in fibrosity between different aneuritic stumps and, if so, would be of clinical importance.

Schwann cell reaction promptly follows the fragmentation of the axis cylinder. There are indications that the Schwann cells participate in the digestion and perhaps even phagocytosis of the tube content during the initial phase of degeneration.^{*, 31, 119, 41} In fact, direct intake of degeneration products might account for their rapid hypertrophy. However, the main share of the clearing of the tubes is soon taken over by the macrophages, which appear on the scene in large numbers.¹¹⁸ Their exact origin has never been traced. Many are undoubtedly transformed blood monocytes. Mobilized histiocytes might enter through the wound surface if the latter were left exposed. However, since macrophages are just as numerous in sleeve-spliced nerves²⁰⁰ (in which immigration from the surroundings is precluded), this source cannot be important. A third conceivable source lies in a possible

* In recent tissue culture studies (still unpublished), Schwann cells from peripheral nerve were actually observed in the act of digesting ingested myelin.

conversion of some Schwann cells and endoneurial cells of the nerve itself into macrophages. Although actually observed in tissue culture,¹⁹¹ the occurrence of such a transformation in nerves *in situ* has not yet been demonstrated. The problem is of considerable practical importance since the ratio between Schwann cells and macrophages in the distal stump has a bearing on the subsequent neurotization process.¹⁹¹

The final fate of these macrophages is uncertain. From the standpoint of successful nerve degeneration, it would be desirable to have them move on after ingesting the debris of the tubes so as to make room for the cords of hypertrophied Schwann cells. As a rule, this occurs. However, under certain still undefined conditions, the macrophages remain trapped inside the old tubes, thus preventing the expansion of Schwann cells and barring the entry of regenerating nerve fibers. I have seen aneuritic tubes crammed with immobilized macrophages many months after transection,* and since this condition may well become a permanent obstacle to neurotization, further exploration of the origin, behavior and fate of macrophages as a basis for improved control over their activity in nerve regeneration is urgently needed.

Mitotic activity of the expanded Schwann cells sets in within a week²³ and the enlarging strands (a) fill the tubes,^{21, 82, 119} except where "ovoids" and macrophages resist replacement; (b) appear in intertubal positions,³¹ partly from former unmyelinated fibers, partly perhaps after escaping through "leaks" in tubes; and (c) spill from the open ends of the tubes into the surroundings, initiating what is often referred to as the "glioma,"^{41, 119} or "Schwannoma"¹⁰⁹ of the peripheral stump.

All these component processes of Wallerian degeneration are interdependent so that interference with any one of them may alter the course of all, which in turn may seriously affect the subsequent neurotization processes for which they are to prepare the proper setting.

Neurotization, i.e., the supply of new axis cylinders, definitely has been shown to be solely a function of the proximal stump. The concept of "autogenous" nerve regeneration,^{14, 150} has been conclusively disproved.^{31, 119} The role of the converted ("degenerated") distal stump is merely that it presents to the outgrowing axons conditions of growth far superior to those offered by any other known environment. This explains why the exploration of the properties of degenerated nerve must be carried on as an integral part of all research on nerve regeneration. First, the degenerated stump does not invariably attain that state of optimum receptiveness for regenerating fibers of which it is intrinsically capable, and knowledge of the reasons for this variability may suggest measures by which that optimal condition may be brought about more consistently; this is particularly evident in the case of nerve grafting in which the often reported inferiority of distal nerve fragments, when used as grafts, contrasts with their effective neurotization when left in place. Unsuspected differences in local conditions can account for such variety of results as that one distal nerve fragment becomes a superb

* Compare also figure in Hammond, Nonidez and Hinsey.⁶⁹

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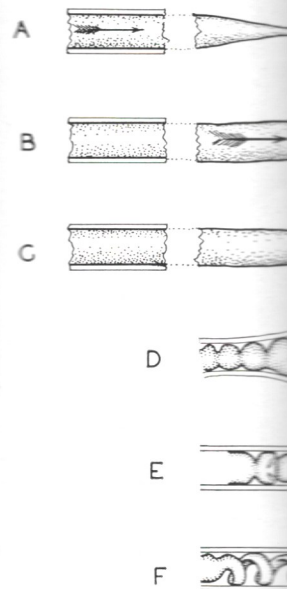


FIG. 1. Diagrams illustrating the progression of outgrowth showing progressive transitory terminal filopodia, convection of axonal substance by the damming up of axons, telescoping, and coiling of the center of the tubes (level of convection continues to move axonal substance neck" leads to the various forms shown in the graphs.

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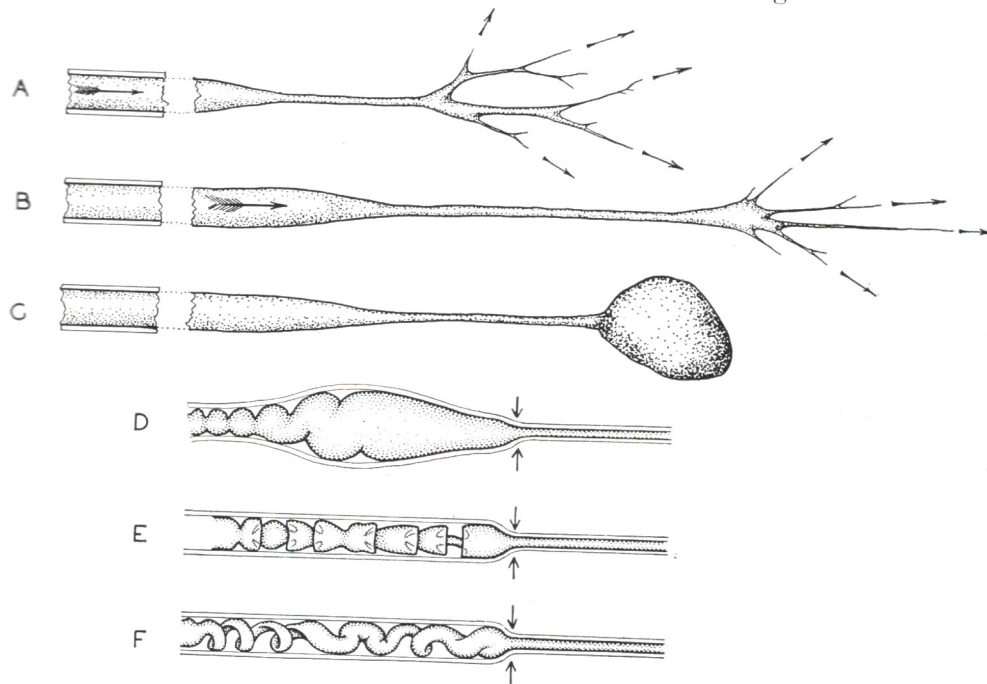


FIG. 1. Diagrams illustrating normal and impeded outgrowth of axons. *A, B*, Two successive stages of outgrowth showing progressive elongation by interfacial tensions (simple arrows), which draw out the transitory terminal filopodia to fine points, followed by lateral enlargement resulting from centrifugal convection of axonal substance by intraaxonal pressure (feathered arrow). *C*, Terminal bulb, produced by the damming up of axonal substance in front of an unsurmountable obstacle. *D, E, F*, Ballooning, telescoping, and coiling of the axonal substance just proximal to a constriction which reduces the diameter of the tubes (level of constriction indicated by arrows). Centrifugal growth pressure in the axon continues to move axonal substance distad, and the damming up of this substance in front of the "bottle-neck" leads to the various forms of contortion shown in these schematic reproductions from photomicrographs.

NERVE REGENERATION

The outgrowth of new axons from the proximal stump is the cardinal process in nerve regeneration. As such it has monopolized much of the attention in the past, an attitude accentuated by the preferential use of silver impregnation methods, which visualize the axis cylinder practically to the exclusion of all other structures. It is well, however, to bear in mind that the distal nerve stump, into which the new sprouts grow, makes some substantial, even though less spectacular, contributions to their growth, and that the

full restoration of a nerve is a compound process involving the participation not only of the axis cylinders but also of those accessory elements of their surroundings on which they depend for nutriment, guidance, protection, myelination, maintenance, and perhaps other as yet unrevealed influences. Consequently, nerve regeneration must never be dealt with in terms of the growing axon alone, but always in reference to the environmental conditions under which growth takes place.

The so-called outgrowth of the regenerating axon is primarily protoplasmic movement of the amoeboid type.^{71, 74, 102, 177} The tip of the axon becomes mobile and extends into the environment by means of fine filamentous pseudopodia ("filopodia").^{114, 143, 144, 145} As it proceeds, it spins out the axoplasm behind it to increasingly greater length (Fig. 1A, B). The motive force

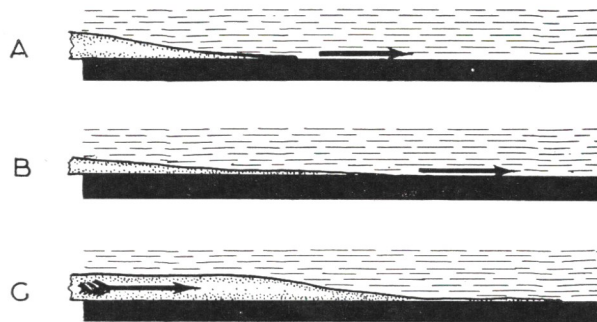


FIG. 2. Pull-push mechanism of nerve fiber advance. Drawn out by interfacial tensions (simple arrow) along solid-liquid interface, fiber receives inflow by intraaxonal pressure (feathered arrow).

actuating this elongation has often been ascribed to pressure inside the neuron (Held's⁷⁷ "vis a tergo"; Cajal's³¹ "formative turgor"; Young's²⁰⁵ "outflow"). Evidence of such pressure has been found in the familiar terminal bulbs of arrested nerve fibers (Fig. 1C),^{31, 41, 205} and more recently in the swelling of locally compressed nerve fibers immediately proximal to the level of compression (Fig. 1D, E, F).^{192, 197} On physical grounds, however, such pressure cannot of itself account for the extension of the filopodia. It is necessary to assume some external pull, which is obviously exerted by interfacial tensions¹⁸⁵ (Fig. 2).

Nerve fibers can extend only along phase boundaries between two media and cannot move into a homogeneous medium in the manner of plant roots.^{72, 177} They are drawn out along a solid-liquid interface (Fig. 2A, B) by forces roughly comparable to those causing oil to spread along a water-air interface. Flow of axoplasm into these pseudopodia, presumably sustained by intraaxonal pressure, then fills them up (Fig. 2C). The advance of the axon is thus due to a pull-push mechanism, with interfacial tensions exerting the pull, and internal pressure pushing from behind. Of the several filopodia appearing concurrently, usually only one receives the main inflow, while the

others are withdrawn terminal branching.

This pull-push mechanism in pure inorganic systems, as thiamin or biotin observation suggests, is not a new axoplasm. The observation suggests that the proximal part of regeneration is the increased assimilation of the fiber. It grows in width. The body.²⁰² In reality, but this brief sketch nerve fiber "growth" to support it.

The linear character orient the outgrowth two questions: what mines which pseudopodia sorbed. Once the tip the fiber follows and be straight, while although some bends the problem of what different theories: (3) Chemotropism.^{26, 27, 93, 155} These ions,^{38, 74, 185} and only

Contact Guidance applies itself blindly to the tip is, therefore, environment plus such make for adhesion. The original conceptions,^{73, 80} is incomplete. As stated before, filopodia are a directional factor to singlowed. This directional factor shows the surface. Extensions show that nerve fibers are microscopic or submicroscopic. Additional evidence

Both cellular and

others are withdrawn.^{144,145} Inflow divided over several filopodia gives rise to terminal branching.^{144,185}

This pull-push advance of the fiber tip can proceed for some time *in vitro* in pure inorganic media containing no nutrients or growth implements, such as thiamin or biotin, other than those possibly stored in the nerve cell.²⁸ This observation suggests that early "outgrowth" is a physical process of elongation, rather than a true "growth" process involving chemical synthesis of new axoplasm. The same conclusion has been reached from the observation that the proximal diameter of nerve fibers decreases during the early stages of regeneration.⁶⁷ Only secondarily does the physical elongation then entail increased assimilation and thereby initiate actual growth for the consolidation of the fiber. After the fiber has reached its full length, it continues to grow in width. This growth has been shown to proceed from the central cell body.²⁰² In reality, the situation is even more complex than here outlined, but this brief sketch will suffice to indicate the intricacy of the mechanism of nerve fiber "growth," and, accordingly, the diversity of provisions required to support it.

The linear character of nerve growth raises the question of what factors orient the outgrowing fibers. Concretely, this problem resolves itself into two questions: what guides the advancing pseudopodia, and what determines which pseudopodia are to become consolidated and which are to be resorbed. Once the tip has been caused to move in a given direction, the rest of the fiber follows automatically. If the tip moves straight, the fiber course will be straight, while an erratic course of the tip will result in contorted fibers, though some bends may later be straightened out by elasticity. Answers to the problem of what guides the fiber tip have been proposed in essentially four different theories: (1) Contact guidance^{40,74,80,177,185}; (2) Least resistance¹⁷¹; (3) Chemotropism ("neurotropism")^{30,31,53,54,107,167}; (4) Galvanotropism.^{26,32,93,158} These have been discussed more fully on previous occasions,^{38,74,185} and only the most pertinent points will be repeated here.

Contact Guidance. "Contact guidance" implies that the growing tip applies itself blindly to the substratum with which it is in contact. The course of the tip is, therefore, determined solely by the configuration of its contact environment plus such physical and chemical properties of the latter as will make for adhesion or non-adhesion and intimacy of the resulting contact. The original concept, that nerve fibers follow gross mechanical structures,^{73,80} is incomplete. It has to be extended to the submicroscopic field.¹⁷⁷ As stated before, fibers can grow only along surfaces; but it takes some additional factor to single out a particular direction along that surface to be followed. This directive factor is presumably provided by the fine structure of the surface. Extensive experimental evidence has been presented,^{177,185} to show that nerve fibers follow the direction of oriented fibrillar units of microscopic or submicroscopic dimensions present in their contact substratum. Additional evidence will be cited below.

Both cellular and non-cellular surfaces may serve in the capacity of con-

tact substrata. But in either case, the line of advance is determined by some linear structures ("pathway structures") within that surface. Fibrin fibers are excellent climbing ropes,^{177,200} and so are Schwann cells.^{96,119,200,205} It is significant that oriented interfaces orient fibroblasts¹⁷⁶ and Schwann cells¹⁷⁷ just as they do nerve fibers. They can, therefore, orient nerve fiber growth either directly or through the intermediary of a correspondingly oriented spindle cell which the nerve fiber then follows. Moreover, nerve fibers already in existence form, under certain conditions, preferential pathways for other advancing nerve tips, which fact accounts for the building up of nerve bundles around pioneering fibers ("fasciculation").¹⁸⁵

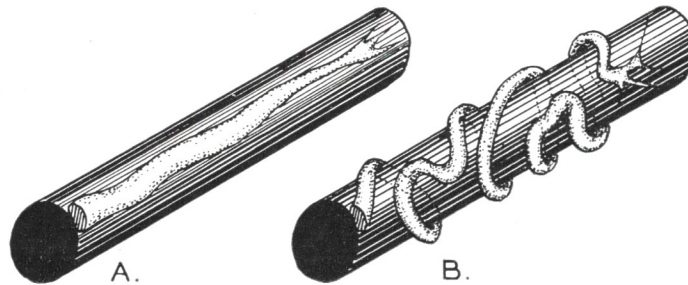


FIG. 3. Straight polarized advance of axons in the direction of the axis of a fiber serving as substratum (A); contrasted with the random winding course (B) to be expected if either simple "stereotropism" or "least resistance" were the determining factors. [A winding course is observed only (a) if the substratum has a spiral surface organization (e.g. in Perroncito's coils), or (b) if the fiber is arrested in its advance, yet keeps increasing in length (e.g., Fig. 1, F), or (c) if a fiber retracts.]

Contact guidance, as here described, implies that the nerve fiber tip reacts solely to cues of its immediate environment. Agents acting from a distance can, therefore, influence the course of a nerve fiber only to the extent to which they affect the physical pathway. Selective affinities between different types of nerve fibers and different kinds of pathways are a possibility worth keeping in mind,¹⁶⁶ although the evidence in favor of selective outgrowth is still incomplete.

Least Resistance. The theory that nerve fibers simply take the course of lowest mechanical resistance—one of the earliest concepts,¹⁷¹ and one still sporadically encountered in current literature⁶⁹—is partly incorrect and, for the rest, non-committal. Let us recall, for instance, that nerve fibers do not grow into homogeneous fluids, even though the mechanical resistance of liquids is negligible; also that nerve fibers, given an appropriate pathway structure, can grow from a softer into a denser medium,¹⁷⁷ unless the latter is impenetrable (see below). Moreover, "least resistance" cannot explain why fibers which extend along a solid-liquid interface follow definite directions, rather than just move about at random, as the all-round absence of obstacles would enable them to do. Axons on a fibrin thread in serum, for instance, follow a straight longitudinal course (Fig. 3A), and do not wind themselves

around the thread. It is stated that nerve fibers take something more positive course. What serious effects have when applied to Elinger tubes, introduced gaps" (see below). The posed between the nerve fibers with a "set for lack of proper nerve Fiber growth needs po

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* This statement is based on observations of nerve fiber configurations in tissue culture. T

around the thread (Fig. 3B).^{*} Thus, while it is unquestionably correct to state that nerve fibers are deflected by mechanical obstructions, it takes something more positive than mere lack of obstacles to define their actual course. What serious consequences superficial thinking in these matters may have when applied to practical problems, is well illustrated by the notorious Edinger tubes, introduced during the last war for the bridging of nerve gaps⁸⁵ (see below). These were tubes, filled with gelatin or agar-agar, interposed between the nerve stumps. The idea was to present the outgrowing nerve fibers with a "soft" medium. Yet, no fibers grew in—as we now realize, for lack of proper interfaces—and the method proved a sad failure.^{81,151,157} Fiber growth needs positive support, not just absence of handicaps.

Chemotropism (neurotropism). The theory that growing nerve tips are attracted toward distant sources of chemical emanations by a mechanism of chemotaxis was first suggested by Cajal³⁰ and later specifically applied to nerve regeneration.^{31,53,54,107,167} It endows the nerve tip with the capacity to perceive and discriminate different chemicals and to orient itself uphill along the concentration gradient leading to their source. Particularly strong attractive potency was ascribed to peripheral nerve in the state of degeneration. It seems that this theory is based wholly on the misinterpretation of experiments undertaken to prove it. Not only are those earlier experiments amenable to other than chemotactic interpretations,^{40,91,185} but more recent experiments, carried out under more critically controlled conditions,²⁰¹ have conclusively disproved the existence of any chemical attraction or chemotactic orientation of regenerating nerve fibers by tissues in general, and degenerating nerve stumps in particular. Results formerly attributed to "neurotropism" can be ascribed to the orienting effects of tension exerted by the nerve stumps on the intervening union tissue serving as pathway for subsequent nerve growth, plus the accentuating effect produced by fiber aggregation¹⁸⁵ ("fasciculation").

This rejection of the theory of "neurotropism" applies only to the thesis of an attraction of nerve fibers towards distant destinations. That the adhesion of nerve fibers to their like, as well as the effectiveness of synaptic and peripheral connections, might be determined by chemical affinities operating after contact is established, is merely a special aspect of the contact guidance theory discussed above.

Galvanotaxis. The theory that fiber growth is oriented by electric fields,^{26,32,93,158} is contradicted by the observation of nerve fibers growing simultaneously in opposite directions.^{144,177} Moreover, all experimental tests of the theory have been consistently negative,^{60,94,177,203} except for one supposedly positive experiment,⁹² in which, to all appearances, mechanical effects were mistaken for electrical ones.¹⁷⁷

^{*} This statement is based on extensive studies on nerve growth along surfaces of different configurations in tissue culture. The results of these studies will be reported more fully on a later occasion.

Nerve Outgrowth. According to the foregoing survey, the tip of the regenerating axon is guided in its advance by structural characteristics of the substratum with which it is in contact. The growing axon traces patterns of its surroundings. Random patterns, such as are presented, for instance, by nerve scars, lead to correspondingly confused nerve growth. Conversely, orderliness of nerve growth presupposes the presence of well aligned parallel pathways. Degenerated nerve offers such pathways in abundance both along the inner and outer surfaces of the tubes and along the strands of Schwann cells. In the gap, however, beyond the limits of degenerated nerve, oriented nerve growth can be enforced only if we provide the nerve fibers with a new system of pathways of the proper constitution and proper orientation. The guiding role of Schwann cells is still controversial. According to some authors,^{41,96,119,205} they precede the axons, laying down the tracks, as it were. According to others,^{31,69} most axons sprout out independently. Observation under especially favorable conditions²⁰⁰ has shown that, generally speaking, Schwann cells have a head start over the axons and that the axons, perhaps for this very reason, tend to cling to the Schwann cords. At the same time, examples of freely outgrowing axon tips were observed, even though relatively rarely. Obviously both forms of outgrowth occur, with axon orientation being effected, in one case through the mediation of Schwann cords, previously oriented by the pathway structure, in the other case by the pathway structure directly.

Parallel guide lines in the pathway structure are not only prerequisite for the orderly parallel outgrowth of Schwann cells and axons, but are also instrumental in keeping fibrous connective tissue from intruding into the path of the nerve. Fibroblasts align themselves along linear structures,^{73,176} just as do Schwann cells and axons. Consequently, the same oriented structural pattern that guides the nerve fibers also deflects extraneous connective tissue cells and collagen fibers into a longitudinal course along the surface of the nerve, which is a useful course. For here the connective tissue is led to build a new sheath over the gap, instead of penetrating into the path of the nerve where it would scarify and impede regeneration. It may be impossible to prevent scar tissue from forming near a wound, but it is perfectly feasible to render it innocuous by directing it into a course parallel to the nerve where it can do no harm. A parallel pathway structure, therefore, both guides nerve growth and protects it against interference. How then can such favorable pathway structures be established?

Fibrillar structures consist of an oriented array of linear colloidal particles.^{56,128} Under random conditions, such particles clump into a spongy framework. If aligned in parallel orientation by some external orienting force they join end-to-end and flank-to-flank, building up larger linear units. The formation of fibrin fibers, for instance, occurs in this manner.⁹ The most potent and common force to produce such parallel alignment is mechanical tension.^{9,42,176,177} Not only has its orienting effect on colloids long been

known, but its molding effect has been experimentally tested. Fibers aggregate along the lines of tension to produce fibrils and fibers of high tensile strength. Tensional stresses also cause shrinkage. An example of this is the cracking of drying soil. The contraction, is a common

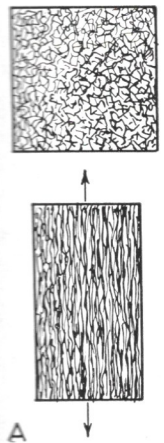


FIG. 4. Effects of tension on tissue structure. (top), when stretched (middle), and after shrinkage (e.g., syneresis) of the tissue (bottom). (A) Tension patterns (to be compared with the shrinkage pattern (B), detached on three

ment to problems of nerve regeneration, however, by the tensional forces also by the elastic and contractile stresses will develop only in response to the deforming forces which it is not prevented from attachment of a tissue to a substrate stress pattern (Fig. 4).

In conclusion, a systematic orientation upon the tissue during the setting down of pathway structures created affect not only the density of the resulting structure but also the direction down to earth, from the tissue to the attractive influences" t

known, but its molding action on tissues has been amply demonstrated and experimentally tested. Rod-shaped ultramicros become oriented and aggregate along the lines of prevailing tension. Parallel tensions, therefore, produce fibrils and fibers of corresponding parallel orientation (Fig. 4A).

Tensional stresses arise either from external stretch or from internal shrinkage. An example of stresses arising from shrinkage may be seen in the cracking of drying soil. Local condensation of fibrous tissue, the familiar scar contraction, is a common source of such internal stresses and is very perti-

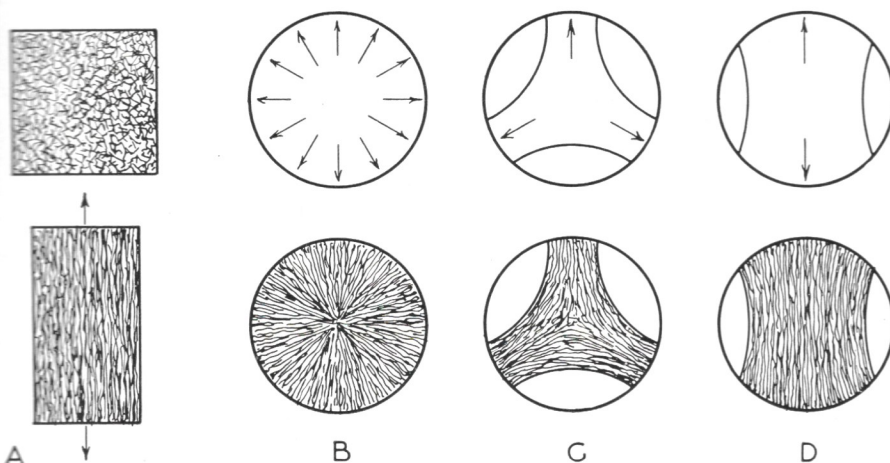


FIG. 4. Effects of tension on fibrous colloids. *A*, Colloid with anisodiametric elements in chaotic arrangement (top), when stretched, assumes orientation in the direction of the prevailing tension (arrows). *B*, *C*, *D*, Tension patterns (top row) and resulting fibrillar orientation (bottom row), arising from the shrinkage (e.g., syneresis) of a colloidal matrix firmly attached to its frame along the whole circumference (*B*), detached on three sides (*C*), and detached on two sides (*D*).

nent to problems of nerve suture. The stress pattern is not determined solely, however, by the tensions imposed on the tissue or generated within it, but also by the elastic and plastic properties of the different tissue components. Stress will develop only in those directions in which the tissue does not yield to the deforming force. And since the tissue will yield in all directions in which it is not prevented from yielding by firm attachments, the points of attachment of a tissue under tension are critical parameters of the resulting stress pattern (Fig. 4B, C, D).

In conclusion, a stress pattern lasting sufficiently long to impose its orientation upon the tissue matrix becomes thereby a primary factor in the laying down of pathways for nerve fibers and spindle cells. Conditions thus created affect not only the orientation of growth, but also its rate, as well as the density of the resulting new tissue. To have brought these problems down to earth, from the speculative language of "trophic stimuli" and "attractive influences" to the factual treatment in terms of technology and

physico-chemistry, has been an essential step toward rational understanding and control of the mechanics of nerve regeneration and tissue repair.

Advance of healthy nerve fibers and sheath cells can proceed as long as there is an unobstructed pathway; hence, failure to advance in the proper direction will have to be ascribed to either lack of pathways or diversion of existing pathways or inadequacy of the physical and chemical conditions along the pathway. Physical inadequacy results from excessive density of the tissue to be penetrated. In order to appreciate this point, one must visualize the actual physical setting in which nerve regeneration takes place. Axons and Schwann cells invade the tissue of the gap, and axons invade the distal stump. If the tissues to be invaded were solid and compact, invasion would be physically impossible because one physical body (nerve fiber) can move into the space occupied by another physical body (distal stump) only by replacement, that is, to the extent to which it can force out of place an equal volume of the latter. And since only liquids, or solids suspended in liquids, are freely displaceable, the capacity of any tissue for receiving new cells and nerve fibers will be limited by its content in liquids and the ease with which these can be shifted (capillarity, viscosity, etc.). The less liquid space a tissue contains, that is, the denser it is, the fewer fibers will be able to penetrate, and the slower also will be the advance of those which have entered; just as it takes more time to drive a nail into hardwood of dense texture than into softwood of spongy texture.

Under certain conditions, an invading tissue makes room for itself by liquefying (digesting) the invaded tissue as it goes along. Some proteolytic activity has occasionally been observed near nerve fibers growing *in vitro*^{177,185} and signs of it can be seen around arrested growth cones.⁴¹ But it is uncertain whether it normally occurs during regeneration and, even if it did, experience has shown that it would be impotent to proteolyze dense collagenous scar tissue. The possibility that the growing axon may absorb some substance directly from adjacent Schwann cells so that it could expand at their expense, cannot be excluded, but no definite information on this point is available.

Without proper room for continued expansion, nerve fibers, even though they may have reached the periphery, cannot swell from the small diameter (1μ or less)^{13,31,67,82,133,134} at which they grow out, to the large dimensions of the mature fiber. Therefore, both in the interest of primary outgrowth and of later size increase, due consideration must be given to the space requirements of the new nerve fibers, and this is just as important as is emphasis on proper orientation of the pathway. Tendons have perfect orientation, but are far too dense to serve as conduits for nerve growth. The same is true of heterografts,^{86,140,190} and alcohol- or formalin-fixed grafts^{67,86,119,139} of nerve or muscle, inasmuch as these are progressively transformed into tough fibrous connective-tissue strands, assuming a texture not unlike that of tendons and equally unfit for adequate nerve growth. While sporadic nerve fibers can be

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found in the crevices of such grafts,¹⁴⁰ this fact should not be overrated,⁶⁷ and the misleading practice of publishing pictures of such finds as evidence of "successful" regeneration⁵⁹ should be abandoned. Such trickle of fibers as is present in these cases is apt to be functionally irrelevant, particularly as many of the fibers passing through dense tissue are in danger of being pressure-blocked.

It seems that the ratio between solid and liquid content of tissues has not been properly recognized heretofore as constituting a limiting factor in neurotization. Its recognition makes it imperative to see to it that the union tissue forming between nerve stumps maintains proper liquidity. Provisions to this end will be outlined below.

Nerve Fiber Branching. Fibers have no intrinsic tendency to branch. Branching occurs always in response to local conditions of the environment.^{177,185} It may occur terminally, that is, at the growing tip, or collaterally, that is, along the stem of a fiber.^{143,144} Terminal branching presupposes, first, forking of the fiber tip, and second, continuation of growth along both branches of the fork. The former is a function of the pathway structure, any branching point of which may call forth the extension of filopodia in both directions. If both branches are successful in drawing supporting inflow of axoplasm, the divided flow may produce permanent twinning; if the competition is unequal, the winning branch will proceed and absorb the weaker one. Collateral sprouts have been seen to arise at points where a fiber has been exposed to mechanical or chemical irritation.^{127,144} These sprouts have a better chance of growing if the growth of the main fiber stem is blocked, comparable to the taking over of the growth dominance by side branches in decapitated trees. Statistically, the incidence of branching will be proportional to the abundance of crossings in the pathway structure, which varies inversely with the orderliness of the latter. The more confused the substratum, the more profuse will be the branching of nerve fibers entering upon it. This explains the excess of branches in nerve scars and neuromas. Along well oriented and unobstructed structures fibers advance without branching, the growing tip apparently consisting of a single filopodium. The branching of Schwann cell cords in the glioma seems to follow similar rules.²⁰⁰ The systematic ramification of regenerating axons in skin and muscle is partly guided by the pattern of the preserved degenerated branches of the original supply,²¹ although occlusion of tubes may cause omission of some branches.²⁰⁶

Fiber Resorption. During the early phase of the regeneration process self-amputation and withdrawal of young and short fiber branches is common.^{21,145} Concerning the later resorption of already established fiber branches, or even whole fibers, however, very little is known.⁴¹ There has been much speculation as to the possible reduction of "functionally inadequate" fiber connections²⁰⁵ (e.g. those producing "axon reflexes"), but tangible evidence to support that assumption is still lacking. Some slow regression in human amputation neuromas has been reported, but the majority of use-

less branches survive many years without change.⁴¹ Experimental results with peripherally unconnected fibers confirm the durability of regenerated axons irrespective of their peripheral connections.²⁰¹

Volume of Reinnervation. By this is meant the number of branches actually attaining peripheral connections, expressing the degree of completeness of reinnervation of receptor and effector organs. It depends on the following factors¹⁹⁵:

(1) Size of the fiber source in the proximal stump.
 (2) Amount of branching in the scar. Such branching can amplify the proximal source.³¹ A given muscle or skin area might thus be saturated with nerve branches from a nerve smaller than that which had furnished the original supply.^{39,103,181,198} However, the clinical exploitation of this fact,¹⁸ recommended by Dogliotti,³⁹ remains problematical in view of certain complicating features,^{4,195} which may offset the advantages of stepped-up branching.

(3) Number of fibers admitted into the peripheral stump. If sufficient time is allowed for regeneration, the number of regenerated fibers in the distal stump approaches more closely the normal fiber quota of that nerve than could be expected by mere chance.^{35,63,103} When a distal nerve stump is confronted with an overabundant supply of nerve branches, it admits only a restricted number roughly corresponding to its own original content.¹⁹⁶ Perhaps the capacity of the distal stump diminishes with increasing delay of reinnervation.⁸² Just how the distal stump controls the volume of neurotization, remains to be determined. Since fibers regenerate both inside and between old tubes, and often several to a single tube,^{21,82,119,196,205} the number of degenerated tubes cannot represent the limiting factor. However, the fact as such is clear and condemns to failure attempts to force into a nerve significantly more nerve fibers than it can hold. It is interesting to note how many basic facts of nerve growth such as the one just outlined have been ignored in the devising of such methods of nerve repair as nerve flap transplantation, nerve implantation, etc., now outmoded.^{8,130,155}

(4) Number of fibers persisting. This implies the possibility of secondary resorption of some fiber branches after they have grown through the distal stump.^{21,205} To what extent this occurs, and whether as a result of spatial, nutritional, circulatory or functional inadequacies, is still obscure.

(5) Density of terminal connections. The capacity of a tissue to receive functional nerve supply seems to be strictly limited. Spatial conditions permitting, any number of nerve fibers may be crowded into an organ. But from that pool only a limited number of endings will be admitted to form functional connections passing impulses. A muscle fiber, in general, accepts only a single nerve branch and then insulates itself against further innervation.^{46,55} For skin, likewise, there seems to be a definite saturation density beyond which innervation cannot be driven. This is indicated by the fact that as soon as a skin district is denervated, collateral supply from the sur-

rounding zone moves in, and its restraining action prevents further growth. In view of these facts, surgical attempts at expansion have no useful purpose.

Maturation. In nerve regeneration is only the first of a series of changes in the mature nerve fiber. Formation of myelin sheath cells, myelination, and such functional changes after connection has been established. The regenerated nerve never resumes its original form for the nerve fibers as well as the myelin sheath cells and Schwann cells. The amount of connective tissue assumed is more irregularly spaced. The question of function is the question of maturation. Some functional impairment may be anticipated²⁰⁵ on the one hand and on the other hand the properties of the fiber on the other hand. It is determined how serious such impairment is, the much more profound the intermingling and connection with the original end-organs.

The growth in width of the nerve is usually¹³⁴ and apparently within a normal size range may be expected. It is less distinct than in normal growth. It is determined by the conditions of the peripheral channel into which the nerve expands. Under such conditions, the mode of expansion is determined. The distal part of the nerve is a restricted zone, for instance, the expansion of distal fibers is determined by prerequisites from the proximal end. A local constriction of the nerve will be impaired.

This fact can explain why the nerve tends to remain under a certain size. It does not expand to the large size of the tube would be the case.

* Unpublished oscillography (Weiss and H. Maylander) have demonstrated that along the regenerated distal por-

rounding zone moves in,^{147,175} presumably because of the cessation of some restraining action previously exerted by the area's own supply. In view of these facts, surgical attempts at "hyperneurotization"⁷⁴⁸ of muscle can serve no useful purpose.

Maturation. In nerve regeneration, as in ontogeny, the outgrowth of the axon is only the first of a series of developments leading to the formation of a mature nerve fiber. Further elaborative developments include the realignment of sheath cells, myelination, formation of nodes, increase of fiber diameter, and such functional adaptations as may occur after peripheral connection has been established. Common experience indicates that a regenerated nerve never resumes the precise features of a normal nerve. This holds for the nerve fibers as well as for the accessory constituents. Endoneurial cells and Schwann cells remain present in excessive numbers, the endoneurial connective tissue assumes relatively more space, the nodes of Ranvier are more irregularly spaced. Of more significant bearing on the restoration of function is the question of the restitution of the normal fiber size spectrum. Some functional imperfections as a result of incompletely recovered fiber size may be anticipated²⁰⁵ from the known correlation between fiber diameter on the one hand and conduction velocity and associated physiological properties of the fiber on the other.⁴⁹ However, it has never been actually determined how serious such imperfections are, particularly in comparison with the much more profound disturbance of functional relations caused by the intermingling and consequent misconnection of fibers with other than their original end-organs.

The growth in width of the regenerated part of a nerve fiber occurs gradually,¹³⁴ and apparently progresses in proximo-distal direction.⁶⁷ The normal size range may be approximated, but grouping into different size classes is less distinct than in normal nerve.^{13,67} The caliber which will finally be attained is determined by no single factor. The original size of the fiber, the peripheral channel into which it grows, the number of branches, the nutritive conditions, the mode of peripheral connections, all these may influence the result. The distal parts of fibers which have regenerated through a constricted zone, for instance, remain thin and atrophic.²⁰² Evidently, continued expansion of distal fiber portions requires continuous supply of some growth prerequisites from the nerve cell body; and when this supply is throttled by a local constriction of the axon, growth of the part lying distal to that point will be impaired.

This fact can explain why large fibers regenerating into small distal tubes tend to remain undersized,^{82,121} while small fibers entering large tubes do not expand to the large size of the latter.¹²¹ In either case the dimensions of the tube would be the limiting factor: in the former, the distal tube would

* Unpublished oscillographic studies of regenerating rat nerves (carried out jointly with H. Davis and H. Maylander) have demonstrated a gradual decline of impulse velocity, and hence, fiber diameter, along the regenerated distal portion of a fiber.

resist expansion,⁸² in the latter, the proximal tube would limit the supply to the distal portion. Moreover, growth pressure may reasonably be assumed to be the lower, the smaller the cell body exerting it. The size of fibers advancing endoneurially between tubes may similarly be determined as the resultant of growth pressure and peripheral limitations of space, nutrients, etc. Secondary atrophy, i.e., shrinkage without disintegration, may occur in fibers which have failed to effect peripheral connections,²⁰¹ but the evidence is still meager.

Myelination has been shown to be a property of the neuron, although myelin cannot be produced except with the collaboration of the Schwann cell.^{143,144} Whether the regenerated portion of a fiber does or does not acquire a myelin sheath, depends, therefore, on whether its stump of origin is myelinated or not. Myelination of fiber parts whose growth has been impaired, remains poor.^{61,202}

Specificity of Fibers. The problem of whether fibers can return selectively to their old periphery has remained controversial, in spite of considerable experimental treatment. Functional selectivity in regeneration has sometimes been claimed without clear commitment as to where and how it operated. It might operate (a) by restricting the ingrowth of nerve fibers to tubes of the corresponding type only; (b) by governing the establishment of peripheral connections; (c) by causing inappropriate connections to be dissolved; (d) by modifying the central relations of a misregenerated neuron.

Experimental and clinical evidence contradicts specificity within the major divisions of the peripheral system, inasmuch as any motor fiber is known to regenerate with equal ease into any motor nerve—witness the common crosses between the hypoglossal or spinal accessory and the facial nerve—and the analogous is true of the sensory system. As the functional implications of misregeneration are being made the subject of an extensive review under preparation, the problem need not be discussed here further.

The case is not equally clear with regard to the cross regeneration of motor into sensory channels and *vice versa*. Sensory-motor cross connections can be achieved experimentally,^{20,178,179} but whether and to what extent their occurrence in normal regeneration is restricted,¹⁵ either by differential facility of growth (contact specificity) along homologous and non-homologous pathways or by selective resorption of non-homologous terminal connections, is still a matter of conjecture. Penetration of motor fibers into sensory pathways and *vice versa* is undesirable mainly because it wastes part of the limited neurotization space of the distal stump on functionally useless elements.¹⁶⁰ Although antidromic impulses—afferent in motor fibers,^{104,105} efferent in sensory fibers¹⁰—appear as by-products of normal nervous activity, it is doubtful whether they have the proper characteristics to act as sensory or motor excitations in the case of crossed end organs. Consequently, even if the random mixing of motor and sensory pathways in regeneration should prove to be of general occurrence, it would cause no conspicuous functional disturbance.

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Trophic Restoration. A discussion of the regression of the denervated tissues and their recovery during reinnervation is beyond the scope of this survey.

Functional Restoration. This implies not simply the reconnection between the central nervous system and the receptors and effectors, i.e., recovery of general sensation and motility, but the recuperation of discriminative functions, i.e., proper discrimination and localization of sensory stimuli, and coordination of muscular activity. The literature on this subject is copious, yet contradictory and inconclusive. Its major deficiencies are lack of standard criteria in the assessing of functional recovery and often failure to take into full account the complexity of functional changes attending denervation and reinnervation. In the motor sphere alone, hypothetical "central reorganization" has often been credited with improvements of motor performance that were obviously due to trick movements, changes in muscle balance, compensatory contraction of unaffected muscles, mechanical transmission of pull, etc. It is difficult to tell from the conflicting evidence how much actual reorganization the centers can accomplish, when confronted with situations in which the majority of neurons have entered into new and unpredictable terminal connections.

A comprehensive discussion of these problems will be presented elsewhere. For the time being, it may be stated that the more critical reports on function after experimental and clinical nerve regeneration indicate a rather limited capacity for "reeducation" of misregenerated fiber connections.* The idea that the regulatory powers of the central nervous system, with or without conscious effort and practice, are great enough to make new and orderly impulse patterns emerge from a system of utterly confused connections, finds no support in known facts. A special kind of reconditioning which new peripheral relations produce in the nerve centers of embryonic or larval animals,^{180,184} apparently disappears in the course of development^{148,149} and has therefore (contrary to an earlier assumption¹⁸⁰) no bearing on the clinical situation. In general, the more aberrant the morphological pattern of regeneration (owing to branching, straying, and shunting to the wrong endings), the less orderly will the functional activities be after recovery. And if one considers the mechanics of nerve regeneration, one realizes that full restoration of normal function, as it existed prior to the injury, is wholly beyond attainment. Only statistically can one try to approach this goal by taking measures that will allow the maximum possible number of nerve fibers to restore connections of the old type or, at least, one functionally similar. The recommendation of some neurosurgeons to match the fascicular topography of the stumps in suturing as best as feasible,¹⁷³ thus seems to make good sense physiologically. It is partly invalidated by the practical difficulties of identifying the orientation of dislocated stumps, of recognizing their fascicular pattern, and of suturing accurately without distortion; moreover,

* Compare, for instance, the articles by Ford and Woodhall⁵² and Coleman.³³ An extensive bibliography will accompany the forthcoming review by Sperry.

the greater the gap, the less physiological congruity will there be between the cross sections because of the existence of intraneural plexuses.¹⁵⁵ However, the main factor to offset even the most careful matching of stumps is the profuse straying and branching of the regenerating fibers in the suture scar, and unless this can be avoided, there is no sense in trying to coapt the stumps macroscopically.

The Interdependence of Regeneration Phenomena. The survey of nerve regeneration presented in the preceding pages, even though highly condensed, conveys an idea of the complexity of the processes involved. The functional restoration of a nerve is a composite product of many and diverse interlocking phenomena, and no one of them can be singled out as the master key to the control of the others. While the orderly restitution of the axon remains the central objective, this cannot be attained except in the proper setting, which, in turn, depends on the behavior of the other tissue components. The more our knowledge of this intricate interdependence grows, the more judiciously shall we be able to deal with the practical problems of nerve repair. For example, certain measures that might be devised to stimulate the growth rate of nerve fibers might turn out to promote even more an obstructive fibrosis so that the net effect on nerve restitution would be adverse. Or one might try to amplify an undersized nerve source by causing the fibers to branch profusely during regeneration,^{18,39} only to find out eventually perhaps that the number of branches any one neuron can maintain in good functional repair is limited. Numerous similar examples could be cited to illustrate that no single step of nerve regeneration can be viewed as an isolated entity. The intimate interrelationship among the various steps is a reality which one can ill afford to neglect in the practice of nerve repair.

METHODS OF NERVE REPAIR

Measures of nerve repair can accomplish their ends only to the extent to which they conform to the realities of nerve regeneration. In the light of these realities, as we now know them, many successful empirical procedures of the past find a splendid *ex post facto* justification, while, conversely, many past failures can now be traced to the fact that some peculiarities of the tissues involved or some of the subtle interrelationships just mentioned had been disregarded. It has become fairly plain by now that there can be no physical or chemical agent, no manipulation or drug, that would be universally beneficial to all component processes of nerve regeneration alike. What aids one, may hamper another. Any method of nerve repair will, therefore, have to be examined in all its consequences, and rated according to the balance of the good and harm it does. Only by wisely improving this balance sheet, can nerve repair be improved, by small steps, insignificant perhaps individually, but telling in their cumulative effects.

The capacity of a nerve to regenerate is subject to constitutional biological limitations. As in other tissue systems, the growth potential is determined by the properties of the tissue itself,¹⁸² and growth rate cannot be

raised above a certain maximum. Part of the elongation of the nerve is limited only by elasticity and compensatory growth which occurs before, if mammalian nerves are cut, free advance of about 4 mm per day, output of which they are capable of covering at much faster rates than that the nerve had not been substituted.

Methods of nerve repair have been devised, but at providing the nerve with its own potentialities for repairing. All we can do is to provide the prerequisites, and by keeping the nerve in a surprising that attempts at repair have had no success in the past. Vitamin B group¹⁶² and other factors in controlled experiments, either in animals or under thiamin deficiency, have produced excessive nerve growth after central lesions,¹⁷⁴ but the hope of doing much for the repair of the nerve, it seems that the most actively promoted is to avoid the cause of the press it.

Concerning the natural process of central wound healing, the nerve tends to run counter to the surgeon's plan. Part of the surgical effort is to create a conflict. This can be done (a) by direct suture, and (b) by direct apposition. Nerve restitution will not occur unless the nerve grows along, rather than across the gap. Nerve outgrowth, and the restoration of conduction and impulse conduction, becomes a matter of principle for the surgeon. Yet, the surgeon's aim is to alleviate the conditions, such as infection, and he must consider the pattern at the junction of the nerve, taken into account.

The perviousness of the nerve within wide limits, with its vascularization, macrophages,

raised above a certain maximum. This need not apply to the purely physical part of the elongation of the regenerating nerve fiber, the rate of which is limited only by elasticity, viscosity, and the like. It does apply, however, to assimilatory growth which supplements and supports the elongation. Therefore, if mammalian nerve fibers have never been seen to exceed a daily rate of free advance of about 4 mm. at best,⁶⁵ this presumably defines the maximum output of which they are capable under optimal conditions. Whenever recoveries at much faster rates are reported, there is reason to suspect either that the nerve had not been severed or that nerve sources closer-by had substituted.

Methods of nerve repair must, therefore, aim not at "stimulating" nerve growth, but at providing it with the conditions under which it can best realize its own potentialities. In last analysis, it is the nerve that has to do the repairing. All we can do is facilitate the job by supplying the necessary prerequisites, and by keeping out handicaps. In view of this situation, it is not surprising that attempts at "stimulating" nerve growth directly have had no success in the past. Particularly, the claimed stimulating effect of the Vitamin B group¹⁶² and biotin⁶⁸ has not been substantiated in critically controlled experiments, either *in vivo*^{50,101} or *in vitro*.^{27,29} While nerve regeneration under thiamin deficiency may be impaired,^{28,108} thiamin excess does not produce excessive nerve growth, although it does seem to facilitate recovery after central lesions,¹⁷⁴ which involves no growth. There seems to be little hope of doing much for nerve regeneration along that general line. At present, it seems that the only way in which nerve regeneration can be effectively promoted is to avoid the natural and clinical hazards that tend to depress it.

Concerning the natural hazards, it must be borne in mind that the general wound healing tendency of the body, by leading to cicatrization, happens to run counter to what would be in the best interest of nerve healing. Part of the surgical effort must, therefore, be devoted to eliminating this conflict. This can be done (a) by holding scar-provoking factors to a minimum, and (b) by directing the unavoidable remainder into a course where nerve restitution will be harmed least, for instance, by forcing scar tissue to grow along, rather than into, the nerve. A nerve scar not only distracts and bars nerve outgrowth, but also is apt to interfere with subsequent maturation and impulse conduction in regenerated fibers. Its avoidance thus becomes a matter of prime concern and has always been recognized as such by the surgeon. Yet, the surgical intervention in itself may intensify rather than alleviate the condition. Trauma, introduction of foreign suture materials, infection, and hemorrhage affect the volume of the scar, while the stress pattern at the junction determines its prevailing orientation. Both must be taken into account.

The perviousness of the distal stump to regenerating fibers may fluctuate within wide limits, with pressure, past history of Wallerian degeneration, vascularization, macrophage activity, and perhaps stretch, being the major

variables. Depending on the condition of the distal stump, the rate and volume of nerve fiber regeneration will vary, which in turn will determine how soon and how well the muscle will be reinnervated.

Escape of fiber branches at the suture line will not only produce painful neuromas, but may, if excessive, drain the growth potential of the whole nerve and thus reduce its capacity for functional recovery. The point that such fiber escape should be precluded is therefore well taken.

These examples may suffice to illustrate our contention that the way to improve nerve repair is not by trying to "stimulate" nerve growth, but by eliminating the handicaps which would ordinarily be in its way. These handicaps operate on a microscopic rather than macroscopic plane, and we can avoid them only after we have learned to recognize them.

SUTURELESS TUBULATION

Reunion of severed stumps by means of a fitting cuff of artery or other suitable material without the use of sutures has certain unique advantages for nerve regeneration, which other methods do not provide to an equal degree. This method, referred to for brevity as "tubular splicing" or "sleeve splicing," has thus far been tested in laboratory animals only, but the lessons of the experiments are unquestionably applicable to clinical practice. The experiments, which have been described in detail elsewhere,^{186,189,193,200} will be reviewed here only briefly. However, some supplementary information not otherwise published will be added.

The main purpose and effect of sleeve splicing is to prevent the formation of a "suture line." Ordinarily, the suture line represents a zone in which the organization of the nerve is definitely and permanently abnormal. Here the nerve fibers are forced to branch profusely and to stray and commingle at random, with the consequences outlined above, while fibrous tissue penetrating from the sheath and from extraneous sources may make the link so compact as to interfere with growth, maturation, and perhaps conduction, of the regenerated fibers. In contrast, the union tissue of sleeve-spliced nerve stumps assumes the same organization as the rest of the nerve, so much so that in successful cases the transition between the former stumps is perfectly smooth.

Sleeve splicing owes its success to three main facts: (1) absence of sutures; (2) transmission of tension between the stumps and the newly forming union tissue; (3) confinement of fluid within the gap and the union tissue. A thorough histomechanical analysis²⁰⁰ of the healing process after end-to-end union with and without tubular splicing has demonstrated that if the tissue linking the nerve stumps is to become a well organized nerve segment, rather than a scar, longitudinal stress and high liquid content are indispensable prerequisites.

This may be explained here briefly by the example of an arterial splice, supposing that we are dealing with a case in which the nerve stumps can be closely approximated without stretch. The nerve ends are joined by placing

them inside a fitting cuff but are left separate and are filled with blood. The cuff undergoes a remarkable contraction of importance for the outcome. The following events occur:

(1) The fibrin of the blood clots between the stumps (Fig. 5, 1).

(2) The erythrocytes are squeezed out of the framework with liquid.

(3) The clot detaches

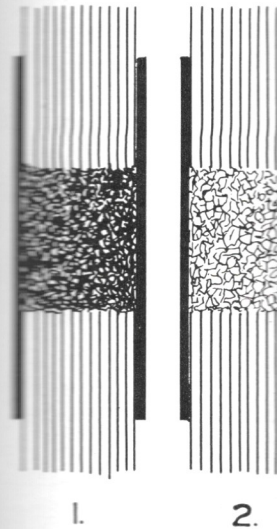


FIG. 5. Transformation of a blood clot. The two phases of the process represent the stages of the process. 1, Blood clot between the stumps. 2, Shrinkage of clot (plain arrow). 3, Tensional orientation of fibrin. 4, Tensional orientation of unstretched fibrin. 5, Outgrowth of fibrin.

ing to the nerve ends between the stumps and tension acting on the stresses arise in the clot.

(4) These longitudinal stress patterns in general are predominantly longitudinal threads which span the

(5) Fibrinolytic activity of leukocytes and possi-

them inside a fitting cuff of artery. The cut surfaces are not brought in contact but are left separated by a gap of a few millimeters, which becomes filled with blood. During the days following the operation, this blood clot undergoes a remarkable transformation, the course of which is of crucial importance for the outcome of the ensuing processes of nerve regeneration. The following events occur in rapid succession²⁰⁰ (Fig. 5).

(1) The fibrin of the blood clot in the gap fuses firmly with the nerve stumps (Fig. 5, 1).

(2) The erythrocytes of the clot disintegrate, leaving a spongy fibrin framework with liquid-filled meshes behind (Fig. 5, 2).

(3) The clot detaches itself from the wall of the sleeve, while firmly stick-

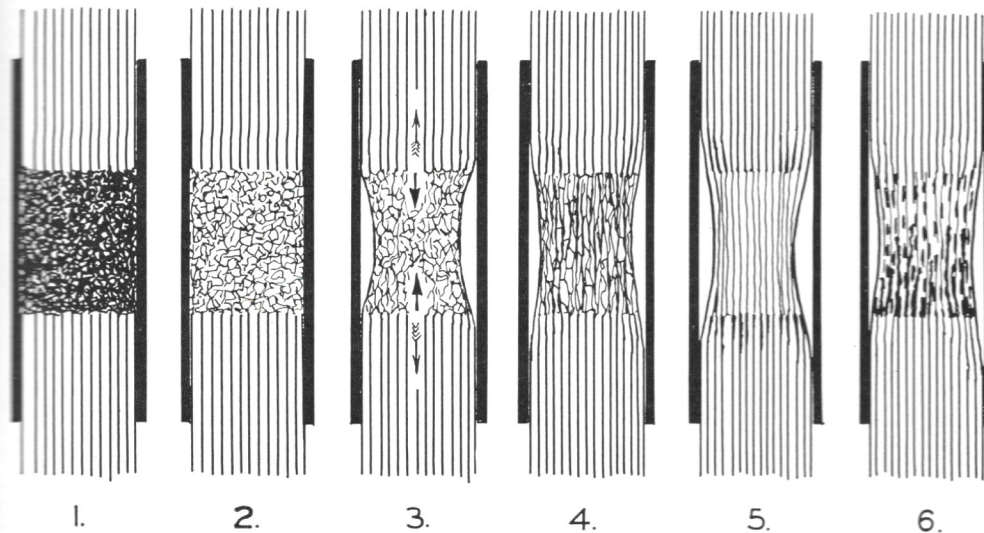


FIG. 5. Transformation of the union tissue between sleeve-spliced nerve stumps. The illustrated phases of the process represent operative, rather than chronological, steps. Chronologically the various phases overlap. 1, Blood clot in gap. 2, Fibrin formation. 3, Lateral detachment of union tissue; syneretic shrinkage of clot (plain arrows) and traction from stumps (feathered arrows) lead to longitudinal tension. 4, Tensional orientation of the fibrin network (corresponding to Fig. 4, *A* and *D*). 5, Liquefaction of unstretched fibrin. 6, Outgrowth of sheath cells and axons through the oriented fibrin matrix.

ing to the nerve ends (Fig. 5, 3). It thus constitutes a mechanical link between the stumps and hence becomes subject to longitudinal traction. Any tension acting on the nerve transmits itself directly to the clot. Similar stresses arise in the clot from the shrinkage attending its syneresis.

(4) These longitudinal stresses act in the manner described above for stress patterns in general. They force the fibrin elements into a predominantly longitudinal orientation, resulting in the formation of straight fibrin threads which span the gap from one nerve end to the other (Fig. 5, 4, 5).

(5) Fibrinolytic enzymes, liberated presumably from disintegrating leukocytes and possibly also exuded from the nerve stumps, destroy all

finer cross links of the fibrin reticulum, while sparing the heavier, longitudinally oriented threads (Fig. 5, 5).

Through this chain of processes the nerve stumps become reconnected within less than a week by a system of straight parallel fibrin strands. Except for these threads, the space of the former gap is still filled with fluid, partly fibrinolysate, partly perhaps nerve exudate,¹⁸⁷ which the impermeable sleeve prevents from dissipating.

It is in this setting, then, that the outgrowth of sheath cells, axons and capillaries takes place. As these elements are bound to move along the existing solid-liquid interfaces, the fibrin threads serve them readily as climbing ropes (Fig. 5, 6), and since the latter span the original gap in straight lines, the most direct and unconfused transit of the regenerating nerve fibers and accessory tissue components from stump to stump is made possible. As a result, the outgrowing fibers and cells glide over the fibrin rails of the union tissue with great ease, thus retracing the parallel longitudinal lines of the fibrin pattern which, in turn, are a product of the prevailing mechanical stresses. The abundance of interstitial liquid, on the other hand, insures ample space for the advancing tissue mass in the sense indicated previously.

As one can see, the effects of sleeve splicing on subsequent nerve regeneration are subtle and manifold. Specifically the following features stand out.

(1) By insulating the junction, the sleeve prevents the young union tissue which fills the gap from adhering to any structures but the nerve stumps themselves.

(2) By the tensions transmitted directly from one stump to the other through the union tissue, the latter is molded into parallel fiber strands corresponding to, and continuous with, those of the nerve stumps.

(3) This pathway system is free from obstructions, and interlacing of its elements remains at a minimum. Consequently, neither the regenerating axons nor the sheath cell cords are subject to the extensive branching and straying typical of ordinary nerve scars.

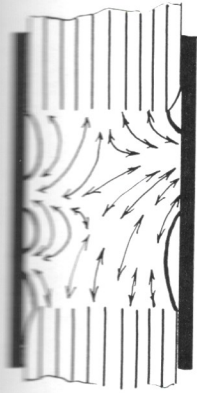
(4) The longitudinal organization of the union tissue directs the growth of the intraneural blood vessels straight across the gap and thus effects prompt vascular reconnection between the stumps.

(5) Fibrous connective tissue surrounding the nerve is likewise forced into a longitudinal course, where it develops into a useful sheath around the junction, instead of penetrating into the interior, where it would deflect and block nerve fiber growth.

(6) By suppressing the formation of communications between the inside of the nerve and the surrounding tissues, the sleeve not only precludes the intrusion of extraneous scar tissue into the nerve, but also the escape of regenerating fibers (neuromas) and sheath cells (gliomas) from it.

(7) Acting as a tight container for the liquid in the union tissue, the sleeve insures the necessary space for the passage of nerve fibers in adequate numbers and for their later unimpeded lateral expansion.

(8) Owing to the lines, the regenerating fibers, the regenerating sheath cells, and the regenerating capillaries, as far as is initially preserved, in order to evaluate the results of the sleeve for further advances in



A

FIG. 6. Examples of distortion of the matrix (B) after sleeve splicing (A). Distorting effect of pores of the matrix (C) on the orientation of the matrix.

to assess the various features of the sleeve for final functional performance of a sleeve-spliced nerve. Another means is spectroscopy, which compares the former over the latter. This discussion aims to find methods. (The various methods are listed.)

1. *Insulation.* To insure that the sleeve has been the purpose of the repair (see below). In many cases the resorption or perforation of the sleeve is more than perfunctory insulation. The sleeve operates so much as a barrier that it forces scar growth into the gap, though a closely fitting

(8) Owing to the straightness and parallel arrangement of the guide lines, the regenerating fibers traverse the gap in essentially the same assemblage in which they emerge from the proximal stump. Hence, functional grouping, as far as it is expressed in topographical grouping, is at least partially preserved, in contrast to its complete disruption at ordinary suture scars.

In order to evaluate the method of sleeve splicing, and as a possible guide for further advances in the technique of nerve reunion, it seems advisable to

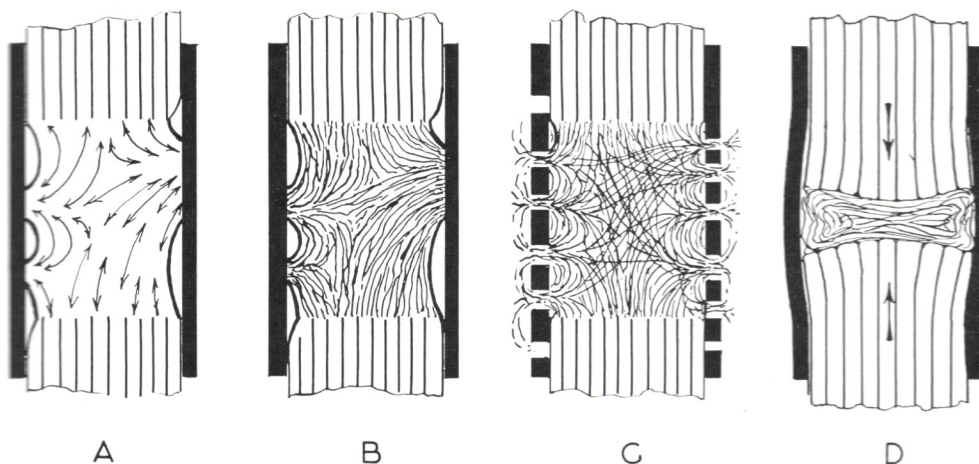


FIG. 6. Examples of disorganization of the union tissue. *A, B*, Stress pattern (*A*) and resulting structure of the matrix (*B*) after incomplete lateral detachment of the union tissue from the sleeve wall. *C*, Distorting effect of pores or holes in the sleeve wall. The tissue is drawn toward the breaks. *D*, Transverse orientation of the matrix as a result of pressure from the nerve ends (arrows).

assess the various features listed above critically in regard to their significance for final functional restoration. While the contrast between the orderliness of a sleeve-spliced union and the confusion at junctions effected by other means is spectacular, this does not in itself prove the superiority of the former over the latter in practical respects. The following point-for-point discussion aims to facilitate a comparison of sleeve-splicing with other methods. (The various points will be dealt with in the order of the above listing.)

1. *Insulation.* To insulate a suture line against ingrowth of fibrous tissue, has been the purpose of many wrapping and tubulation attempts of the past (see below). In many cases, these attempts were half measures in that the resorption or perforation of the materials used failed to provide more than perfunctory insulation. The sleeve as used in sleeve splicing does not operate so much as a mechanical shield as by creating conditions which will force scar growth into a longitudinal instead of transversal course. Even though a closely fitting sleeve precludes the intrusion of extraneous tissue,

the epineurial sheath inside the sleeve is an ever present source of fibrous tissue, and if it fails to cause trouble in sleeve-spliced nerves, this is because the longitudinal stress pattern diverts the proliferated material into a longitudinal course along the surface of the nerve. Accordingly, any intervention or postoperative occurrence that upsets the longitudinal stress pattern is bound to defeat the purpose of shielding.

Most common failures of this kind arise from adhesions between union tissue and the covering cuff (Fig. 6A, B). Such adhesions produce tension lines directed from the surface into the interior of the gap; hence, they lay down direct inroads for the penetration of epineurial tissue into the nerve (Fig. 6B). The inner lining of the cuff must, therefore, be of such a nature that the blood clot will either not adhere to it from the very first, or, at least, become detached shortly after by fibrinolysis. Arterial tubes satisfy this condition. So do sleeves of tantalum metal,¹⁹³ the use of which will be described below. On the other hand, coverings made from materials capable of fusing with the fibrin of the blood clot would fail in this respect.

It is equally futile to expect a longitudinal stress pattern to develop after sheathing the union with cuffs that either are porous or, though initially tight, undergo rapid erosion and resorption (Fig. 6C). Any hole or fissure in a sleeve is apt to become, first, an anchor point for the stress pattern; secondarily, a center upon which fibrin fibers will converge; and ultimately, an entrance point for extraneous connective tissue as well as an escape route for regenerating sheath cells and axons. Materials sufficiently resistant to withstand resorption for several weeks may, however, be acceptable; for during that period the union tissue will have assumed its longitudinal orientation, a continuous fibrous sheath will have covered the junction, and after the sleeve has thus served its purpose, it may be dispensed with.

For similar reasons, a coat of blood plasma such as is used in the so-called "plasma suture"^{64, 207} of nerve cannot be expected to serve as a real sleeve in the sense here emphasized. For the surface coat of plasma and the blood clot in the gap are bound to merge into a single mass, particularly as some suture plasma is apt to seep in between the cut surfaces. The syneresis of such an irregular mass will produce an irregular tension pattern, leading to corresponding irregularities of the subsequent nerve and scar growth. The marked contrast in microscopic appearance between junctions after plasma suture and after sleeve splicing bears out this contention.*

In conclusion, only if there is no adhesion between clot and sleeve, can a strictly longitudinal tension pattern develop.

2. *Tension.* Mechanical considerations intimate that the most potent factor in the creation of tensions at the junction is the syneretic shrinkage of

* A semidiagrammatic illustration of a number of suture lines after plasma suture may be found in the article of Gutmann and Sanders⁶⁷; for illustrations of sleeve-spliced junctions, see the figures in Weiss,^{186, 189, 190, 193} and Weiss and Taylor.^{199, 200} An improved picture of a plasma union was presented by Tarlov¹⁶⁵ at the 1944 meeting of The Harvey Cushing Society.

the clot itself. If the nerve tends to retract, the tendency of the clot, by its syneresis, to contract, and the tendency of the nerve to retract, are in the same direction. No such stresses can be expected to develop between the nerve stump and the union tissue. The first of the two postulates can be satisfied by the union tissue itself. The second, that the union tissue be placed under sufficient tension to keep the nerve ends from retracting, is satisfied by the strain on the union tissue. The union tissue will show less of this tendency to retract if it is applied more than just the tension of the nerve traction along the nerve. The union tissue, being the longitudinal organ,

The optimal size of the union tissue, in terms of syneretic effectiveness with respect to nerve fiber regeneration, is an optimum length of approximately 1 cm. Even though successful unions can be made with specially prepared blood clots, the less strict will be the results.

If the nerve ends are separated by a mediary blood clot, the rule to avoid flanging of the nerve ends, the rule of the union tissue produces a defect (Fig. 6D). The union tissue orientation tends to divert the nerve ends from guiding them lengthwise. The nerve ends are not in immediate contact with the clot, whenever they press against it. Obviously, it is the fate of the nerve ends under pressure.

Sutures at the nerve ends, and the use of nerve stamps, are objectionable. The chief defect is that they prevent the nerve ends from retracting. Even if foreign body reaction is avoided, the suture material; even if the nerve ends are in contact with the neurium, and even if we assume that the suture represents a potential pathway for regenerating fibers, in the long run the consequence of suturing

the clot itself. If the nerve stumps are sufficiently taut to resist the contractile tendency of the clot, the desired longitudinal stresses arise in the latter. No such stresses can be expected between slack nerve stumps. From these facts two postulates can be derived. First, there should be a small blood clot between the nerve stumps not only as a cementing agent but primarily as a foundation for the union tissue, to orient the fibrin bridge between the stumps by its syneresis. Second, the nerve stumps must not be allowed to yield to the contraction of the union tissue. Whether this latter provision requires that the union be placed under active tension, remains to be seen. It may be sufficient to keep the nerve just free from slack. The tendency of freshly transected nerves to retract after the operation will presumably place enough strain on the union tissue to produce satisfactory orientation. Older lesions will show less of this tendency, but even in these it may be unnecessary to apply more than just the gentlest stretch. Later, as the union becomes firmer, traction along the nerve may be allowed to increase as a means of accentuating the longitudinal organization of the union.

The optimal size of the blood gap is an empirical problem. Too short, its syneretic effectiveness will be low; too large, it presents increasing hazards to nerve fiber regeneration. The hundreds of cases thus far studied indicate an optimum length of approximately once or twice the diameter of the nerve. Even though successful regeneration through much larger gaps, filled with specially prepared blood, has been obtained (see below), the longer the gap, the less strict will be the tensional organization of the union tissue.

If the nerve ends are pressed against each other, with or without intermediary blood clot, the result may be serious. It has long been an empirical rule to avoid flanging of the nerve ends in end-to-end sutures.⁸ The soundness of the rule becomes evident if one realizes that any axial pressure against the union tissue produces stresses directed at right angles to the nerve axis (Fig. 6D). The union tissue will therefore assume a prevailing transverse orientation tending to deflect and stop the regenerating nerve fibers instead of guiding them lengthwise to the distal stump. Even if the nerve cross sections are not in immediate contact, but are left separated by a small blood clot, whenever they press toward each other, the result will be the same.²⁰⁰ Obviously, it is the fate of many end-to-end sutures to create just this kind of pressure.

Sutures at the nerve junction, that is, stitches placed near the rims of the stumps, are objectionable for a variety of reasons, but their most serious defect is that they prevent the formation of a longitudinal stress pattern. Even if foreign body reactions can be wholly avoided by the use of inert suture material; even if the stitches can be strictly confined to the epineurium, and even if we disregard the fact that each thread or wire stitch represents a potential path of penetration for fibrous tissue and of escape for regenerating fibers, interference with the stress pattern is an unavoidable consequence of suturing. For one thing, the drawing together of the stumps

gives rise to that undesirable transverse pressure pattern just mentioned. Moreover, since all traction between the stumps is transmitted through the rigid suture threads, it bypasses the union tissue at the suture line and deprives it of the beneficial influence of longitudinal tension. The tensional chaos prevailing at a junction that has been protected by sutures against traction finds its visible expression in the disorganization of the resulting "suture line."

Therefore, from the theoretical as well as the experimental standpoint, omission of sutures near the cut surfaces of nerve stumps seems a prerequisite to optimal regeneration. In practice, forcible approximation of the stumps is often a necessity. However, this can still be effected without suturing at the very level of the junction, simply by placing stay sutures or sling stitches through the nerve stumps at such a distance from the ends that the junction itself remains unaffected. Only the excess tension on the stumps should be taken up in this way, leaving enough available for the free nerve ends to straighten the young union tissue in the gap (see below).

In conclusion, sleeve splicing can have the desired effect only if the nerve stumps are not slack and if the union tissue is enabled to act as the sole mechanical link between the stumps. This implies omission of any sort of rigid sutures between the nerve ends.

3. Growth Orientation. By aligning the fibrin strands lengthwise, the longitudinal stress pattern gives rise to two important effects: it prepares the shortest direct route across the gap for the outgrowing sheath cells and axons, and it removes a most potent incentive to axon branching, namely, intersections in the fibrin matrix. This fact is plainly evident in the microscopic pictures of sleeve-spliced nerves.²⁰⁰ It is fully borne out by actual fiber counts, which have yielded substantially similar figures for the proximal stump, the region of the junction, and the distal stump.¹⁸⁶

While sleeve splicing thus definitely reduces branching and straying of fibers to negligible proportions, the question remains as to how much significance one should attach to this fact. The answer will depend on how serious a drawback to nerve regeneration profuse branching and straying of nerve fibers at an ordinary suture line really is. That it may affect functional restoration, will be shown below. What concerns us here is whether it affects morphological repair of the nerve.

The problem can be treated only in statistical terms. If regenerating axons remained unbranched, the proportion of fibers reaching the distal stump would be inversely related to the frequency of diversions in the scar. On the other hand, since intersecting pathways also increase the incidence of axon bifurcation, loss by diversion might be compensated by amplification of the fiber mass by branching. Nerve regeneration as a whole might, therefore, suffer no net numerical loss, provided that all axon branches possess the same power of growth, no matter how many of them there are to a single neuron. However, there is good reason to doubt this premise. While our factual information on this point is still very fragmentary, numerical studies

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5. Prevention of Sc been stressed as imp while the disorienting is a definite handicap. later phases introduce those nerve fibers that

on nerve regeneration,¹⁹⁵ as well as the realization that the axon grows essentially by synthesis from the cell body,^{82,202} seem to indicate that the growth energy of a neuron is not unlimited. Consequently, the vigor of growth of a branch which must share the limited resources of the neuron with a number of sister branches is likely to be less than that of an unbranched sprout. When, as occasionally happens, more than one branch of the same neuron reaches the periphery (e.g. in nerves showing so-called "axon reflexes") it is quite conceivable that such multiple branches may never grow to full size. Consequently, if a given neuron can support only a limited amount of axoplasm, the dissipation of a major fraction of it in the form of abortive branches in the scar would evidently stunt the growth of their sister branches that happen to proceed into the periphery. Such branches will presumably grow more slowly, be more easily arrested by obstacles, and retain small caliber.

In conclusion, the oriented pathway system produced by the longitudinal stress pattern is of dual benefit to nerve regeneration: in a positive way, by guiding sheath cells and axons across the gap; in a negative way, by reducing the incidence of branching and straying, which, statistically speaking, affect regeneration adversely.

4. *Vascularization.* The blood supply of peripheral nerves is furnished by a continuous system of anastomosing longitudinal vessels fed by arteries which enter at a few points along the course of the nerve.^{2,12} In view of the continuity of the longitudinal neural vessels, any one of these collateral sources may be eliminated without harm to the nerve.³ Therefore, the fact that a splicing sleeve over a nerve junction might permanently bar one such source from reentry into the nerve is wholly irrelevant. The union tissue can derive abundant vascularization from intraneural channels and does not require local extraneural contributions. On the contrary, where local ingrowth of blood vessels occurs, as, for instance, in many unshielded junctions, it is frequently attended by fibrosis and strong adhesions. Moreover, it has never been established whether or not in such cases a direct vascular reconnection between the severed nerve stumps is effected, nor can the possibility be ignored that lack of vascular continuity might under certain circumstances impair nerve function.

In contrast, sleeve splicing facilitates vascular reconnection between the stumps. When invading the union tissue, the vascular sprouts join the other regenerating elements in assuming a predominantly longitudinal course.^{200,201} Circulation between the two stumps is thus restored promptly, sometimes within a matter of days.

5. *Prevention of Scars.* Ingrowth of scarifying connective tissue has often been stressed as impeding the advance of regenerating nerve fibers. But while the disorienting effect of the scar during the earlier regeneration phase is a definite handicap, its progressive condensation and contraction during later phases introduces even more serious difficulties by tending to strangle those nerve fibers that have passed through. As was mentioned above, local

constriction of a nerve makes it impossible for those parts of the nerve fibers distal to the affected level to regain proper caliber and myelination.²⁰² Such underdeveloped fibers are not necessarily unfit for functional use, as regenerated fibers generally resume functional activity long before they have fully matured.⁶⁷ However, again in statistical terms, recovery may be assumed to suffer in proportion to the weakness of the fibers. Moreover, excessive scar contraction is apt to produce pressure block of conduction through the strangled region.

In conclusion, by deflecting scar tissue into a longitudinal course, where its contraction cannot encroach upon the nerve, sleeve splicing eliminates a serious hazard.

6. *Suppression of Neuromas and Gliomas.* Nerve fibers and Schwann cells moving into a random scar are not stopped short but may keep on roaming for some time without direction, giving rise to the bulging terminal neuromas of the proximal stump and gliomas ("schwannomas") of the distal stump. The character of these swellings depends largely on the accidental configuration of their environment. If the scar tissue is frayed and radiates into the adhering tissues, nerve fibers and Schwann cells escaping from the nerve ends will infiltrate the surrounding parts. Where the nerve ends have become capped by a fibrous layer, and particularly when edema fluid has accumulated, the trapped nerve fibers are forced to keep circling inside.¹⁸⁷ Either mode of distraction leads to two undesirable effects: hyperaesthesia or pain at the site of the neuroma, and dissipation of nerve fibers which would otherwise be available for regeneration.

Fiber counts of regenerated nerves from which fibers have escaped during the passage from the proximal into the distal stump¹⁹⁵ have shown that such losses are irretrievable and may lead to a corresponding deficit in the peripheral reinnervation. Unprotected suture lines offer opportunities for such fiber escape, not in the crude manner in which it is sometimes stated, by leaving "holes" open for the exit of nerve fibers, but because they favor the formation of fibrous and vascular connections between the interior and exterior of the nerve. As was stressed before, nerve fibers do not grow through openings as such, but only along solid supporting structures. A suture line may, therefore, be macroscopically faultless, with the epineurium tightly closed all around the nerve, and yet, the connective-tissue strands and vessels which will penetrate along the suture threads and between the apposed rims of the epineurium will establish escape routes from the interior. To prevent fiber escape becomes, therefore, a matter of preventing the formation of pathways linking the inside with the outside of a nerve. A splicing sleeve serves this purpose provided it is and remains unperforated and impervious to connective tissue until the union tissue has become organized and the mass of regenerating fibers has passed the critical level. The same considerations apply here as discussed above under point (1). Sleeves from materials undergoing rapid resorption will be no insurance against local fiber escape unless they can force the replacing connective-tissue elements to array themselves

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The suppression of Schwann cell overgrowth in sleeve-spliced nerves is remarkable. It seems that Schwann cell cords growing forth from the cut ends of a nerve tend to proceed until they meet other Schwann cells and then stop. Chances of encounter are obviously lower in the tangle of a suture scar than along the straight rails of a sleeve-spliced union. Therefore, Schwann cell proliferation is excessive in the former case, while in the latter it stops after repopulating the straight lines of the union tissue.²⁰⁰

In conclusion, sleeve splicing, properly executed, does benefit nerve regeneration by preventing fiber dissipation at the site of the lesion.

7. *Confinement of Liquid.* The necessity of according regenerating nerve fibers sufficient space in which to expand was stressed above. The transformed (degenerated) distal stump provides space approximately equivalent to its own original fiber complement. With an increasing period of denervation, it shrinks⁸² and becomes correspondingly less favorable for neurotization. Even with this limitation, spatial conditions in the distal stump are essentially adequate. This is not necessarily true, however, of the union tissue between the stumps. Originally, the liquid content of this tissue is high,⁹⁶ fed by syneresis, hemolysis, fibrinolysis,²⁰⁰ and endoneurial fluid.¹⁸⁷ However, unless this liquid is prevented from oozing out, the solid constituents at the junction will become increasingly denser, occupying more and more of the space that should be kept open for the passage and later expansion of nerve fibers. Thus, even without intrusions from extraneous sources, the tissue at the junction would act like a scar, that is, contract and impede fiber growth.

In conclusion, by providing for the retention of fluid in the young union tissue until the nerve has become reconstituted, impermeable sleeves perform a distinct service to regeneration.

8. *Functional Reorganization.* Functional order, i.e., motor coordination and sensory discrimination, can be restored only to the extent to which either the process of regeneration brings fibers back to their own old terminations or the central nervous system can adjust its functions in accordance with the new pattern of connections. As the former condition is practically unattainable, functional recovery must rely largely on functional readjustments (see above). Critical observers describe the scope within which these can be achieved as rather limited. Much will depend on how much of the original orderliness of the peripheral nerve fiber pattern can be preserved during regeneration. Even though most fibers will end at new terminations, there may be a distinct advantage in leaving them at least assorted in groups, on the premise that it should be easier for a given central cell mass that used to act as a unit to change its functional role as a whole, than for it to reassign to each one of its elements a new role.

While the criss-crossing of fibers at ordinary suture lines dissolves all for-

mer functional grouping of the regenerating fibers, sleeve splicing permits them to retain some degree of their original associations. This has been proven both by histological^{186,200} and physiological¹⁸⁹ studies of nerves regenerated after sleeve splicing: Individual fascicles of the proximal nerve have become reconnected with circumscribed muscle groups, proving that their fiber complement has remained essentially assembled during the transit into the distal stump.

In conclusion, greater orderliness of connections is rendered possible by sleeve splicing. On theoretical grounds, this should facilitate functional re-education.

The preceding discussion has outlined eight crucial features to which sleeve splicing owes its success. It should be remembered, however, that all conclusions are based on experiments in animals only. So far as these experiments go, the method has proved its value. Not only in small animals,¹⁹⁵ but

TABLE 2*

Specimen	Nerve	Graft	Splicing Artery	Time Months	Muscles	Weight			
						Operated Side (O) g	Control Side (C) g	Difference (O-C) g	Percentage Recovery
cat #3	tibial	+	f.-d.	6	gastrocn.	13.9	14.7	-0.8	95%
	peron.	+	f.-d.	6	peron.	5.7	6.3	-0.6	90%
cat #6	tibial	+	live	12	plantar flexors	36.5	38.5	-2.0	95%
	peron.	+	live	12	dorsi-flexors	16.4	16.7	-0.3	98%
monkey #21	tibial	-	f.-d.	6	plantar flexors	56.0	52.0	+4.0	108%

* The table lists all cases in which terminal muscle weights have been determined. "f.-d." is abbreviation for "frozen-dried."

in cats and monkeys as well,^{189,190} have paralyzed muscles after reinnervation through sleeve-spliced nerves recovered nearly their full volume (in terms of the corresponding muscle of the control side), even with the interposition of a frozen-dried nerve graft (Table 2). Failures have occurred, but they could always be traced to faulty technique.¹⁸⁶ When properly executed, sleeve splicing yields sure success in all animal groups thus far tested:

TECHNIQUES OF NERVE REUNION

Artery Cuffs. The clinical success of sleeve splicing will depend on how closely its crucial features can be reproduced in the human subject, as well as on the readiness with which cuffing material of the proper specifications becomes available.

Arteries, while perfecting the final solution for human use, previously, one of which was to surround the nerve so as to prevent its being subjected to the same stresses by subjecting the arteries of rats, cats, rabbits, and dogs. These arteries have, on the other hand, been frozen-dried arterial sleeves just as elastic and handleable as fresh arteries can be kept for many months. A wide store of assorted sizes of sleeves can be made a stored sleeve re-

These advantages are not to be compared between frozen-dried sleeves and those of live arteries. Since the union tissue are not as strong as the pattern of regeneration and through such breakage and get lost in the volume of regeneration, the use of potentially painful hypodermic needles to be considered. A biochemical species of sleeve furnishes the most suitable material in point of size, and yield from a single specimen of arteries are less desirable. They have proved too flabby.

Tantalum Cuffs. In the case of arteries, more easily prepared than the agent. As will be shown, the use of tantalum for nerve tubulization is a possible solution. Success in full elasticity could be obtained with a thin foil. By proper handling, cylindrical rolls of tantalum in intimate contact by their ends, tightly sealed even to the point of elasticity and friction.

Technically, such cuffs can be made much the same manner as a sleeve junction is straight and smooth. Biologically, their presence are inert and provoke

Arteries, while performing excellently in laboratory animals, may not be the final solution for human nerves. Some difficulties have been pointed out previously, one of which is that fresh arteries sometimes tend to tighten around the nerve so as to produce constriction.¹⁸⁶ This can be circumvented by subjecting the arteries before use to quick-freezing and drying. Nerves of rats, cats, rabbits, and monkeys spliced with frozen-dried and rehydrated arteries have, on the whole, shown excellent functional recoveries.^{189,190} Frozen-dried arterial sleeves become permanently incorporated. They are just as elastic and handle just as easily as fresh arteries. The fact that they can be kept for many months in the dry state makes it possible to keep a store of assorted sizes on hand. Short rehydration is all that is needed to make a stored sleeve ready for use.

These advantages are partly offset by certain weaknesses. Adhesions between frozen-dried sleeves and their surroundings are more extensive than those of live arteries. Similarly, local adhesions between their inner wall and the union tissue are not uncommon, with the result that the otherwise straight pattern of regeneration suffers sporadic disturbances. Local erosions occur, and through such breaks an appreciable number of fiber branches may penetrate and get lost in the wall of the sleeve. While this need not affect the volume of regeneration, it might leave the junction clinically in a state of potentially painful hyperaesthesia. Moreover, there is still the supply problem to be considered. As the freezing-drying procedure does not abolish the biochemical species differential, only human arteries can be used. Aorta furnishes the most suitable sleeve, but only fetal or early infant aorta qualifies in point of size, and since the sleeve must be free of side branches, the yield from a single specimen is rarely more than three pieces. Other types of arteries are less desirable because of their heavy muscular wall.¹⁸⁶ Veins have proved too flabby, at least in animal experimentation.^{186,189}

Tantalum Cuffs. It thus became advisable to look for a substitute for arteries, more easily procurable, without being less effective as a splicing agent. As will be shown below, none of the materials suggested in the past for nerve tubulization could qualify. The favorable results obtained recently with the surgical use of the metal tantalum,^{25,132,152} however, pointed to a possible solution. Success came after a method was discovered¹⁹³ by which full elasticity could be conferred upon the otherwise soft and pliable tantalum foil. By proper heat treatment, the foil can be fashioned into fully resilient cylindrical rolls with overlapping edges. The overlapping parts stay in intimate contact by their own elastic pressure so that the tubes remain tightly sealed even though their width is adjustable within the limits of elasticity and friction.

Technically, such tubes can be applied for sutureless nerve reunion in much the same manner as arterial sleeves, provided the nerve bed at the junction is straight and can be kept straight during the healing process. Biologically, their performance is equal to that of arterial sleeves.¹⁹³ They are inert and provoke little, if any, foreign body reaction. As the union tissue

does not adhere to the metal, its longitudinal orientation is assured, while the epineurial and other potentially scar-forming tissue is forced to grow down along the inner wall, where it reconstitutes a smooth cylindrical sheath over the junction. Dissipation of fluid from the interior is prevented. No scar can form at the level of the junction. Vascular connection between the nerve stumps is restored promptly. If adequately executed, such unions show practically no trace of the former break as early as five weeks after the operation in cats and monkeys. There would seem to be no objection to removing the metal sleeves after this period if this should become clinically desirable.

The main shortcomings of tantalum sleeves are their rigidity, which limits use to straight-way unions, and their opacity, which removes the junction from direct visual control. The search for an even superior material, combining the features of tantalum sleeves with greater flexibility and translucency, will therefore have to be continued.

Special Uses of Sleeves. Aside from the fact that it provides an optimal setting for nerve regeneration, sutureless sleeve splicing has certain practical advantages of some consequence. The duration of the operation is considerably shortened as compared to conventional suturing. Furthermore, even nerves of the smallest caliber, too fine for accurate suturing, can easily be mended with sleeves. Where fascicular topography is recognizable, e.g., in freshly severed nerves, separate reunion of individual bundles can be effected with ease. Nerve lesions at branching points can be treated simply by placing the common proximal stump into one end, and the several distal stumps into the other end of a sleeve. Sleeve splicing, thus, not only simplifies the technique of nerve repair, but also makes repair feasible in cases that would defy other less subtle methods.

Sleeve splicing is of particular significance in connection with the use of nerve grafts for the bridging of large defects. Much of the blame for the failure of grafts can unhesitatingly be ascribed to the fact that they were improperly joined to the nerve stumps. If a single scarified suture line after end-to-end suture can impair nerve regeneration, two junctions are bound to be even worse.¹¹ The suggestion has been made to resect and resuture, in a second operation, the distal suture line.³⁶ It would seem preferable, however, to avert the formation of suture line partitions and scars in the first instance, a goal which can at least be approximated by sutureless sleeve splicing. As has been shown experimentally, sleeve-spliced nerve stumps become linked in the same orderly fashion whether they contain axons or not. Axon-free ("aneuritic") segments, such as a graft and the distal nerve stump, thus become joined by longitudinal cell strands which afford unimpeded passage for the regenerating axons when they later arrive at this level.²⁰⁰ The conditions for strict orientation of the union tissue between graft and nerve stumps are not quite so favorable as between directly opposed nerve ends because of the unavoidable slackness of the graft. Even so, the experimental tests in rats,¹⁹⁹ cats and monkeys,¹⁹⁰ using frozen-dried nerves as grafts, have demon-

strated the adequacy of the method. It simplifies the use of cable grafts. The cable can be introduced without need of attachment.

The Problem of Gaps. The problem of gaps under which the nerve ends are joined without force. This is not a new problem. The length of the gap between the ends, the loss of substance, subsequent atrophy, and reunion, the length of the gap, the gain the lost distance, the extreme flexion of joints, and the methods being practiced.^{8, 116, 117} Further stretching of the sleeve splicing. Where sutureless splicing is used after healing, it is not a problem. In such cases, the method of shutting the nerve fibers together is of holding the stumps in position.

Sleeve splicing does not require gripping power. Sutureless splicing, on the other hand, would cancel most of the advantages found in the combination of sutureless and suture for holding purposes.¹⁹¹ Both nerve stumps are held in position, and then drawn together a few millimeters of each end. This is the usual method, and the syneresis of the nerve are relied upon for the organization of the union. The union is not to be enclosed in tissue, but is secured by the approximation of sutures, but the former method is mobile. The practical difficulty is the same.

Data on two points are available: a judicious action in nerve repair, and the nerve ends in a sleeve.

As for the former problem (see also *Iders*¹³⁹ for a review), as for the rupture of nerve fibers and the slightest extension. An excessive stretch.^{78, 113, 161} The period of an individual

strated the adequacy of the sleeve-splicing technique in grafting. It also simplifies the use of cable grafts, as the multiple strands which are to make up the cable can be introduced one by one into the sleeve without further need of attachment.

The Problem of Gaps. The preceding discussion presupposes conditions under which the nerve ends can be brought and held within close range without force. This is not commonly attainable unless by special measures.¹¹⁶ The length of the gap between the nerve ends is determined by the original loss of substance, subsequent elastic retraction of the stumps, and in secondary reunion, the length to which the fibrotic ends have to be resected. To gain the lost distance, mobilization and rerouting of the nerve course, extreme flexion of joints, progressive stretching, and even shortening of bones, are being practiced.^{8,116,130,139,155} Of these measures those that do not require further stretching of the mended nerves are fully compatible with sutureless sleeve splicing. Where stretching becomes necessary, either at the time of the operation or after healing, the weakness of the link must be taken into account. In such cases, the customary suturing, even if not fully successful in shutting the nerve fibers in and scar tissue out, serves the very real function of holding the stumps in apposition.

Sleeve splicing does not of itself provide a substitute for this latter function, since sleeves must not be fitted too tightly, and hence, have only limited gripping power. Suturing of the nerve ends inside the sleeve, on the other hand, would cancel most of the benefits of the method. A solution has been found in the combination of sutureless sleeve splicing with a "remote" suture for holding purposes.¹⁹³ A single loop of thread or wire is stitched through both nerve stumps at points as remote from the ends as is technically feasible, and then drawn just tight enough to bring the cut surfaces within a few millimeters of each other. The free ends are then united by a sleeve as usual, and the syneresis of the blood clot and the slight "give" of the loop in the nerve are relied upon to allow the longitudinal stresses needed for the organization of the union tissue to develop. The tension loop is, of course, not to be enclosed in the sleeve, but to be left on the outside. Another way of securing the approximation of the stumps would be by separate stay sutures, but the former method seems preferable because it leaves the nerve mobile. The practical details of these problems remain to be worked out.

Data on two points, inadequately known at present, are indispensable for judicious action in nerve repair: the amount of stretch which nerve can tolerate without damage, and the maximum acceptable length of the gap between the nerve ends in a sleeve.

As for the former point, the clinical literature is inconclusive (see Sanders¹³⁹ for a review), as some authors approve of degrees of stretch certain to rupture nerve fibers and blood vessels, while others caution against even the slightest extension. Animal experiments have revealed the dangers of excessive stretch.^{78,113,161} During the embryonic as well as the postnatal growth period of an individual, nerves are definitely under natural stretch, as they

are "in tow" by the growing organs.¹⁸⁵ This stretch, however, is exerted very gently and is distributed evenly over the length of the nerve. These conditions, notably the steadiness and evenness of pull, are difficult to reproduce artificially during regeneration. The usual clinical precaution to apply postoperative stretch by small and gentle steps, is well taken. Nevertheless, there is an elasticity and plasticity limit beyond which stretch cannot be carried without serious damage.

In this connection, two points of great practical significance call for attention: the scale on which nerve stretch is to be measured; and the differential effect of stretch on the proximal and distal stumps. As for the former point, it is evident that stretch must be referred to the length of the portion actually undergoing extension. The stress produced by lengthening a five-inch segment by one inch will be twice that of lengthening a ten-inch segment by the same amount. Extensibility of a nerve will, therefore, have to be expressed in percentage of free length, and not in absolute units. If the maximum stretch limit were, say, ten per cent, gradual lengthening by one inch of a stump free to yield over a length of more than ten inches would cause no damage. However, that same one-inch extension would be fatal to another nerve stump that is free for no more than two inches, as this would amount to a fifty per cent extension, which is more than that short nerve piece could stand. In other words, stretch by only a fraction of an inch in one case may impose a much greater strain upon the nerve than stretch of more than one inch in another case.

What length of the nerve is free to yield will often be difficult to ascertain. While liberal mobilization at the time of the operation may free the nerve ends for a sufficient distance up and down, this makes allowance for only such stretch as will be exerted in uniting the ends. All additional stretching, however, to be applied later in the interest of straightening the joints and lengthening the nerve, deals with a wholly unpredictable situation in that adhesions formed in the meantime between the nerve and its bed will have reduced the free portions, which can yield to the stresses, to an unknown fraction of the original length.

The second point to be considered is that a degree of stretch which would rupture the nerve fibers in the proximal stump, might not interfere with nerve regeneration at all when applied to the distal stump. The pathway function of the latter could hardly be assumed to suffer from the rupturing of some neurilemmal tubes and the separation and extension of some Schwann cell columns. The ratio according to which a given amount of stretch will apportion itself between the two stumps will vary with the site of the lesion relative to the moving joint, the fixation of the stumps by adhesions, and the coefficients of extensibility, which will presumably differ for the two stumps. Thus again, each case presents a problem of its own. Moreover, aside from its effects on the nerve fibers, overstretch presents the danger of intraneural hemorrhages from ruptured vessels.

Obviously the technique must be taken into account. The preceding situation in its complexity is usually in removing nerve s

The Irreducible Gap
nerve ends, discussed earlier, which a gap becomes decidedly indispensable in deciding induced by other methods. The length of the gap and the analysis and of the resulting rate of vascular penetration retention of liquid; collagen orderliness and density of is obvious that even the from adequate for quantities them are tentative and s

With this reservation at about one to two times gaps do not seem to insist is not to imply that in successfully spanned, but with the length of the normal blood. The aspect the nerve stumps. Conclusion, pure blood plied led to the following results

Ringer's solution was and serum. The union t than under standard conditions contend with appreciable use of pure blood plasma been weak, possibly due to the fibrin pattern density of the union tissue for nerve regeneration. compactness of the tissue expansion of regenerated fibrous fibrotic foci, with those seen in ordinary signs of constriction on impairment of the recovery

* Most of these experiments

Obviously the technological realities of each case must be duly taken into account. The preceding remarks could no more than briefly expose the situation in its complexity. Continued systematic research would aid materially in removing nerve stretching from the realm of adventure.

The Irreducible Gap. The beneficial effect of a short gap between the nerve ends, discussed earlier, raises the question of the critical length beyond which a gap becomes detrimental to nerve regeneration. This knowledge is indispensable in deciding what to do about gaps that cannot be further reduced by other methods short of grafting. The main factors varying with the length of the gap and the volume of blood which fills it are: degree of syneresis and of the resulting tension; fibrin orientation; intensity of fibrinolysis; rate of vascular penetration; rate and extent of Schwann cell immigration; retention of liquid; collagen deposition; and in further consequence, the rate, orderliness and density of axon passage. With so many variables involved, it is obvious that even the many hundreds of cases of our experience are far from adequate for quantitative evaluation. Any conclusions drawn from them are tentative and subject to future revision.

With this reservation, however, the critical length of the gap can be set at about one to two times the diameter of the nerve. Proportionately longer gaps do not seem to insure optimal conditions for nerve regeneration. This is not to imply that in individual cases much greater distances cannot be successfully spanned, but statistically the prospect of success will decline with the length of the gap. The value just given refers to gaps filled with normal blood. The aspect changes when other fillings are interposed between the nerve stumps. Comparative studies with sleeves filled with Ringer's solution, pure blood plasma, and whole blood of varying composition, have led to the following results.*

Ringer's solution was evidently replaced by a coagulum of nerve exudate and serum. The union tissue forming in this matrix becomes more compact than under standard conditions, and the regenerating nerve fibers have to contend with appreciable fibrosis. This was even more pronounced after the use of pure blood plasma.²⁰⁰ In this case, syneresis of the clot seems to have been weak, possibly due to the absence of blood platelets, and the orientation of the fibrin pattern, consequently, remained incomplete; above all, the density of the union tissue exceeded by far the range considered favorable for nerve regeneration. Collagen deposition was fairly extensive, and the compactness of the tissue left no adequate liquid spaces for the lateral expansion of regenerated nerve fibers. As a result such unions showed numerous fibrotic foci, with arrested, circling and tortuous nerve fibers, not unlike those seen in ordinary suture scars, though on a smaller scale. There were signs of constriction on many fibers, which implies, as was explained above, impairment of the recovery of fiber caliber. The unfavorable performance of

* Most of these experiments have not been previously published.

blood plasma as a gap filling must evidently be ascribed partly to poor syneresis, but mostly to the fact that it lacks the vast liquefied spaces which develop in whole blood as a result of hemolysis and fibrinolysis.

In an attempt to reduce the consistency of the clot in the gap even below that of whole blood, experiments were undertaken in which fragments of the proteolytic buffy coat (leukocytes) of centrifuged blood were added to whole blood in the splicing sleeve. In these experiments, gaps measuring several centimeters were left between the nerve ends and filled with the modified blood. Three cat nerves so treated gave fair functional regeneration; muscle volume had returned to 71 per cent of normal in one animal which died after $4\frac{1}{2}$ months, an interval too short for full recovery. Histologically, the picture was not nearly as good as with short gaps. The nerve fibers had regenerated in rather tortuous courses, and there was definitely more fibrous tissue between them than is common in ordinary sleeve splicing. This may have been due in part to the measurable constrictions which these long arterial sleeves gradually developed, putting the tissue inside under pressure. Or it may be that blood columns of such length, even with added leukocytes, do not undergo enough liquefaction, hence, become fibrotic. At any rate, these cases demonstrate that fairly satisfactory regeneration can be obtained with gaps as long as twenty times the diameter of the nerve. Nevertheless, the potential dangers of long gaps, particularly in larger nerves, are too great to take the risk clinically as long as other methods, including grafting, can be applied.

Plasma Union. The use of blood plasma for linking ends of severed nerves^{64, 67, 141, 163, 164, 165, 207} shares one important feature with sleeve splicing, namely, the omission of sutures near the lesion. In facility of execution, it rates about intermediate between sleeve splicing and ordinary suturing. Satisfactory results have been reported in animals. Judged by the standards outlined previously in this article, plasma suture compares with sleeve splicing about as follows.

Insulation against extraneous tissue is presumably insured by the action of longitudinal traction on the plasma coat. The resulting orientation effect is fully capable of transforming this coat into a firm sheath around the nerve. Orientation inside the union tissue is likely to be less orderly, as has been already indicated earlier in the text. More specifically, the following circumstances would seem to counteract strict longitudinal organization of the union tissue after plasma suture. In cases in which stretch is required to approximate the stumps, the plasma clot is not firm enough to withstand appreciable tension; its holding power is of the same low order as that of sleeves and hence must be supplemented, just as in the case of sleeves, by a remote tension suture. This measure deprives the union tissue of longitudinal traction and leaves syneresis of the clot the sole tension-producing factor. Since experience has shown that the syneretic shrinkage of pure plasma is very slight in comparison to that of whole blood, only negligible tensions will be generated at plasma junctions unless there is a sizable hemorrhage between the stumps.

Moreover, even the required pattern. Since whether it consists of clot, contains fibrin, i The union tissue will be dispensable for longitudinal pathway structure, w Though a certain amount after it has become re the best plasma junction.

A second and possibly unavoidable source of the nerve. The dense matrix because of lack of fiber expansion, and l paper. It cannot be re is one of the major impediments to nerve fibers regeneration after mere crushing,⁶⁴ line.

For the reasons of sleeve splicing in peripheral yet know whether or how to the features mentioned show up except statistically.

Nerve Wrapping, *operandi* of sleeve splicing the resemblance between and tubulation (vari only a superficial one. but they differ fundamentally satisfactory results obtained with the predominant method practised in the past.

The professed purpose of various descriptions is to escape of nerve fibers; the proximal into the have failed in principle conceptions of the nature from essentially macroscopic or failure are decided by and submicroscopic level that nerve repair can

Moreover, even those tensions that do develop will not have the required pattern. Since the matrix between the nerve stumps, no matter whether it consists of plasma seeping into the crevices or of a hemorrhagic clot, contains fibrin, it will merge with the surface coat into a single mass. The union tissue will therefore fail to undergo the lateral detachment indispensable for longitudinal orientation. Instead, the tensions, and hence, the pathway structure, will assume a pattern radiating in all possible directions. Though a certain amount of self-straightening may occur in the union tissue after it has become repopulated by cells, it is difficult to imagine that even the best plasma junction could ever approach the orderliness of a sleeve junction.

A second and possibly more serious defect of plasma unions is the practically unavoidable seeping of some of the plasma between the cut surfaces of the nerve. The danger inherent in the progressive condensation of this matrix because of lack of fibrinolytic enzymes, lack of liquid spaces for nerve fiber expansion, and heavy collagen deposition, was outlined earlier in this paper. It cannot be repeated too often that compactness of the union tissue is one of the major impediments of nerve regeneration. The observation that nerve fibers regenerate at significantly slower rates after plasma union than after mere crushing,^{64,65,67} may be an expression of some defects at the suture line.

For the reasons outlined, plasma suture does not seem to approximate sleeve splicing in performance. It must be stressed, however, that we do not yet know whether or how much practical significance will have to be attached to the features mentioned. It may turn out that their importance would not show up except statistically.

Nerve Wrapping, Tubulization, etc. From the description of the *modus operandi* of sleeve splicing given above, it should have become plain that the resemblance between this method and past practices of nerve wrapping and tubulation (variously called "tubulization" and "tubularization") is only a superficial one. They all have in common the sheathing in of the lesion, but they differ fundamentally in purpose and execution. Consequently, the satisfactory results of sutureless sleeve splicing do not necessarily conflict with the predominantly unfavorable opinions on those other methods, as practised in the past.^{22,130,139,155}

The professed purpose of wrapping nerve sutures with membranes of various descriptions has been to prevent penetration of scar tissue and escape of nerve fibers; that of tubulation, to pipe the regenerating fibers from the proximal into the distal stump through extensive gaps. Both methods have failed in principle because they were based on crude and inadequate conceptions of the mechanics of nerve and scar growth. Both proceeded from essentially macroscopic considerations, ignoring the fact that success or failure are decided not in macroscopic dimensions, but on the microscopic and submicroscopic level, where the cells move and operate. We now know that nerve repair cannot be compared to a plumbing job, that a semblance

of mechanical insulation of the suture site is no insurance against cicatrization, and that an empty pipeline is no suitable link over a nerve gap.

The literature on the subject is controversial and often difficult to assess because of lack of rigorous standards in executing and describing the operations, and in rating the results obtained. Table 1 on page 401 lists the principal experimental and clinical reports on the sheathing of nerve stumps, either for insulation or for the bridging of gaps. The list makes no claim to completeness. Moreover, the various entries are of very unequal value; some cover carefully controlled work, others constitute rather casual reports. Purely speculative recommendations or warnings have been omitted. In the following comments the individual articles will be referred to by their order numbers as listed in Table 1.

To consider wrapping measures first, most of them were by their very nature unfit to provide the intended safeguards against cicatrization. Some potentially scar-producing tissue, notably epineurium, remains inside of the wrapping. When the material is used in sheet form (split arteries, fascia, etc.), additional connective tissue can enter through the seam or between the overlapping edges. If the wrapping is loose, it admits further connective tissue from the ends. As was stated above, the presence of such connective tissue need not in itself be harmful to the nerve. If it can be forced into alignment along the outside of the nerve, as it is in sutureless sleeve splicing, it will not interfere with nerve regeneration. However, since in practically all wrapping techniques the nerve ends are first of all sutured, and since the very effect of suturing, for the various reasons outlined above, is to favor ingrowth from the surface into the interior of the nerve, loose wrapping over sutures is of questionable value.

If, on the other hand, one tries to minimize connective-tissue penetration by wrapping the nerve tightly, subsequent contraction of the coat may produce constrictions as serious as those of primary scars (27). Wrappings subject to early erosion and resorption are not only ineffectual but may be harmful by prolonging the proliferative period of the surrounding connective tissue. Unfavorable results must likewise be expected from materials that evoke strong foreign body reactions because of chemical incompatibility with the host tissues, or of liberation of irritating substances, or simply by mechanical irritation (rough surfaces; sharp edges and creases; etc.). However, it cannot be denied that wrapping even over sutures may have some merit if it is done with an elastic, non-constricting, non-irritative, non-porous material resistant to resorption and fragmentation (5, 9, 10, 11, 12, 14, 16, 18, 19, 21, 23, 26, 27, 37, 46, 49, 52).

In contrast, tubulation has met rather generally with clinical failure. Closer inspection of the various procedures that have been tried indicates that the execution rather than the principle was wrong. The ideal substratum for nerve regeneration is degenerated peripheral nerve, and no substitute that would even remotely approach it in adequacy has as yet been discovered. Hence, whatever the medium filling the gap between the nerve stumps

may be, growth in it graft. Yet there are d tions, the greater wil by a sufficient volum regeneration in arter potency was report meters, and there is But with increasing l and the slightest ad ress of regeneration.

The most patent

Open space between ble to place cylindri nerve unless they are nerve is almost unav which the operatio Fig. 44). Such space preclude the establi tion of the newly fo able liquid content o

Perforation and cally of solid appear the regenerating Sch tion-like action they these perforations h within the first few the lateral spaces n quick resorption (1 fore, not only fail to

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Collapse. Tubes completely occlud 39, 48).

Inadequate gap used in connection gard for, the requ has been the sugg tine, on the suppo of its "softness." 41, 42, 44). So did fat, fat mixed wit does not afford a

may be, growth in it will be inferior to that in the distal stump or even in a graft. Yet there are different degrees of inferiority, and the better the conditions, the greater will be the length of gap that can be successfully spanned by a sufficient volume of Schwann cells and nerve fibers. For example, good regeneration in arterial sleeves filled with blood with increased fibrinolytic potency was reported above to have occurred over gaps of several centimeters, and there is no reason to question similar reports of earlier authors. But with increasing length of the gap, the hazards to nerve growth increase, and the slightest additional handicap may imperil the whole further progress of regeneration.

The most patent shortcomings were the following:

Open space between the nerve and tube wall. Since it is technically impossible to place cylindrical tubes of wholly or nearly unelastic material over a nerve unless they are wider than the nerve, an open space between sleeve and nerve is almost unavoidable with such materials. Actually, this is the way in which the operation of "tubulization" is usually depicted (Stookey,¹⁵⁵ Fig. 44). Such spaces obviously admit fibrous connective tissue into the gap, preclude the establishment of a longitudinal tension pattern, lead to distortion of the newly forming union tissue and offer an outlet for the indispensable liquid content of the gap.

Perforation and resorption. Tube walls which, even though macroscopically of solid appearance, are full of microscopic pores, are bound to sponge up the regenerating Schwann cells and nerve fibers (28) because of the local suction-like action they exert on the matrix (Fig. 6C). It is immaterial whether these perforations have been present from the beginning or have developed within the first few weeks by erosion. Larger holes act as unfavorably as do the lateral spaces mentioned in the preceding paragraph. Tubes subject to quick resorption (1, 2, 5, 8, 11, 13, 29), or disintegration (6) may, therefore, not only fail to have any beneficial effects, but be actually harmful.

Foreign materials. Tubes from substances foreign and irritative to the body (22, 25, 39, 45, 47, 52) may evoke extensive inflammatory and fibrous reactions, the products of which disrupt the young union tissue and produce heavy scarring in its place.

Collapse. Tubes too flabby to retain their shape collapse and may become completely occluded, thus naturally obstructing nerve regeneration (28, 39, 48).

Inadequate gap fillings. Natural as well as artificial fillings have been used in connection with tubulation, mostly with little knowledge of, and regard for, the requirements of nerve growth. The most spectacular mistake has been the suggestion of Edinger (30) to fill tubes with agar-agar or gelatine, on the supposition that nerves would easily pervade that mass because of its "softness." The method proved a complete failure (34, 35, 36, 39, 40, 41, 42, 44). So did the fantastically unrealistic charging of tubes with dog's fat, fat mixed with lecithin, and brain pulp (39). Of course, no filling that does not afford abundant internal interfaces, such as are formed by the

precipitation of fibers, has any chance of becoming invaded by Schwann cells and axons. Any inert mass that does not have the proper chemical and colloidal properties will become blocked off from the living tissue and serve only as obstruction to growth; while any resorbable mass is apt to become replaced by dense connective tissue. Moreover, not only the original composition of the gap filling, but all its subsequent modifications as well as physical transformations must be taken into consideration. For example, a blood clot can serve as a proper gap filling only if the lysate arising from its liquefaction remains collected. The more this fluid is allowed to become dissipated, the denser and more impervious to cell and axon growth will the matrix become. This finds expression in the common experience that hemorrhage between the nerve ends is followed by fibrosis (26, 28, 39, 48). Such fibrosis could presumably be checked by providing for an adequate degree of enzymatic liquefaction of the clot and by safeguards against loss of the resulting fluid.

Some reports on tubulization speak of the space between the nerve ends as having been left "empty" (34, 35, 38, 44). As explained earlier, this is physically absurd. There must have been either air or saline solution or blood or a serous exudate present at first, but since the subsequent fate of these fortuitous fillings, undergoing absorption, transformation, replacement, etc., has remained unknown, it is impossible to determine just what conditions nerve regeneration had to contend with in these instances.

Constriction. Just as is the case with simple wrappings, tubes that are subject to gradual shrinkage may strangle the covered nerve segment, either during regeneration or later (27). Tubes that provoke heavy scar formation along their outer surface without being able to withstand the constrictive force of this scar, likewise cause strangulation.

Most of the well-intentioned measures listed above have suffered not merely from poor execution, but primarily from lack of a clear conception of how they were to attain their objectives. This is excusable in view of the fact that at the time when they were devised analytical understanding (not to be confounded with histological description) of nerve regeneration was still in a very rudimentary state. It would be less excusable, however, to continue to violate or ignore the rules that in the meantime have been revealed to govern nerve regeneration. It has been the prime purpose of this article to show that these rules cannot be formulated in terms of the macroscopic manipulations of the surgeon, but only in terms of the micromechanics of the nerve fibers, their accessory cells, and their environment. The macroscopic aspect of wrappings, scars, "obstructions," "neuromas" and the like, as such, is irrelevant and often misleading. The microscopic and submicroscopic levels are the domain in which the real mechanisms of nerve repair operate, and it is to those levels that one must look for the decision between success and failure. Devices or manipulations that look quite promising from a purely macroscopic viewpoint, often are revealed by micromechanical analysis to be

wholly absurd; hence, their use is a waste of time and effort.

The only safe procedure is for the surgeon to become familiar with the biology of the tissue in question, to come to view his manipulations in their physical and chemical consequences; to understand the mechanical consequences of tubulization as invariable results of the microstructural changes that will occur; to learn to calculate the mechanical changes, and to understand the interrelations of Schwann cells with another and with the nerve itself.

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wholly absurd; hence, will lead to equally absurd results in practical application.

The only safe guide in the long run would, therefore, seem to be for the surgeon to become so thoroughly familiar with the biology and microtechnology of the tissues concerned with nerve repair that he will automatically come to view his macroscopic manipulations in terms of their micromechanical consequences; that he will cease to think of tubes as pipes, of sheaths as shields, of connective tissue as necessarily obstructive, or of dense vascularization as invariably desirable, and will instead turn his attention to the microstructural frame in which cells and nerve fibers move. He will then learn to calculate the play of tensions, fiber coagula, synergetic and proteolytic changes, and to recognize their sources; to understand the intricate interrelations of Schwann cells, axons, macrophages, and fibroblasts with one another and with their matrix and to let his actions be guided by this insight.

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