EXPERIMENTS ON THE MECHANISM OF NERVE GROWTH

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TWENTY-NINE FIGURES

INTRODUCTION

The extreme elongation of its cytoplasm makes the neuron uniquely suited for the study of the relative roles of nucleus and cytoplasm in cell growth. For the last 6 years, one of the authors (P. W.) has carried on research on this problem with results that have shed new light on protoplasm synthesis in general. Cursory reference to these results was made on previous occasions (Weiss, '43b, p. 19; Weiss and Davis, '43, p. 277; Weiss, '44a, '44b, '47), but a full account has been purposely held up pending the conclusion of additional experimental tests. These have now been completed, but at the same time, the volume of significant data has grown to such proportions as to call for monographic treatment. Since the prospects for such a monograph are still rather indefinite, it seemed desirable to present at least a condensed record of the main results with illustrative examples. This summary account is given in the present paper.

The volume of an axon may be many hundred times that of the cell body (perikaryon) containing the nucleus. How this comparatively enormous mass can be maintained in the adult nerve fiber, and restored in the regenerating nerve fiber, is a basic problem of growth. The axon is enveloped by sheath cells over its full length and is in active exchange with sur-

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rounding blood and endoneurial fluid. The general notion is that the axon thus draws from its environment nutrients, which, in the presence of certain undefined "trophic influences" of the nucleus, can be assimilated in situ to form new protoplasm. In this view, the axon would grow over its entire length from local resources. Contrary to this supposition, the experiments to be reported here have furnished evidence that the axon grows from its base in the nucleated cell body; that synthesis of new protoplasm occurs exclusively at the nuclear site; and that this is equally true for the incremental growth of immature or regenerating axons and for the anabolic renewal of the cytoplasm in mature axons. The evidence rests mainly on experiments in which the "supply line" between nucleus and the more outlying parts of the axoplasm was throttled in various degrees by a constricting ring of artery placed around the whole nerve.

METHODS AND TERMINOLOGY

Most experiments were done with nerves of white rats (totaling over 300), with corroborating results in rabbits, chickens, and monkeys. Since the nerve fibers were examined individually, the actual number of test cases amounts to more than 100,000. In this report, only those observations will be considered which have been fully verified throughout the course of our research and which can be safely repeated. The following terminology will be used:

Perikaryon: the nucleated body of the nerve cell. Axoplasm: the cytoplasm of the axon (A, fig. 1).
Plasma membrane: the outer surface film of the axon, usually closely adhering to the inner surface of the myelin sheath. Neurokeratin: the protein framework of the myelin sheath. Schwann cells: the sheath cells enveloping the myelin sheath, but lying inside the fibrous sheath of the nerve fiber (S, fig. 1).

*In an earlier phase, Dr. A. Cecil Taylor collaborated in these experiments and his assistance is gratefully acknowledged.
Neurilemmal tube: the fibrous sheath which contains the nerve fiber (including the sheath of Henle or Key-Retzius); abbreviated "tube" (T, fig. 1).

Internode: the segment of a nerve fiber lying between two nodes of Ranvier.

Ovoids: the oval vacuoles in nerves undergoing Wallerian degeneration, containing axon and myelin debris.

Nerves were studied either in paraffin sections or in teased preparations. The former were either fixed in Bouin's solution and impregnated with silver according to Bodian or fixed in formalin and stained with osmic acid. Teased preparations were made either from living fibers immersed in Ringer's solution or from fixed specimens impregnated with silver according to Bielschowsky. The stained fibers were teased in glycerin and embedded in the same medium or in Gurr's water mounting medium. Nerve fiber calibers were measured in tracings of either teased fibers or cross sections of whole nerves, either by planimetry or by diameter determinations as described previously (Weiss, Edds and Cavanaugh, '45).

Since all experiments deal with constricted nerve fibers and their characteristic deformations, it will be expedient to use standard designations for the different regions of the experimental nerves in their relation to the constricted zone (fig. 2). An arrow marks the proximo-distal direction of the nerve. The constricted zone is referred to as C, and the parts of the nerve lying proximally and distally to it as P and D, respectively.

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**Fig. 1** Diagram of the components of a nerve fiber. A, Axis cylinder. M, Myelin sheath. S, Schwann cell. N, Nucleus of the Schwann cell. T, Fibrous sheath or "tube."
P is subdivided into $P_a$, $P_b$ and $P_c$, indicating different degrees of axon alteration: heaviest alteration in $P_c$, minor disturbance in $P_b$, and normal unaffected appearance in $P_a$. Similarly, in the distal stump, the part near C may be distinguished as $D_a$ from the more peripheral part $D_b$. All illustrations are oriented with the proximo-distal direction from left to right or from top to bottom.

![Diagram](image)

**Fig. 2** Designations of critical zones in constricted and control nerves. Explanation in text.

**RESULTS**

The diagram, figure 3, summarizes the essential features of the experiments for the case of single regenerating fibers. Rows A to E review the main phases of ordinary nerve regeneration after simple crushing. Interruption of the axon B leaves a proximal stump connected with, and a distal stump severed from, the perikaryon; the distal stump disintegrates. In C, the proximal stump begins to elongate by the active advance of its amoeboid tip. This elongation, usually referred to as "outgrowth," is primarily a phenomenon of protoplasmic movement, rather than growth in the sense of increase of protoplasmic mass. Its mechanism has been dealt with in previous publications (Weiss, '41, '44c, '45) and need not concern us here. The regenerating branch is originally only 1 micron or less in diameter, its cross section therefore only a small fraction of that of the old fiber stem (e.g., less than 1 hundredth in the case of a 10 $\mu$ fiber). In D, the advancing tip has reached and become connected with the peripheral end organ, and the new axon portion has gained in width. This
enlargement continues long after terminal connections are reestablished, until finally, in E, the whole fiber has recovered nearly its original caliber. This increase of the originally slender protoplasmic filament to a multiple of its size (in the above example, 100 times), is an expression of extensive growth, that is, production of new axoplasm. This phase of the regeneration process has been our basic test object. We can alter it controllably by narrowing the space available to the fiber for expansion, as is shown in rows F to I of the diagram.

Reduction of the lumen of the fiber tube, as in F, does not interfere with the distad advance and terminal reconnection of a regenerating fiber, which is slender. But as soon as this fiber, as it continues to expand, attains the dimensions of the constricted zone, a remarkable difference appears between those parts lying at the distal and at the proximal sides of

**Fig. 3** Diagram of stages of nerve regeneration without and with constriction. Explanation in text.
the narrow neck. The distal segment ceases to grow and remains permanently undersized, while the proximal segment not only continues to enlarge, but near the entrance into the constricted zone, enlarges excessively. Two stages of this process are shown in rows G and H. One gets the impression that a column of axoplasm is pressing distad and becomes dammed up where its channel narrows. This impression is strengthened by observations on released constrictions: some of the dammed material then moves into the distal portion, which widens accordingly, as illustrated in row I.

With this schematic description of the basic experiments as background, we may now turn to a more detailed account.

1. The effect of constriction

Short segments (cca. 1 mm long) of artery with a lumen smaller than the nerve diameter were used to produce constriction (Weiss and Davis, '43; Weiss and Taylor, '44b). When distended, these rings can be slipped over the cut nerve end and placed in the desired position. Their subsequent contraction produces the effects commonly referred to as nerve compression or constriction. Since this compression is localized, it is physically absurd to compare its effects with those of static pressure acting uniformly on an enclosed system, as is occasionally done (Denny-Brown and Brenner, '44b). The difference lies in the fact that localized pressure produces a shift of substance from the compressed to the non-compressed area. The extent of this movement depends on the physical properties (mobility, elasticity, etc.) of the compressed system and its restraining environment; in the present instance, the axon and its tube, the former more plastic, the latter more rigid. The arterial collar, in closing down on the nerve, squeezes underlying nerve content into the adjacent free portions. This involves, besides blood and endoneural fluid, neuroplasm and myelin (see below, section 6). For the individual nerve fiber, this implies a gradual reduction of diameter throughout the constricted zone, and
slight dilation at either end of the constriction. As the yielding nerve content accommodates itself to the arterial lumen, the local pressure declines and eventually disappears. So-called "pressure block" of nerve conduction produced by arterial cuffs (Weiss and Davis, '43) is due not to maintained "pressure," but to the displacement of axonal substance.

Constriction thus acts stepwise: local increase of pressure causes escape of substance into zones of lower pressure thus producing reduction of diameter, which, in turn, automatically relieves the excess pressure. The high plasticity and elasticity of nerve fibers is clearly demonstrated by their incomplete redistension after temporary constriction of only a few hours duration. The salient permanent feature of constriction is thus the local narrowing of the fiber tube which it entails.

The degree of narrowing suffered by a given nerve fiber varies not only with the degree of compression of the whole nerve, but also with (a) the topography of the fiber; (b) the consistency of the surroundings; (c) the fiber diameter. The compressing force reaches maxima at the ends of the constricting sleeve, but with the short rings used in our studies, this longitudinal differential is negligible. In more detail, these relations are as follows.

(a) Topographic variation of the compression effect. The effect is not evenly distributed over the whole cross section of the nerve, but is strongest at the surface and declines rapidly toward the interior. This gradient can be readily demonstrated by the following model experiment imitating the conditions of nerve constriction (fig. 4): A bundle of rubber tubes to represent nerve fibers is arranged in concentric circles. Their lower ends are closed, the upper ends connected to glass manometers. They are partly filled with indicator fluid. Constriction of the bundle is produced by a metal bracelet with overlapping edges. During the constriction, the water columns rise in direct proportion to the volumes of fluid displaced, which, in turn, are proportional to the respective reductions of diameters. The diagram shows the average displacement for 15 separate determinations. Dis-
regarding the minor deviations due to inequalities in the packing of the tubes and in the constricting force, it is evident that the tubes, and comparably the nerve fibers, are the more compressed, the nearer the surface they lie. The innermost elements are least affected. This gradation has been verified in our experimental nerves and will be dealt with in section 2.

Fig. 4 Model of differential displacement of the content of a bundle of tubes under local compression. The water levels indicate the average displacement in 12 outer, 6 intermediate and 1 innermost tube computed from 15 separate experiments.

(b) Variation of the compression effect with the proportion of interstitial tissue. Since interstitial fluid around a nerve fiber can shift more easily than the content of the fiber, it acts as a shock absorber. The nerve sheaths (epineurium, perineurium) likewise have a cushioning effect. Accordingly, a given amount of constriction will affect the nerve fibers the more, the smaller the interstices, that is, the denser the packing of the fibers, and the less the amount of enveloping tissue.
(c) Variation of the compression effect with fiber size. In general, the larger fibers of a constricted nerve suffer a greater proportional reduction of diameter than do the smaller ones. Originally deduced from the fact that the

![Graph](image)

**Fig. 5** Constriction in fibers of different size. Cross section of the constricted part of fiber plotted over cross section of the normal proximal stem of the same fiber, for 47 teased fibers.

![Graph](image)

**Fig. 6** Degree of constriction in relation to fiber size. Relative loss of diameter of fibers of different size classes plotted over original diameter, for the same fibers as in figure 5. Class 1 includes fibers between 1.6 and 2.4 μ; subsequent classes follow in steps of 0.8 each.

largest fibers are the first to cease conducting during progressive constriction (Gasser and Erlanger, '29; Weiss and Davis, '43), the greater relative involvement of the larger fibers has now been confirmed by direct measurements. The graphs reproduced in figures 5 and 6 are based on a sample of 47 individual fibers teased from 3 different nerves (fixed
and stained) after constrictions of long standing. In figure 5, the cross section of the constricted part of each fiber is plotted over the cross section of a far proximal, normal segment of the same fiber. One notices a remarkable uniformity of the calibers in the constriction irrespective of the original fiber caliber. What fluctuation there is, can be ascribed to local conditions such as outlined in (a) and (b), rather than to original fiber size. The fact that fibers of all sizes thus approach a common lower size level, implies that the larger ones have lost relatively more than the smaller ones. This is directly shown in figure 6. This graph gives the average percentage reduction of cross sectional area in the constriction (100 \( \frac{P - C}{P} \), where P and C are the cross sectional areas at P and C, respectively) for the various size classes of fibers. It can be seen that there is a steady increase in the degree of constriction with increasing caliber. The same conclusion can be reached from comparing the constriction of the total cross section of a nerve with the reduction of its 150 largest fibers; in a sample case (R256), these reductions amounted to 20% and 65%, respectively, demonstrating the relatively heavier involvement of the larger fiber classes.

2. Standard experiment

As the standard experiment, we choose the one diagrammed in figure 2 F-I; i.e., placing a constriction around one of a pair of nerves (tibial and peroneal), at the same time initiating regeneration by a crush farther proximally. After intervals varying from 4 to 35 weeks, the regenerated nerves are examined for recovery of fiber size at different levels. Figure 7 shows samples from a typical case (R259; 35 weeks p. op.) at the indicated levels P and D at identical magnifications. The 2 left panels represent the regenerated fibers of the unconstricted control nerve. By comparison, the right panels show the great disparity of size of the regenerated fibers in the constricted nerve proximally and distally to the
constriction. The fibers are very much undersized at level D, while excessively dilated at P_c.

Figure 8 shows the histogram of the 150 largest fiber cross sections of the case illustrated in figure 7 at corresponding levels (P_c lies a little closer to the constriction in fig. 8 than in fig. 7). One notes the inverse relation between fiber sizes proximal and distal to the constriction. Histograms of all other cases are essentially similar to this one. Instead of presenting them in detail, we have computed the total areas of the 150 largest fiber cross sections at levels P_c, P_c, and D_s for 7 pairs of nerves. These data are summarized in the
All show marked surpluses and deficits of fiber volume at levels $P_s$ and $D_u$, respectively.

The subnormal size of fibers distal to a constriction was first noted by Weiss and Taylor ('44b). It was recognized then that since the distal diameters of such fibers showed no

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**FIBER SIZE CLASSES**

Fig. 8 Histograms of the 150 largest fibers at the indicated levels of the nerves illustrated in figure 7. (35 weeks p. op.)
further increase after 4 weeks of regeneration up to 15 weeks, their growth was not just retarded, but fully arrested. This conclusion has been substantiated by the present series with observations extending over an even longer period, up to 35 weeks (see figs. 9 and 18). The distal parts of the fibers never widen appreciably above the caliber imposed upon them in the constricted zone, and this size deficit is permanent. Actual values will be given below. Myelinization was found to vary directly with the diameter; that is, the distal segments of reduced size possess a correspondingly thin myelin sheath (see figure 4 in Weiss and Taylor, '44b). Control series, in which the constricted nerves were either left disconnected from their peripheral fields or were reconnected by arterial splices, proved that as far as the part of the nerve lying

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Fig. 9 Total areas of the 150 largest fiber cross sections of 7 pairs of constricted and control nerves at levels P₁, P₂, and D₃. Regeneration times indicated at bottom of graph — 1 unit = 1000 μ². Note (1) the greatly reduced fiber size in the constricted nerves at level D₃, showing no increase with longer regeneration periods; (2) the excessive fiber size at level P₂, tending to increase with time; (3) over-all reduction of fiber size during earlier weeks due to atrophy of disconnection, followed by recovery.
distally to the constriction is concerned, the results were essentially the same in both conditions.

The explanation of these results is unequivocal. The constriction divides the nerve into 2 parts of unequal growth—an unimpaired proximal one, and a distal one whose size is limited in direct proportion to the reduced lumen of the fiber at its narrowest point. This relation indicates that the distal portion depends for its continued growth on something that is supplied from the nucleated central portion and transported distad at a rate proportional to the cross section of the tube. The local narrowing at the constriction acts as a “bottleneck” which throttles the proximo-distal transfer and thus reduces the rate of supply to the farther distal levels. The material in question might be axoplasm as such or some growth accessory the concentration of which limits the rate of local synthesis of axoplasm. However, a reduced rate of supply could not of itself account for the fact that the deficit in size is a permanent one, for the distal fiber part would merely be slower in reaching normal dimensions, but ought eventually to grow to the full size. The fact that it does not, indicates that fiber size is the stationary product of 2 opposite processes, one consuming axoplasm at a uniform rate and the other replacing it at the rate at which supplies become available. Constriction evidently does not affect the rate of consumption, which will depend on local factors, but only reduces the rate of replacement. In consequence, the distal fiber volume comes to a steady state at a lower size level than proximally.

The decision of whether what moves down the fiber is axoplasm as such or merely growth accessories for its local synthesis, is of fundamental importance, because it contains the answer to the general problem of whether a cell grows from a circumscribed source, especially the nucleus, or ubiquitously throughout the cytoplasm. While the distal growth deficit in constricted nerve fibers can be reconciled with either view, the character of the compensatory surplus on the proximal side of the constriction, the “damming”
phenomenon, points to the perikaryon as the sole production site of neuroplasm.

3. Damming

A. Axon. Figures 7 and 8 illustrate the excessive size assumed by the fibers proximal to the constriction. In contrast to the distal deficit, the proximal surplus is not uniformly distributed over the length of the fiber, but is accumulated immediately in front of the bottleneck (zone P₁; fig. 2). The oversized fiber diameters grade off proximally, and a few millimeters back from the constriction, the calibers are again normal. As will be shown presently, the local widening is only one of several different expressions of a local piling up of axoplasm, a phenomenon henceforth to be referred to as "damming." Together with the accumulation of fluid in the endoneurial spaces between the nerve fibers (Weiss, '43a; Weiss, Taylor and Edds, '45), it leads to the characteristic bulging of the nerve in zones P₁ and P₂.

For details of the damming process, the nerve fiber must be studied in profile views. This has been done in longitudinal sections of nerves as well as in whole mounts of teased nerve fibers. For the latter purpose, the nerves were first fixed and silver-impregnated according to Bielschowsky, then transferred to glycerin, stripped of the epineurium and teased into small strands of from one to several fibers. This technique has the advantage of preserving single fibers in full continuity from far proximal through the zones of damming and constriction far into the distal portion. Because of the possibility that the arterial collar might contract further on fixation and alter the chronic appearance of the nerve fibers, it was carefully slit and removed from many of the specimens prior to fixation. However, this precaution proved unnecessary, as no difference was noted between nerves fixed with and without the cuff. A description of the essential features of damming follows:
Figure 10 shows in a composite picture the principal appearance of nerve fibers in the zone of greatest damming, P. In the constricted zone, C, the fibers are thin and have a perfectly smooth contour; their myelin sheath is inconspicuous, sometimes imperceptible (see Denny-Brown and Brenner, '44b). The constricted parts of the fibers contrast sharply with the portions lying immediately proximally, which are greatly bloated and otherwise distorted. The transition between the 2 zones is remarkably abrupt (fig. 11).
Morphologically, the alterations in P, can be classified in 4 groups: (1) ballooning; (2) beading; (3) telescoping; (4) coiling. Individual fibers may show any of these alone or in combination.

1. Ballooning. The axon is greatly distended into irregular balloons measuring up to 50 μ in diameter, separated by narrower portions. The fact that the balloons actually represent dammed up axoplasm rather than fluid deposits inside the sheath, is well demonstrated by the behavior of the neurofibrils. These are usually uniformly dispersed through the whole width of the balloons and follow contorted meandering courses strictly conforming to the configuration of the axon (see the second fiber from the top in fig. 10). In many cases, balloons herniate through the plasma membrane and form blind pouches. The most distal balloon tapers into the constricted zone. Proceeding proximad, one notices a rapid decline in the size of the balloons grading over into the second and milder aspect of damming — beading.

2. Beading. The axon has approximately its normal average diameter, but instead of a smooth cylindrical outline...

Fig. 12. Samples of beaded fibers taken at 3 different distances from the constriction, the top one being the closest. Case R33 (10 weeks p. op.). × 400.
shows a rhythmic fluctuation of width, giving a beaded effect (bottom fiber of fig. 10). Beading of axons may result from various causes, e.g., inadequate fixation, retraction during fixation near the cut end of a nerve, vacuolization of diseased neurons. While the beading observed in dammed fibers differs in several respects from any of the foregoing, the common

![Graph showing linear decline of beading with distance from constriction.](image)

**Fig. 13** Linear decline of beading with distance from constriction. Average distance between beads in micrometer units is plotted over the distance of the respective sampling levels from the constriction (C) for 3 nerves, 10 weeks p. op. Size of samples: R32 (full line), 1379 beads; R33 (dotted line), 1698 beads; R30 (double line), 1136 beads.

feature is the alternation of wider and narrower portions of the axon. In the case of dammed fibers, the narrow portions are the result of local detachment of the axon from the tube, with extrusion of liquid into the intervening space, exaggerated by additional shrinkage during fixation. The portions
where the axon has remained attached to the sheath then appear as "beads" linked by the shrunken portions (fig. 12).

The spacing of the beads shows a striking over-all regularity. While unrelated to such preformed marks as the incisions (clefts of Schmitt-Lanterman), it is directly related to the distance from the constriction. This relation is a linear function. The number of beads per unit of length, or more pertinently, the incidence of lateral detachment, at a given level is inversely proportional to the distance between that level and the constriction; the closer to the constriction, the

Fig. 14 Photomicrographs of telescoped fibers. Top: Sectioned specimen R29 (18 weeks p. op.), × 400. Bottom: Teased fibers from R116 (19 weeks p. op.), × 330. (Note also large balloon at extreme right.)
shorter the beads (fig. 12). Figure 13 shows this linearity for 3 sample nerves. The quantitative relation between the beading pattern and the site of constriction not only confirms the reality of the phenomenon, but contains a valuable clue as to the mechanics of the damming process. This will be discussed later.

3. Telescoping. This is best visualized as beading, combined with a lengthwise compression of the axon (fig. 10, top fiber; and fig. 14). Thus the axon portions that have remained attached to the sheath are shoved together accordion-fashion and partly double over the retracted intermediate portions. The result resembles the configurations assumed by a rubber tube that is being stripped off a closely fitting rod.

4. Coiling. The axons describe spirals (fig. 15) of the sort that one obtains by accommodating a flexible rope in a cylindrical container which is too short. The direction of the spirals varies from fiber to fiber and often changes in the same fiber after a few turns. It must be emphasized that in contrast to the coiling seen at the free ends of freshly cut

![Image of coiling of small fibers proximally to constriction. R202 (11 weeks p. op.). × 400.](image-url)
nerve fibers (Young, '44a), coiling in our cases concerns un-
interrupted fibers.

Ballooning and telescoping are exclusively observed in
larger fibers, coiling predominantly in smaller ones. All of
these features are confined to the fiber portions lying prox-
imately to the constriction, that is, on the side of the peri-
karyon. On rare occasions, a recurrent fiber can be found
which has doubled back in its distal course and grown prox-
imal, entering into the constriction from the distal side. Such
fibers show signs of damming at the distal end of the sleeve,
which for these fibers, having grown in reverse, is actually
the end nearer the perikaryon.

B. Myelin sheath. The configuration of the myelin sheath
follows essentially the contour of the ballooned and telescoped
portions. The thickness of the sheath corresponds generally
to the caliber of the particular fiber, except for extremely
bloated portions, around which the myelin is greatly thinned.
It remains thin at the transition into the constricted zone
and throughout the latter zone itself.

C. Internodes. It was first established by Leegaard (1880)
that while the average length of internodes increases with in-
creasing fiber diameter in primary fibers, internodal length
of regenerated fibers is relatively constant irrespective of
caliber. This fact has been confirmed in recent reinvestiga-
tions of internodal distribution (Young, '45b; Hiscoe, '47).
The average value for regenerated fibers in the rat was found
to be cca. 300 μ (Hiscoe, '47). In constricted regenerated
fibers, however, the length of the internodes is constant only
up to a level about 3 mm proximal to the constriction. Between
this level and the constriction, most internodes shorten pro-
gressively in direct proportion to their closeness to the con-
striction. This relation is illustrated in the graph, figure 16.
Internodal distances were measured in a number of teased
fibers from standard experiments (regenerated and con-
stricted), and the values obtained were averaged for the last
identifiable internodes immediately proximal to C, then for
the next to last, the third last, and so forth, proceeding back-

wards from the constriction. Plotting the average length of these successive internodes over their average distance from the constriction (fig. 16), one readily recognizes the progressive foreshortening in \( P_s \) and \( P_c \) from a normal value of about 300\( \mu \) in the farther proximal stem of the fiber (\( P_s \)). Only fibers definitely affected by constriction have been included in this computation. It is noteworthy that the effect shows the same linear increase with increasing proximity to the constriction that was observed in beading (fig. 13).

**Fig. 16** Average lengths of consecutive regenerated internodes plotted over their distance from the constriction.

**D. Nodal damming.** Superposed upon the phenomena described in the preceding, is a characteristic regularity which repeats itself within each internode of the affected zone (\( P_s \) and \( P_c \)). It concerns the distances between the points at which the axon has become detached from the myelin sheath, as revealed by the spacing of consecutive discs (in telescoped segments) or beads (in beaded segments). This spacing is closest at the proximal end of the internode and widest at the distal end, with a fairly regular gradation from one end to the other. The last bead just before the node is not only longer than the more proximal ones, but it often tends to
balloon. Then, on the opposite side of the node the spacing is again very close, widening toward the next node, and so forth. Figure 17 shows typical examples. This polarity of the inter-node reflects the highly asymmetrical condition of the axon relative to the node. It is evidently an expression of some sort of mechanical "valve” action of the node and will have to be considered in connection with the interpretation of

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**Fig. 17** Samples of polarity of damming at nodes of Ranvier. Top: Balloonned fiber (sectional specimen), R202 (11 p. op.). × 710. Middle: Mildly beaded portion of fiber (teased specimen), R97 (24 weeks p. op.). × 630. Bottom: Almost normal part of fiber (teased) of R116 (19 weeks p. op.). × 630. Nodes marked by arrows.

damming. It indicates that passage across the node meets with some resistance, although certainly not with a complete barrier as recently conjectured by von Muralt (’46). In practical respects, the nodal polarity is a very sensitive indicator of damming even in the mildest forms of constriction, and a reliable marker of the proximo-distal direction in isolated nerve fibers.

**E. Damming in different fiber types.** Since only fibers above a certain size are measurably affected by constrictions of the
kind practiced in our experiments, the question whether dam-
ming occurs at all in unmyelinated fibers, which are below
that size limit, remains open. For the larger somatic types,
however, it is evident that damming occurs in both motor and
sensory fibers. The mixed nerves, with which we have worked,
contain large numbers of both, the sensory fibers being
slightly more numerous than the motor ones. If only one or
the other type were capable of damming, a conspicuous num-
ber of undamaged fibers would have to be observed in each
instance. This was decidedly not the case, as every larger
fiber showed signs of damming. The fact that in both fiber
types, damming occurs at the cell-near side of the constric-
tion, proves that it bears no relation to the direction of physi-
ological impulse conduction.

F. Interpretation. Only the most notable morphological
manifestations of damming have been reported here. Many
more variations of the same theme have been recorded. Taken
together, all observations lend themselves consistently to one,
and only one, interpretation, as follows. The nerve fiber is
subject to a force which presses the column of axonal sub-
stance constantly and steadily forward in the direction of the
periphery. Normal nerve fiber tubes have the proper width
for this translatory movement to proceed unimpeded at the
given rate; that is, the channel is wide enough at any one level
to let pass down the full volume of substance received from
above. When the width of the channel is reduced, as in con-
stricted nerve fibers, a local bottleneck is created which re-
tards the rate of passage. If the supply from proximal
continues at an undiminished rate, the portion immediately
proximal to the bottleneck will receive more substance per
unit of time than can be disposed of, and a surplus will begin
to pile up, comparable to a traffic jam.

The physical effect on the axon is that of a longitudinal
compression starting from the level of resistance at C and
proceeding backwards. The axon buckles under this com-
pression, and telescoping, beading, and foreshortening of the
internodes are the morphological results. Smaller fibers are wound into coils. In large fibers, the tube wall evidently yields to the increased lateral pressure, thus giving rise to ballooning. The common factor in all these features is the accommodation of a given length of axon in a shorter length of tube than it would normally occupy. As this accommodation takes place, the excess pressure to which it was due, is relieved; in the case of balloons, for instance, by the widening of the containing tube.

The impact of this process is greatest where the axonal movement meets sudden resistance in the form of a reduced lumen, that is, right in front of the constriction. It is here, therefore, that the most extensive deformations of the axon are observed. Since the deformation makes room for part of the surplus, the next proximal portion has less of an overload to sustain, part of which again goes into physical deformation, while the rest rebounds on the next proximal level, and so on up the fiber with declining strength. In this manner, the excess pressure is gradually consumed in its ascending rebound, a fact clearly reflected in the linear decline and eventual subsidence in centripetal direction, of such visible effects as beading and shortening of internodes.

While the existence of a translatory movement of the axon can thus be deduced from its physical manifestations under restraining conditions, the nature of the moving force remains obscure. Hydrostatic pressure built up by the perikaryon, as assumed by Young ('44a), furnishes no adequate explanation. Such turgor would communicate itself to the whole system and, depending on its rate of propagation, produce either uniform distension of the whole tube or, at slower rates, greater widening near the base than near the tip. The fact that in reality the effect follows exactly the reverse order, namely, from tip to base, makes it necessary to postulate a dynamic (as opposed to static) pressure, with a moving force driving the axoplasm in centrifugal direction, in the manner of a peristaltic wave.
4. Persistence of damming

The manifestations of damming described in the previous chapter are not transitory features but persist in the nerve fibers so affected throughout the life of the animal. They do not regress, but on the contrary, become more accentuated with time. It will be remembered that in our standard experiment, all fibers passing through the constricted zone are regenerated branches, hence very thin during the early postoperative period. Even the constricted tubes are still oversized for these young sprouts (fig. 3, F). Not until a fiber has grown in width beyond the diameter of the bottleneck, does damming set in. As the fiber continues to enlarge, so does its dammed portion, and the latter keeps on swelling even after the main stem of the fiber has reached its final size (fig. 3, G and H). This is brought out by comparing damming intensities at different intervals.

Figure 18 presents such a comparison. It shows the histogram (of the 300 largest fiber cross sections) of two pairs of nerves, one pair studied 6 and 8 weeks (average 7 weeks), and the other, 35 weeks after the operation. There is a slight deficit in the size of the proximal fibers (Pₕ) of the younger set, which is presumably due to the transitory atrophy of neurons following transection (Gutmann and Sanders, '43). The average difference is a little more than 1 μ. In contrast, the size difference in the dammed portions (Pₗ) is much more pronounced, the average being about 3 μ higher in the older than in the younger pair, and the maximum size being 30 μ in the former, as against 21 μ in the latter. It may be noted incidentally, that the distal fiber population (Dₜ) has made no appreciable gain in size during the additional 28 weeks (an average of only about 1 μ), which illustrates the statements made in chapter 2.

Further evidence of the increase of damming with time is contained in measurements of individual teased fibers. By planimetry, it was possible to determine the actual volumes of the ballooned portion of a given fiber (Pₕ and Pₗ) and of an
Fig. 18  Histograms of the 300 largest fibers in 2 pairs of nerves constricted for different lengths of time, as indicated.
equal length of its unaffected proximal stem \((P_a)\). Designating the volume of portion \(P_b + P_c\) as \(V_{s+b}\), and the volume of a proximal sample \(P_a\) of equal length as \(V_s\), the degree of damming can be expressed by the quotient \(\frac{V_{s+b}}{V_s}\). The resulting values for representative fibers from three nerves with regeneration times of 7, 12 and 18 weeks, respectively, are given in table 1 below.

**TABLE 1**

*Increase of damming \(\frac{V_{s+b}}{V_s}\) with time.*

<table>
<thead>
<tr>
<th>CASE</th>
<th>NUMBER OF FIBERS MEASURED</th>
<th>REGENERATION TIME</th>
<th>DEGREE OF DAMMING</th>
</tr>
</thead>
<tbody>
<tr>
<td>R,61</td>
<td>26</td>
<td>7 weeks</td>
<td>1.9</td>
</tr>
<tr>
<td>R,13</td>
<td>6</td>
<td>12 weeks</td>
<td>2.4</td>
</tr>
<tr>
<td>R,116</td>
<td>12</td>
<td>18 weeks</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*In each case, fibers with the most extreme degree of damming were selected.*

The figures of the table show clearly that the dammed axoplasm increases not only in amount, but also in proportions, as more time elapses. Eventually, however, the dammed portion, too, becomes stationary. One of the factors in determining this final equilibrium is undoubtedly the limited distensibility of the tube. Another factor that may have to be considered is a possible increase of permeability of the distended surface, permitting intraaxonal fluid to exude, thus relieving some of the local pressure.

The fact that the deformation of constricted fibers is permanent, raises the question as to the functional adequacy of such fibers. Is a 10\(\mu\) fiber, for example, which widens to 30\(\mu\) in \(P_c\) and then narrows to a constant 3\(\mu\) for the rest of its peripheral course, of functional use? Preparations of teased nerves have proved that practically all larger fibers of a constricted nerve are permanently deformed in some degree. Therefore, any major function mediated by such nerves must involve deformed fibers. No detailed study of muscle function has been made. In gross observation,
however, motor coordination of the experimental limbs appeared essentially unimpaired. There is no doubt that finer exploration would reveal local variations of conduction velocity and threshold paralleling the variations of diameter. Yet these irregularities seem to cause no marked disturbance of the functional performance. Similar observations, showing the coordinated function of muscles innervated by regenerated fibers of greatly reduced caliber were recently made in monkeys (Matson, Alexander and Weiss, '48).

5. Quantitative correlation between constriction, damming and distal size deficit

In order to test the quantitative dependence of proximal damming and distal size deficit upon the degree of constriction, we make use of the fact that the constriction effect shows a definite gradient from surface to core. As explained earlier and illustrated in figure 4, fibers lying nearest the surface will suffer the greatest, those lying innermost, the least compression. Consequently, if we subdivide a cross section through the constricted part into a series of concentric zones, average fiber size will vary from a minimum in the outer ring to a maximum in the center. In practice, instead of measuring all fibers of a cross section, we merely sample a sufficiently wide median strip. By using proper landmarks, we then select topographically corresponding median strips at levels $P_1$ and $D_0$ and determine their fiber size patterns for comparison with the pattern in the constriction. Data for 3 nerves have thus been computed and are summarized in figure 19. Each median strip was subdivided into five equal parcels, as indicated in the accompanying diagrams. All nerve fibers were measured and counted, and the average fiber size for each parcel was calculated. In the graphs, these values are given in percentages of the average of the central zone. Broken lines represent the individual nerves, solid lines the mean values for all three of them.
Fig. 19 Surface-core differential of average fiber size at the 3 indicated levels for 3 constricted nerves, R,56 (35 weeks p. op.), R,59 (35 weeks p. op.) and R,68 (29 weeks p. op.). For further details, see text.
The middle graph confirms our premise, that in the constriction itself, there is a systematic decline of fiber size from interior to surface. This feature is essentially duplicated in the bottom graph, which represents the distal nerve. The parallelism between these two graphs proves that there is a direct proportionality between the diameters of the constricted and of the free distal portions of a fiber. By contrast, the shape of the top graph, representing the dammed portions, is essentially inverted. The fibers of the outermost zone, which are the narrowest at C, are the widest at P.. Hence, statistically speaking, the more the lumina of fibers are reduced in the constriction, the more they become dilated by excess axoplasm at the level of damming.

The inverse relation between constriction and damming can only be demonstrated for larger groups of nerve fibers, but is obscured in individual fibers by random fluctuations of local conditions. This conclusion is based on complete measurements of numerous individual teased nerve fibers. For each such fiber, the amount of ballooning was calculated from planimetric measurements of profile tracings of both the entire ballooned stretch (P_s) and a corresponding length of the normal stem (P_n) of the same fiber. These values were then related to both the absolute size of the constricted portion (C) and the relative size of the latter in proportion to its original caliber (P_c). Figure 20 presents these data for 26 individual fibers of the same nerve (R61). The only regularity recognizable in this graph is that the maxima of ballooning tend to increase from right to left, that is, with increasing degrees of constriction. This is in accord with the general statements made above for groups of fibers. Otherwise, however, the individual values are scattered irregularly.

The measurements of individual fibers have also made it possible to compare in one and the same fiber the surplus of proximal axoplasm with the distal deficit. The proximal surplus is determined by subtracting from the total volume of the ballooned portion the volume of a normal proximal por-
tion of equal length of the same fiber; the distal deficit, similarly, by subtracting from the latter value the volume of a corresponding length of the free distal portion. For a given sample fiber, it was thus calculated that the excess of axoplasm piled up at the constriction would be enough to fill the distal portion up to its normal size for a distance of no more than 2 mm. This is only a fraction of the total distal length of

Fig. 20 Relations between degree of constriction and intensity of ballooning. Ordinates give the excess volume of the balloononed area relative to the original size of the fiber \( \frac{B-P}{P} \), where \( B \) is the total volume of the balloononed portion, and \( P \) the volume of an equal length of the normal stem of the same fiber. Abscissae give \( (\bigcirc) \) the absolute sizes of the fiber cross sections in the constricted zones, as well as \( (\bullet) \) these sizes relative to normal fiber diameter \( \frac{C}{P} \).
these fibers, which amounts to several centimeters. Evidently, the distal deficit is considerably greater than the proximal surplus. It would be of interest to know whether at least the ratios between them are similar for different fibers, but this could not be determined in our cases, as the distances between constriction and end organs, which vary from fiber to fiber, were unknown.

6. Damming studied in live fibers

The bulk of evidence obtained from fixed and stained preparations has left no possible doubt that the axonal deformities described above as signs of damming, are true features of the living fibers and not histological artifacts. The strict localization of the phenomenon relative to the constriction, its constancy of appearance under a variety of treatments, the quantitative correlation of damming with various biological variables (e.g., postoperative period), and many similar facts, could be cited as evidence. Nevertheless, it seemed desirable to repeat the observations in living untreated fibers, in order to establish just how faithfully the fixed preparation portrays the living fiber.

For this purpose, the constricted nerves were excised, transferred into Ringer’s solution and split lengthwise. One half was fixed for histological preservation, the other half carefully teased under the microscope into bundles containing only a few fibers each. Sealed between slide and cover glass, such fresh fibers remain essentially unaltered for varying periods, which at best last for a fraction of an hour. The appearance of normal fibers under these conditions has been described and illustrated on a previous occasion (Hiscoe, ’47).

Fibers from constricted nerves show all the principal features observed in the fixed preparations. Although the photographic records are far inferior to direct microscopic inspection because of the thickness of the bundles, we reproduce here a few examples (fig. 21). They show fibers either at the point of entry into the constricted zone or immediately
proximally to that point. In numerous live specimens of this kind, the main forms of damming — ballooning, beading and telescoping — have been verified; only coiling could not be positively identified, presumably because it occurs only in small fibers, which are difficult to trace amidst the tangle of lines. As was to be expected, there were certain differences of morphological detail between the live and the preserved

Fig. 21 Photomicrographs of untreated live fibers proximally to constrictions, immediately after teasing and transfer to Ringer’s solution. Note convolutions of axon and thin myelin on balloons. These configurations can be readily distinguished from the severe alterations appearing in all teased fibers within a relatively short period after removal from the body and consisting mainly of myelin extrusion and vacuolization of the axis cylinder. Top: R19 (constricted for 27 weeks, regeneration time 12 weeks; see section 8A). × 700. Middle and bottom: R115 (regeneration time 17 weeks, constricted for last 14 weeks; see section 8B). × 630.
fibers, but these were wholly subordinated to the basic feature of close correspondence between the two states.

7. Acute effects of constriction

Having established the reality of the described morphological phenomena, closer scrutiny must be given to their interpretation as a case of axoplasmic damming. Alternative explanations must be ruled out. One such alternative is the possibility that the observed deformities are residues of the acute deformation which the nerve fiber suffers in the act of constriction. We, therefore, reexamined the histological effects of acute nerve constriction, which had already been studied by previous investigators, particularly Stroebe (1897), Cajal ('28), Denny-Brown and Brenner ('44b). As mentioned in chapter 1, the cuff placed around the nerve causes an actual displacement of substance from within the compressed zone into the adjoining free portions, which thereby become dilated. One could imagine that this dilation of the tubes creates a mould which would impress its shape on any axons subsequently regenerating into such tubes, as for instance, those of our standard experiment. The various deformities ascribed to clamming would then simply be replicas of a condition produced by the process of constriction itself, rather than a growth phenomenon. In order to test the matter, we constricted nerves with arterial rings as usual, but examined them shortly thereafter. A graded series with periods of constriction lasting from a fraction of an hour to 2 weeks was studied both in living and preserved specimens.

The first marked effects appear only after several hours. They consist of a narrowing of the fiber in the constricted stretch. Measurements during this period show that the axis cylinder loses width faster than the myelin sheath, indicating a more plastic state of the former. The substance squeezed from this zone escapes into the uncompressed portions of the tube to either side and widens them accordingly. The displacement is about the same in the ascending and descending direc-
tions, and we have detected no sign of polarity. Even after a day, the constricted parts of the fibers have not yet been reduced to their minimum diameters as judged from the size range of the long-term experiments (figs. 5, 20). The slowness with which the axon of an intact nerve yields to compression, contrasts with the rapid deformation obtainable in isolated fibers and presumably indicates a state of very high hydrostatic pressure in the endoneurial fluid, already deduced from previous studies (Weiss, '43a).

After a day or 2 the system has become stationary and now consists of a narrow constricted portion flanked by 2 bulbous enlargements. Superficially, these might be taken to resemble the widenings seen in the long-range experiments, but closer study reveals some crucial differences. Above all, the contour of the fiber, even in the widened parts, remains smooth and shows none of the characteristic configurations of damming. It is conceivable that more sudden compression of a fiber might simulate damming figures more closely, but this point is not at issue, as the rate of compression in our standard experiment was on the same slow order as that of the acute experiments dealt with here. Moreover, the widenings caused by the actual pressing out of substance from the constriction do not reach the dimensions of our ballooned fibers. Also in this connection, it should be remembered that the ballooned portions of constricted fibers continue to swell even after several months (fig. 18).

Wallerian degeneration in constricted fibers is somewhat modified. As has long been known, constriction may act like a complete crush and cause degeneration of the distal remainder of the fiber. This does not occur in all cases, however, and fibers may survive in toto in spite of a permanent stricture. Demonstrated originally for sectioned material (Weiss and Davis, '43; Denny-Brown and Breiner, '44a), this has now been confirmed in single teased fibers, whose course could be followed through the constriction. The long-range changes of such fibers will be reported in a later chapter. In those fibers which do undergo degeneration, the fragmentation of
the axis cylinder is somewhat delayed in the constricted zone as compared with the free distal portion (see Weiss and Burt, '44). In the free portion, the morphological signs of degeneration appear more or less simultaneously over its full length, and despite special efforts, we have been unable to find evidence that the visible changes start at the proximal end and proceed distad. This confirms the general conclusions of most of the earlier histological studies of this problem. Physiological tests, on the other hand, have shown excitability of distal nerve stumps to subside in proximo-distal sequence (Rosenblueth and del Pozo, '43). Evidently, there is no parallelism between the loss of physiological properties of nerve and the appearance of gross histological signs of degeneration.

In general, our observations on short-term constrictions have confirmed the findings of earlier investigations (Stroebe, 1897; Cajal, '28), but have produced no alternative explanation of our long-term results. There was one other potential source of error, however, that had to be taken into account, and that was the following. During later stages of Wallerian degeneration, ovoids and axon remnants are gradually resorbed and removed by macrophages. In constricted fibers, this removal is markedly delayed in the parts of the fiber adjoining the constriction. Therefore, a fiber which had been severed higher up, as in our standard experiments, retains residues of debris to either side of the constriction, a fact which shows up in a double bulge of the whole nerve (fig. 2). It could be argued that by impressing their shape on the fiber tube, these ovoid remnants might create a mould which when later filled by the regenerating axoplasm would force the latter into a correspondingly beaded and bulging shape. Convolutions of the axon could be explained as detours around such residual bodies. Actually, the slightly tortuous course of some regenerated fibers in zone D. is attributable to this cause. Yet many other of the observed features, such as telescoping, coiling, nodal polarity, declining internodal length, etc., are left unexplained, which seems to rule out
degeneration products as the cause of "damming." Even so, the possibility that the disarrangement of axon and myelin produced by the constriction itself might have contributed to the picture of "damming," was real enough to call for a crucial experimental test. This test was applied in a series of experiments described in the following.

8. Control experiments

A. Delayed regeneration. A constricting sleeve was placed around the nerve as in the standard experiment, but the proximal crush to start regeneration was not made immediately, but from 15 to 17 weeks later. An additional 12 to 20 weeks were then allowed for regeneration. The long delay of the start of regeneration was to insure that the acute local disturbances caused by the process of constriction had subsided by the time the regenerating fibers arrived at that level. The final results were, however, exactly as in the standard series. Figure 22 shows teased sample fibers from such a preparation at the transition from $P_e$ to $C$.

B. Delayed constriction. In this series, the reverse order from the preceding was followed. The nerve was first crushed and the constricting sleeve was not put on until 2 to 4 weeks later. By that time, the nerve had undergone practically complete Wallerian degeneration and contained young and thin regenerating fibers. The constriction thus acted on a system quite different from that of the standard experiment.
There was no appreciable amount of myelin debris left to produce the local congestion suspected in the foregoing chapter of being a "damming" factor. The axons were so thin and so amply cushioned by the Schwann cords and fluid content of the tubes (see chapter 1b) that the constriction had no direct impact on them. They were then allowed to continue their growth for another 1 to 19 weeks.

The final results were fully in line with our original interpretation. During the first few weeks, when the young axons were still thinner than the constricted tubes, they could grow without encroachment. However, as soon as they had reached the dimensions of the tubes and could expand no further, damming at the proximal side set in. Examples of such fibers are reproduced in figure 23; some of the live fibers of figure 21 are also from this series.

Stress must be laid on the fact that in these experiments the mass of regenerating fibers had already passed the level of the constriction when the latter was applied. This rules out any contention that damming might be a residue of
widened fiber tips temporarily halted by an obstruction. Terminal bulbs are a common feature of arrested fibers and, as will be explained below, are merely another manifestation of damming. But our present experiments demonstrate conclusively, as will others further below, that damming is not necessarily confined to the blind end of a fiber as it is after complete obstruction, but can occur anywhere along the fiber course as a result of partial occlusion.

C. Constriction of aneuritic nerves. In this series, the constriction was applied to nerves which had undergone complete Wallorian degeneration and remained uninervated for a period. In a first operation, the peroneal nerve was cut without provisions for reconnection of the stumps. Four weeks later, an arterial constriction ring was placed over the degenerated distal nerve stump, which was then united by tubular splice (Weiss, '43b) to the proximal stump of the freshly severed tibial nerve. The fibers were then allowed from 17 to 25 weeks of regeneration. The results were the same as in the other series, with marked damming of the regenerated tibial fibers where they pass into the constricted segment of the peroneal.

This series also eliminates the objection that the direction in which the constricting sleeve is slipped onto the nerve, may have anything to do with the polarity of the damming phenomenon. In our standard experiment, as well as in series A and B of these control experiments, the sleeves were pulled over proximal nerve stumps from the distal end. One might contend that this manipulation could shove axoplasm back into a pile at the proximal level of the sleeve. In practice, precautions were taken against this eventuality, and furthermore, our studies of the acute effect of constrictions (chapter 7) have directly shown that there is no difference between the upward and downward displacement of axoplasm, regardless of how the sleeve is put on. But even more crucial are the results of the present series, in which the sleeves were put over distal stumps from the proximal end, that is, in the reverse direction, without concomitant shift in the site of
damming from the proximal to the distal side of the constriction.

D. Regeneration through released constriction. The 3 preceding series have demonstrated that damming occurs no matter what the state of the nerve may have been when the constriction was applied, and quite independently of any direct molding effects of the constriction on the bed of regeneration (e.g., deformation, congestion, occlusion, etc., of the tubes). This does not exclude a contributory role of these effects, but it eliminates them as major factors of the damming process. Conversely, one could expect that damming would fail to occur after a constriction of only temporary duration even though the nerve had undergone the acute changes attending constriction. This conclusion was tested as follows. Nerves were constricted as in series A, and 25 days were allowed for the primary changes to take effect. Then the sleeves were removed and regeneration was started by crushing at a more proximal level. After 6 more weeks of regeneration, the nerves were studied.

Most of the fibers showed no evidence of the original constriction and no damming. A minority, however, did show signs of constriction and associated mild damming. Different nerves varied in this respect. It was clear from the preparations that some tubes had not resumed their original width after the removal of the sleeve, thus defeating the purpose of the experiment. This partial irreversibility of the constriction effect will be further dealt with below. It limits the conclusiveness of the results in the present series to those fibers which did recover their width, and in these, there was no damming. The comparatively weak degree of damming in the others corresponds to their partial recovery of diameter.

E. Conclusion. It is evident that these control series have brought no support for alternative explanations of the axonal deformities which we have ascribed to damming. It may be pointed out, moreover, that any of the envisaged alternatives could, at best, have only accounted for the atypical proximal configurations of the axon, with no bearing whatever on its
distal size deficit; whereas our interpretation views both these phenomena as corrolaries of a single common principle — the proximo-distal convection of axoplasm.

9. Damming in uninterrupted adult fibers

From the very first, it was apparent that damming was by no means confined to regenerating fibers, but occurred in constricted primary fibers as well. However, the earlier evidence gained from sectioned specimens lacked full conclusiveness because of the difficulty of tracing individual fibers across the constriction. Although there were many large primary fibers observed distally to the constrictions, and many dammed fibers proximally, it was impossible to prove incontrovertibly that they belonged to the same fibers. This difficulty was later overcome by the study of teased fibers. As teased single fibers could be followed through the constricted zone in their continuity, the presence of damming in old primary fibers could be established with absolute certainty.

As mentioned in chapter 7, a constriction of medium intensity entails Wallerian degeneration of the distal portions of some of the fibers, while other fibers persist. These 2 categories can later be told apart by the appearances of the distal portions, the former being regenerated fibers, which are distinguished by their smaller size, irregularities, short internodes, and excessive number of Schwann cells. The differential between old and regenerated fibers is the greater, the larger the fiber class and the shorter the time elapsed since the date of constriction. Our statements are based exclusively on large fibers whose diameter was markedly reduced in the constricted zone, yet whose distal portions could unquestionably be diagnosed as preserved primary fibers.

The proximal and distal portions of such fibers showed polarized changes relative to the constriction strictly comparable to those recorded for the regenerated fibers. That is, there appeared an excess of axoplasm in $P_\alpha$, with an opposite trend, toward reduction, in $D$. To consider the latter first, it
was to be expected that the distal portions would gradually shrink to the same small size that proved to be the upper limit of growth for the distal portions of regenerated fibers, that is, only slightly wider than their width in the bottleneck. So far, however, we have been unable to ascertain any such extensive atrophy. The reason is perhaps that fibers which did show it, de ipso facto resembled older regenerated fibers too closely to be included in our selection. Only the earlier stages of atrophy could thus be safely identified. They gave evidence of shrinkage of the axis cylinder within a less plastic myelin sheath, the former retracting from the latter in places. Instead of assuming a uniformly reduced diameter, the distal axon thus consists of a succession of alternately unreduced and reduced portions (fig. 24), producing an aspect of irregular beading, which differs both in origin and appearance from the type of beading that is associated with damming. Whether the irregularities are eventually smoothed out and whether and how the myelin sheath adapts itself to the altered dimensions, remains for further studies to determine.

At the proximal side of the constriction, the fibers show the typical damming signs of ballooning, telescoping and beading (fig. 24). The myelin sheath in this region conforms roughly to the bulges and convolutions of the axon. Internodal length is decreased in proportion to the nearness from the constriction. This is shown in the graph, figure 25, in which the length of internodes of 23 affected fibers has been plotted over their distance from the constriction. The points represent measurements of up to 8 consecutive internodes of individual teased fibers. The mean lengths of the first, second, third, etc., internode, in ascending order from the constriction, are represented by heavy circles. Up to 3 mm from C, the mean stays at about 1200 μ, which is typical average for the larger fibers with which we are dealing (Hiscoe, '47). Between this point and the constriction, each successive internode becomes progressively shorter. A comparison of this graph with the analogous plot for regenerated
Fig. 24 Effect of constriction on mature uninterrupted fiber. Case R.210 (4 weeks p. op.). X 300. Three sections from the same nerve fiber are shown; left: damming at P; middle: inside the constricted zone; right: distal part, D₀. In the distal section, note the normal myelin sheath and the slightly irregular outline of the axis cylinder, indicating beginning atrophy.
fibers (fig. 16) shows complete correspondence except for scale.

In interpreting these data, it must be borne in mind that these nodes are not newly formed, but are old nodes of primary fibers. The reduction of internodal length, therefore, signifies an actual shortening of the fiber segments concerned, involving both axon and myelin sheath. As can be seen from figure 23, the degree of shortening bears a linear relation to the distance from the constriction, similar to beading (fig. 13). The shortening of the axon segments is directly linked with their widening (ballooning) and buckling (telescoping), and both phenomena thus appear as merely different aspects of a single event — the longitudinal compression of a moving column of axoplasm, due to the increased resistance encountered at the bottleneck, with resulting lateral bulging and folding.

These results prove that damming occurs not only in fibers that are still growing in size and mass, as was the case in our standard experiment, but in adult, stationary fibers as well.
We are thus forced to the conclusion that the proximo-distal growth of the axonal column is a process which goes on continuously in all living nerve fibers and of which regeneration after severance is only a special manifestation.

10. Tandem constrictions

It seemed of interest to determine whether or not damming can occur in more than 1 place along the same fiber. Nerves were therefore provided with 2 constricting sleeves several millimeters apart. Longitudinal sections showed damming at both levels, though it could again be disputed whether the fibers dammed at the first and those at the second level were the same. Crucial results were, however, obtained with teased preparations, in which individual fibers could be traced through both levels. It is rather difficult to isolate fibers over such great distances without breaking them. However, some attempts were successful, and in favorable cases, damming was then seen in the same fiber at the proximal sides of both the upper and the lower constrictions. Damming at the lower level was, however, less common and less intense, for reasons which can be readily understood. Due to the upper constriction, the fibers are already greatly reduced in caliber as they pass through the lower constriction. The latter is therefore much less effective than the former, producing only little, if any, additional reduction. As a result, the damming effect remains mild. The significant point to note is that it has appeared at all. For this proves that the centrifugal convective mechanism of the fiber continues to operate at the far side of a constriction.

Tandem damming, moreover, confirms the irrelevance of the acute constriction changes for the damming process, discussed in chapter 8. For the fiber stretch lying between the 2 constrictions, the acute changes are essentially symmetrical at the upper and lower ends; yet, damming is confined to the lower. This likewise defeats any attempt to bring damming in connection with circulatory disturbances — a contention un-
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urable on many other grounds (e.g., damming at far end of constriction in recurrent fibers; increase of damming with time; coiling; etc.).

11. Released constrictions

From the results thus far, it has become apparent that the axoplasm even of the mature fiber is in constant translatory centrifugal motion and that the size of the axon at any 1 level is the expression of a stationary equilibrium between the rate at which axoplasm is delivered to that level and the rate at which it is passed on to the next distal level. In channels of uniform width rates of supply and disposal are equal for any 1 level. A sudden narrowing of the channel, as in constricted fibers, curtails downward convection and thus causes damming. This being a stationary rather than static condition, it should follow that reopening the channel to its full width by removing the constriction, would act like the opening of flood gates: the piled up material would be swept distal, so that the fiber would gradually widen and eventually become equalized proximally and distally.

This deduction was tested by experiments in which the constricting sleeves were removed after a period of from 3 to 25 weeks and the nerves were then given a recovery period of from 1 day to 8 weeks in the sleeveless condition before being examined. Some tests were also made to ascertain the extent to which fibers constricted for brief periods only (1–8 hours) could return to their former width upon removal of the constricting agent. We found that only constrictions of very short duration (several hours) were fully reversible, while constrictions of longer duration left a permanent impress on the fibers, due partly to the fact that the tubes adapt themselves to the reduced dimensions, and partly to the cementing action of the endoneurial connective tissue. The size to which the nerve is initially compressed is the resultant of the contractile and elastic force of the arterial sleeve on the one hand and the total pressure and elasticity of the nerve
interior on the other. In the course of time, the former force is partly replaced by the structural rigidity of the fibrous matrix of the nerve, which restricts the fibers to their reduced dimensions even after the sleeve has been removed, and which must be overcome by increased internal pressure if the fibers are to re-expand. Therefore, if in the following, we speak of released constrictions, this refers merely to the removal of the constricting sleeves, with the understanding that this still leaves some residual resistance opposing the expansion of the tubes. This internal resistance is the greater, the longer the nerve has been in the constricted state.

Nerves released about 1 month after constriction, by which time damming had become prominent, behaved as predicted. The dammed up axoplasm forced its way into and through the formerly constricted zone and on into the distal segment. Deprived of the arterial cuff, the narrow tube sections were not rigid enough, despite reinforcement by endoneurial collagen, to withstand the internal pressure of the advancing axoplasm. Their walls yielded and became distended. Since in accordance with local variations of resistance, the axis cylinder bulged out more in some places than in others, it assumed an irregularly varicose and dented appearance. Figure 26 shows tracings of photographs from levels P, C and D of a released fiber (right) and an unreleased fiber (left) constricted for the same length of time; figure 27 shows actual photographs from zone C, released and unreleased. The sharp contrast between released and unreleased fibers, both in regard to diameter and smoothness of contour, illustrated in these samples, is typical.

As the released axoplasm presses on downward beyond the old constricted zone, it widens more and more of the undersized distal part of the fiber. This process varies some-

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Fig. 26 Effect of release of constriction after damming. Tracings of photomicrographs of fibers (tensed) at levels P, C and D, after (right) 25 days of constriction, followed by 4 days in released condition, and (left) 29 days of unreleased constriction for control. All 3 sections of the right figure taken from the same preparation.
Figure 36
Fig. 27 Photomicrographs of constricted zones, 4 days after release (right) and unreleased (left). $\times$ 500. The released fibers are from the same preparation as the fiber in the right half of figure 26.
what depending on whether this part is a regenerated or preserved one. But the essential features are the same in both cases. It is interesting to note that both in zones C and D, there appears now a great deal of secondary damming on a minor scale, with slight bulges of axoplasm to be seen at the proximal sides of many Schwann nuclei, residual ovoids, myelin folds, and similar protrusions into the axonal space. Moreover, the peculiar polar configuration characteristic of nodes in a dammed zone (see chapter 3; fig. 17) and formerly confined to $P_\text{b}$ and $P_\text{c}$, now begins to appear in D as well. Evidently, any structures encroaching on the lumen of the still narrow distal tube, offer continued resistance to the axonal movement, thus establishing countless little bottlenecks with concomitant damming.

![Image](image_url)

Fig. 28 Appearance of former balloon at entrance to zone C (7½ weeks of constriction), 3 days after release. R.222 (teased preparation). $\times$ 630.

As the released axon gains in width in zones C and D, it loses its surplus in $P_\text{c}$. The argentophile substance, which in the dammed state has occupied the full width of the balloons, becomes gradually reduced to a central filament of roughly the normal dimensions of the axis cylinder, as is illustrated in figure 28 (also fig. 26, top right). The myelin sheath at first preserves its shape as an empty shell around the spaces of the former balloons, connected with the shrunken central mass by remnants of the neurofibrillar reticulum. The axis cylinder does not recede from the sheath as a whole, in the manner observed in distal fiber atrophy (see above), but the plasma membrane remains adherent to the sheath, and the change in the distribution of argentophile substance is one within the axis cylinder itself. One obtains clearly the im-
pression that the fibrillar constituents of the axoplasm are rapidly drained from the pockets of the former balloons into the descending axial stream. Since silver impregnations are notoriously erratic, we have always fixed and impregnated a released nerve and its unreleased control from the opposite limb side by side in the same solutions. In such pairs, the dammed portion of the unreleased nerve was usually much more heavily impregnated than the corresponding portion of the released nerve, as is illustrated in the top picture of figure 26. This differential was sufficiently common to support the assumption that the emptiness of the balloons is due to actual loss of argentophile fiber substance rather than to defective impregnation.

During the first 2 days after release, the advancing front of the axoplasmic "flood wave" can be recognized rather clearly by the lumpy appearance and greater width of the axon portion which it has passed, in sharp contrast to the smoother and more delicate appearance of the portion ahead. It was thus possible to measure the rate of advance. All measurements were made in teased preparations. While determinations on a single isolated fiber were often ambiguous, the combined values for numerous fibers of the same nerve proved remarkably consistent. They reveal the rate of the convection during the first 2 days to be of the order of 1–2 mm per day. The extremes thus far noted were about ¼ mm and a little more than 2 mm. The measurements are still too crude to attempt a more detailed correlation between rate of advance on the one hand and such properties as fiber size, distance from cell body, amount of damming, etc., on the other. Much more refined work is needed on this subject. But the general order of magnitude as here indicated seems fairly safely established.

Measurements after periods of more than 2 days were less conclusive. Often the distal fragments of our teased preparations were too short to show the position of the wave front, but above all, the latter became less distinct as it advanced. This is understandable if we remember that according to an
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earlier calculation (chapter 5, last paragraph), the whole dammed up material of a given fiber is only enough to fill a 2-mm stretch of the distal portion up to its normal diameter. Even allowing for the fact that the distal fiber does not increase to normal size right away, it is still evident that the available surplus of axoplasm is fully spent after adding width to only a few millimeters of fiber, that is, on the basis of our measured rate of 1 mm per day, after a few days. After that, the fiber continues to increase steadily, but the convenient mark of the flood wave front has disappeared, and with it the morphological basis of our measurements. However, some favorable cases measured 4 days after release, gave values of the same order as those for the first 2 days, intimating that the wave progresses at a constant rate.

This fact makes it highly probable that the measured rate is identical with the normal rate of the centrifugal convection of axoplasm in undisturbed fibers. This contention would be open to doubt if the dammed up portion itself exerted local elastic pressure in addition to the regular convective force. However, the fact that the tube and the myelin sheath of the balloons retain their shape as the axoplasm is drained from them, disproves the notion that the content of the axon is under elastic pressure from its container. Also, if the downward movement were actuated by elastic forces of the dammed zone itself, its rate should rapidly decline, since the elastic force would diminish in proportion as the local congestion is being removed. Yet, as stated before, indications are that the rate remains essentially constant during the first several days, when most of the dammed material is drained off.

This evidence, though still tenuous, points toward the rate of 1 mm per day as being actually the normal average rate at which axoplasm is propelled downward along a nerve fiber. The advance of suddenly released dammed material is merely a marker to make the continuous movement conspicuous and measurable, but in our view does not materially change the character of the movement.
During the weeks following release, zones C and D continue to widen, straighten and become smoother of contour. At the end of 4 weeks, many of the earlier inequalities of diameter have disappeared and the fibers offer a fairly normal aspect over their full length, although at the former level of damming, one still notices slight irregularities, such as occasional dents or vacuoles in the axon or convolutions of the myelin sheath. Moreover, the formerly constricted zone and the distal segment, while considerably wider than before the release, have not attained the full width of the proximal axon stem.

In order to test whether, when given more time, the fibers would recover further, the following experiments were carried out. Peroneal and tibial nerves were each constricted for 10 weeks. After that the arterial sleeve was removed from the peroneal only, the tibial being left constricted for control. At the same time, a distal sample of the peroneal was fixed for histological study. After an additional recovery period of 8 weeks, the released peroneal and the unreleased tibial were preserved and studied in cross sections. It was evident at first glance that the fibers in the distal peroneal (released) were (a) much larger than they had been at the time of the release, when the first sample was taken; (b) much larger than the distal fibers of the (unreleased) tibial; (c) considerably smaller than their own old proximal stems.

In one specimen, the 150 largest fibers at each sample level were measured and plotted in histograms. The total cross sectional areas of these 150 fibers of the peroneal were (in square micra): 2526 in the distal sample (Dᵢ) taken prior to release; and 14658, 6638 and 6161, at levels Pᵢ, C and Dᵢ respectively, at the end of the experiment. It can be seen that during the 8 weeks following release, the distal fibers have grown from 2526 to 6161, that is more than 2½ times. At that figure, they are, however, still less than one-half of their own proximal size. Their increase is attributable entirely to the release of the constriction, since the comparison with the unreleased tibial, as well as with other unreleased cases (fig.
9), shows that they would otherwise not have enlarged further. The fact that they are still undersized, is readily understood by considering that the formerly constricted zone is likewise only less than one-half of the proximal figure. This failure of chronically constricted tubes to re-expand fully after the removal of the sleeve, in turn, is accounted for partly by the fact that they are tightly cemented by endoneurial connective tissue, and partly perhaps by the limited distensibility of the shrunken tubes themselves. That their plasticity is limited, has already been noted in ordinary nerve regeneration; when regeneration is delayed, the tubes of the distal stump shrink, and when later reinnervated, remain of reduced size (Sanders and Young, '44). In our present cases, the residual narrowness of the fibers at the site of the former constriction, according to chapter 2, places a commensurate size limitation on their farther distal portions.

The incompleteness of size recovery is, therefore, to be ascribed not to reduced growth pressure of the axon, but to the increased resistance with which it has to contend in widening its channel. From this it should follow that size recovery after release will be the less complete, the longer the interval between constriction and release has been, that is, the more firmly the endoneurial tissue under the constriction has become consolidated.

12. The fate of the axonal material

It is not our intention in this article to go much beyond the sheer presentation of the facts underlying the concept of proximo-distal convection of axoplasm. Discussion of the many ramifications of the concept will have to wait for a later monographic treatment. Only a few problems most intimately connected with the described phenomena will be singled out for immediate consideration. The first and most urgent question is what becomes eventually of the axoplasm that steadily moves from the cell body toward the periphery. If this movement were confined to regenerating fibers, the
answer would be simply that the material is being used for formation and subsequent growth in width of the new fiber segments. But since the same movement goes on in mature fibers of stationary size as well, the solution is less obvious. We must conclude that in these stationary fibers, for every quantity of substance received from the perikaryon, an equivalent quantity is lost in some fashion, but where and how, remains to be determined.

For a correct appreciation of the problem, it is essential to bear in mind that the centrifugal movement engages the whole column of axoplasm, rather than merely some diffusible liquid within it. The whole morphological evidence of damming revolves about this cardinal fact; to pick a few reminders at random, let us refer to the configurations of telescoping and coiling, to the foreshortening of internodes, to the convolutions of the neurofibrillar apparatus. These features can only be understood as deformations of a system that has some degree of form stability, but are inexplicable in terms of the behavior of liquid systems. It has been suggested previously, on rather indirect evidence, that there may be a centrifugal transport of substance going on in axons (Cook and Gerard, '31; Parker and Paine, '34). It will be noted that our observations, while partially supporting this view, at the same time qualify it by showing the convection to be of the axon, rather than in the axon. Where then does the axon lose the equivalent of what it steadily gains from its center?

The first possibility is the discharge of axonal substance into the periphery. In the case of muscle, such a discharge has been claimed as an observed fact by Carey and coworkers ('46). In preparations of skeletal muscles impregnated with gold chloride, they describe an extrusion from epimembranous axons through endplates into muscle fibers of "auphilic neurosomes," the extent of the granulations varying with the stimulatory and other conditions of the animal. The known capriciousness of the gold chloride technique puts the reality of these structures in serious doubt; and the results of the
stimulation experiments would be just as valid for artifacts, for the changed physico-chemical state of the stimulated tissue could readily account for the observed variation in the amount and density of the metallic deposits. At any rate, these results are in need of verification by more crucial techniques. Even if correct, they would not answer our problem for the sensory fibers, for which the assumption of a massive substance discharge into the end organ would seem rather absurd.

If axonal extrusion into the periphery can be discounted, the only alternative is metabolic degradation, with removal of the split products by diffusion into the endoneurial spaces and capillaries. We must assume then that certain basic systems of the cytoplasm which undergo continuous katabolic disintegration cannot be resynthesized from local resources but have to be supplied ready-made from the distant source of the nucleated cell territory, and that the steadily descending axoplasm is the conveyor of the requisite supplies. These would come down physically and chemically incorporated in the moving structures of the fiber, presumably the neurofibrillar network to be released locally in accordance with local metabolic rate.

This concept would necessitate a sharp separation of metabolic processes into 2 classes: (1) those in which the whole metabolic cycle of breakdown and resynthesis can take place in isolated samples of cytoplasm; and (2) those in which only the katabolic phase occurs throughout the cytoplasm, while the anabolic phase is confined to the intranuclear or, at best, paranuclear space, so that the cycle cannot be completed unless in the presence of both cytoplasm and a functional nucleus. Metabolic studies of the common tissues cannot differentiate between these 2 types, as the test samples contain both nuclei and cytoplasm. Only in large cells, such as eggs and neurons, can nucleated and non-nucleated fragments of cytoplasm be examined separately. According to extensive investigations of the metabolism of isolated fragments of peripheral nerve, respiration, glycolysis, and the syntheses
involved in these cycles, as well as the synthesis of acetylcholine and other non-protein systems, continue in axoplasm severed from the nucleus (cf. Gerard, ’32). These processes, therefore, belong in our class 1. They occur ubiquitously with the intervention of enzyme systems. The ubiquitous presence of the latter is thereby taken for granted, but there is no evidence that they likewise originate in the localities where they are found to be operative. We may assume that they represent systems of class 2, which have to be supplied from a nuclear source, and we may logically include in this class all basic protein systems.

This would lead us to the contention that all the basic protein systems of a cell are synthesized within, or at least in conjunction with, the cell nucleus, and that, contrary to the systems of class 1, proteins cannot be synthesized by the cytoplasm itself. While their manufacturing site would thus be confined to the nuclear space, they would after release into the extranuclear space, undergo a variety of transformations and degradations, and eventually katabolic decay. The feedback of such cytoplasmic degradation products to the nucleus for reutilization in the reproduction of the basic systems can plausibly be assumed to vary inversely with the diffusion distance involved. Accordingly, nerve fibers, in which this distance becomes excessive, should show only a minimum of direct reutilization of their own protein split products. This deduction seems to find confirmation in the comparatively high values of ammonia liberation from peripheral nerve. Following this line of thought further, we discovered that there is actually a remarkably close correspondence between the rate of protein katabolism, estimated from available biochemical data, and the rate of cytoplasmic replacement, as determined from the experiments with released constrictions. This is shown by the following rough calculation.

According to Gerard (’32, p. 258), "it may be taken as a rough average that at rest in oxygen or nitrogen at 20° a nerve forms 0.3–0.4 mgm per cent per hour" of ammonia. Correction for body temperature would raise the figure to about
1 mgm per cent per hour. Assuming that all nitrogen of the liberated ammonia comes from the deamination of protein, this would correspond to a breakdown of 5-6 mgm of protein per 100 gm of nerve per hour. Since the protein content of nerve is about 4% of wet weight, we can state that of every 4 gm of nerve protein, an amount of 5-6 mgm, or about 1/700, disappears every hour. The total nerve protein would consequently disappear in about 700 hours, or 29 days. This value for the rate of natural protein decay seems reasonable if one considers that the "half-life time" of antibody protein, as well as of the average serum molecule, in the body has been estimated to be about 2 weeks (Schoenheimer, Ratner, Rittenberg and Heidelberger, '42). Now, if nerve protein is spent at this rate, it would have to be replaced at the same rate in order to maintain the nerve in a steady state. The nerves which served for our constriction experiments, measured between 40 and 80 mm in length. To replace this length from its central end in 29 days, would require a steady supply stream of the requisite material at a daily rate of 40 to 80 mm, divided by 29, or about 1.4-2.8 mm per day. One immediately recognizes that this postulated rate of replacement is of the same order as the actual rate of proximo-distal convection of axoplasm (1-2 mm per day) established by our observations (chapter 11).

There are a great many uncertainties in our argument. Its biochemical premises are conjectural, our calculations are only approximative and our measurements of axoplasmic convection crude. The fact that the calculated and observed rates turned out to be almost identical, may, therefore, be regarded as coincidental. Yet, even allowing for an error by a factor of 10, the correspondence between the 2 sets of values, derived from entirely different data, would still be close enough to lend support to our hypothesis that the movement of axoplasm serves the continuous replenishment of the specific protoplasmic, particularly protein, systems of the nerve fiber, the production of which has to be assumed to occur exclusively in the territory in or around the nucleus. We are
thus led, although from a quite different direction, to essentially the same conclusion at which Hydén ('43) had arrived from his analysis of protein metabolism by means of ultraviolet absorption spectra of the various cell parts. In his view, the nucleus of the nerve cell is the site of continuous intensive synthesis of nucleoprotein, with steady discharge of the latter into the cytoplasm. Other related views will be discussed below. However, in contrast to earlier more general suggestions of proximo-distal “travel” of “chemical substances” (e.g., “respiratory ferment or accessories”; Gerard, ’32) in the axon, our observations rather point to the axon column as a whole being in a state of motion as a relatively coherent system.

13. Degeneration

Our results have provided not only confirmation, but an actual detailed visualization of the so-called “trophic” control of the perikaryon over the axon. The disintegration of axon fragments severed from their central nucleus, as in “Wallerian degeneration” of peripheral nerves, has been ascribed to the loss of such “trophic” control; in fact, the idea that this control might be exerted by substances emanating from the nucleus, was deduced from the degeneration of distal stumps. It thus becomes our task to reexamine the problem of Wallerian degeneration in the light of our new facts.

Because of the composite nature of peripheral nerve, Wallerian degeneration is a highly complex phenomenon, in which loss of conductivity, metabolic changes, morphological alteration and subsequent disintegration of the axon, myelin disruption, Schwann cell hypertrophy and hyperplasia, macrophage activity, etc., play different, though interrelated, parts. In view of the essential symmetry of conditions in the proximal and distal stumps for all components but the axon itself, the interruption of the latter must be rated as the prime event setting off the chain following transection. Evidently, something formerly supplied from a proximal source
has dropped out. The question is whether this "something" can be directly identified with the steady axonal supply stream as defined in this article.

There is some evidence that degeneration advances in proximo-distal sequence. With many earlier investigators, we have been unable to discern any such consistent gradient by the common morphological criteria of degeneration. However, subtler physiological tests seem to prove such a gradient for the loss of conductivity (Rosenblueth and del Pozo, '43) and excitability (Groat and Koenig, '46), suggesting a proximo-distally progressing depletion in the nerve of some essential constituents. This course is thus in the same direction as the axonal supply stream. Yet, the time relations of the 2 phenomena are not of the same order. Nerve degeneration proceeds at a rate of millimeters per hour and is complete in most nerve fibers of warm-blooded animals within a matter of days. In contrast, the figures quoted earlier in this chapter indicate a much slower rate of advance (1 mm per day) and metabolic exhaustion (several weeks) of axoplasm. It is impossible, therefore, to attribute degeneration simply to the fact that new axoplasm can no longer be supplied to the distal stump. A purely "trophic," i.e., nutritive, explanation of degeneration seems inadequate, and a dynamic concept is called for. Just what dynamic factors indispensable for the integrity of the axon are transmitted from the cell body, seems still as obscure as ever. It is conceivable that further research may disclose a link between this factor and the propulsive mechanism of the axoplasm, but for the time being, such connection is purely speculative. Our results have, therefore, contributed little to the elucidation of the problem of degeneration other than a warning against an oversimplified concept of nutrient depletion.

In this connection, an observation on degeneration in nerves with chronic constrictions is of interest. When such nerves are transected proximally so that the distal stump includes, in proximo-distal order, (1) a stretch of erstwhile normal nerve, (2) the dammed zone, (3) the constriction, and (4)
the remaining distal portion, the disintegration of the axis cylinder occurs in the following sequence: \((2) \rightarrow (1,4) \rightarrow (3)\). The ballooned portion breaks down very precociously (fig. 29), the normal fiber portions follow, and the constricted portion is greatly delayed, as described previously (Weiss and Burt, '44). These observations, though based on a small number of cases and in need of repetition, emphasize the

![Image](image_url)

**Fig. 29** Precocious degeneration of dammed fiber portions after nerve section. The top and middle pictures are from the same tibial fiber, constricted for \(6\frac{1}{2}\) weeks, and the bottom picture from an unconstricted fiber of the peroneal control. Both nerves had been cut farther proximally \(44\) hours before fixation. Top: Balloons immediately in front of constriction show advanced axon disintegration (note radial structure of neurokeratin of myelin sheath). Middle: Farther proximal sample of same severed fiber stump, with no signs of degeneration. Bottom: Severed control fiber without degeneration. Bodian's silver impregnation. \(\times 630\).

influence of local factors on the rate of degeneration. Especially noteworthy is the fact that \((2)\) breaks down prior to \((1)\), and \((4)\) prior to \((3)\); in both cases a more distal portion preceding a more proximal one.

A dynamic concept of Wallerian degeneration, such as that recently sketched by Young ('44b), seems in general closer to the truth than a trophic concept. However, the very fact
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of the structural consistency of the axon, brought out by our experiments, disproves the fluid nature attributed to the axon by Young, and hence, contradicts his explanation of degeneration in terms of simple surface tensions. While Young has recognized this fact ('45b), a valid theory of degeneration seems still a matter of the future.

14. Regeneration

What bearing has the centrifugal convection of axoplasm, dealt with in these pages, on the so-called outgrowth of the free tip of a regenerating nerve fiber? The rate at which regenerating fibers advance in warm-blooded animals is, under optimal conditions, of the order of a few millimeters per day (maximum of cca. 4 mm). This value is very similar to the one we found above for the rate of the axoplasmic movement in stationary fibers. It could be assumed, therefore, that the process is essentially the same in both cases. According to earlier investigations (see Weiss, '45), the free tip advances along interfaces to which its fine filopodial extensions apply themselves. The pull thus exerted by the tip is then supplemented by the filling up of the fiber from behind as a result of the activities described in this paper. The motile mechanism involved can, therefore, be characterized as a "pull-push" principle (Weiss, '44c). Comparing the fiber to a plastic pipe line, we may say that as the terminal filopodia extend the line, the older fiber portions lying behind pump more axoplasm into the new segment and consolidate it. The major contribution to the work of elongation would thus come from the "push" component. This would limit the rate of the whole advance essentially to the rate of the "pumping" process; which would indeed explain the agreement between our convection rate and standard regeneration rates.

Lewis ('45) has seen evidence of a peristalsis-like contractile wave effecting the elongation of axons in tissue culture, and he has pointed to the presumable identity with our axoplasmic convection mechanism. We are in essential agree-
ment with this view, provided the guiding function of the filopodia is duly taken into account. Pumping from behind without concomitant filopodial extension can but inflate the tip to a bulb, as actually occurs in the familiar terminal swellings of stalled regenerating fibers ("terminal clubs"; Cajal, '28). Such bulbs are simply instances of terminal "damming." This can be averted as long as advancing filopodia provide outlets for the material that keeps pressing distad. Young's ('42a) suggestion that nerve regeneration consists essentially of an "outflow" of axoplasm, is, therefore, only partly correct. Neither is the axon in the implied liquid state nor could the mere passive spilling of axoplasm from its tube under turgor pressure yield the morphological pictures seen in regeneration and damming. The assumption, that hydrostatic pressure generated in the cell body could produce longitudinal orientation and alignment of the molecular chains in the axon (Young, '45b), likewise lacks physical realism. These objections to Young's particular hypotheses notwithstanding, one must appreciate the fact that his basic concept of "pressure from behind" is valid and, above all, that the pragmatic value of his attempts to fill the descriptive symbols of the morphologist with physico-chemical sense, outweighs any defect of an admittedly tentative set of hypotheses.

In conclusion, a consideration of the outgrowth phase of regeneration makes it probable that the same mechanism that actuates the proximo-distal shifting of axoplasm in stationary fibers also acts in propelling regenerating fibers. This fact is reflected in the similar rates of the 2 processes, regenerating fibers advancing at a rate of a few millimeters per day (Young, Gutmann, Guttmann and Medawar, '42). In this connection, it is well to recall the ambiguity of the term "regeneration rate." As stressed on an earlier occasion (Weiss, '45), the advance of the tip of a regenerating fiber is characterized by a succession of brief spurts and stalls. The overall rate of its progress, therefore, varies with the frequency and duration of arrests, which, in turn, are functions of the constitution of the physical substratum. Nerve fibers
under different conditions consequently display quite different overall rates of regeneration, even though their basic rate of actual movement be the same. To treat "regeneration rate" of a nerve fiber as if it were a true "growth rate" is, of course, even more fallacious. To avoid confusion, it is best to keep a realistic picture of the processes involved in mind.

**DISCUSSION**

Most of the past work on nerve growth has dealt with the elongation of the nerve fiber, which is primarily a process of protoplasmic movement (Harrison, '10). The advancing tip spins out a protoplasmic thread, which is at first very thin. Generally, fibers are also still relatively short when they connect with peripheral tissues. The comparatively enormous dimensions of the mature fibers are reached by subsequent growth. Elongation and enlargement are, thus, 2 distinct phases of nerve development. Elongation is a physical process of extension produced by the pull of the free tip or the tug of a terminal organ ("towing," Weiss, '41). Enlargement is the result of the production of new protoplasm at a rate which not only makes up for the thinning that passive stretching entails, but above that, increases the width of the fiber. Elongation creates the cylindrical shape of the fiber, while the scalar growth process feeds additional substance into it. Concurrent at first, the 2 processes later become distinct, as elongation ceases, while enlargement continues. In regeneration, elongation is reactivated, but again is outlasted by enlargement. The factors instrumental in elongation have received due attention, but not so the factors of enlargement, although as determiners of the typical caliber spectrum of nerves, they have very important functional implications (Erlanger and Gasser, '37). Pathological aberrations of the elongation process (neuromas, branching, disorientation, stoppage, retraction, etc.) have been amply studied, but the pathology of fiber size (atrophy, hypertrophy) is still an obscure chapter. Of late, however, a marked turn of interest
has become noticeable, and the true growth process, i.e., enlargement, is beginning to get its share of attention from several directions (Hydén, '43; Sanders and Young, '44; Bodian and Mellors, '45). Our own contributions to the problem date back to 1943 (Weiss, '43b, Weiss and Taylor, '44b), and it is with this phase that the present report is chiefly concerned.

According to the evidence presented, the sole source of new axoplasm lies in the central territory of the neuron, which contains the nucleus. For the elongation of the nerve fiber, this fact has long been acknowledged in the neuron doctrine, which states that the axon receives no protoplasmic contributions from foreign cells. We now find that the enlargement of the fiber demands an even more restrictive formulation to the effect that the axon produces no protoplasmic additions within its own territory, but receives all protoplasmic supply from a circumscribed production site located in or at the nucleus. The evidence is derived from 2 distinct, but supplementary phenomena, (a) the permanent size deficit at the distal side, and (b) the damming of axoplasm at the proximal side, of a fiber segment in which the lumen of the tube has been artificially reduced.

Of the two phenomena, the proximal one is more informative. While distal atrophy could be equally as well explained by a reduced supply from the cell body of some essential accessory factor for local growth as by a reduced supply of fully grown protoplasm, the findings on proximal damming exclude the former explanation. Also, while the distal deficit merely proves the existence of proximo-distal convection, the damming process reveals its very character. For this reason, our major attention has been given to the damming phenomenon, and we have endeavored to establish the underlying facts as securely as possible. The quantitative regularities recorded and the various control experiments (chapters 6 and 8) seem to make our conclusions reasonably safe. These conclusions are the following.
The deformities, designated as damming, are real features of the live fiber. They form wherever a fiber suddenly passes through a narrower cross section, and it is immaterial whether this has been brought about by actively reducing the diameter of a mature wide fiber or by limiting the expansion of a still growing fiber. A technological consideration of the various shapes and spacings of these deformities has revealed that they have precisely the configurations to be expected in a coherent column of viscous substance propelled at a steady rate in a semiplastic sheath, when confronted with an abrupt reduction of the cross section of its channel. Damming, according to this evidence, is caused by growth. The validity of this conclusion has been checked against the alternate assumption, that the observed deformities might be residues of the primary deformation suffered by fibers in the act of constriction (chapter 7). As we have seen, there are definitely such residual effects at both sides of a constricted zone. They are clearly distinguishable, however, from true damming, which is superposed upon them, confined to the proximal side, and furthermore, can be obtained in unadulterated form under conditions in which the acute effects of constriction have been averted (chapter 8C).

From the combined evidence of all experiments, the conclusion that damming really is a sign of continuous axon growth, seems cogent. Damming occurs because the part of the axonal column lying between the cell body and the constriction receives more axoplasm from proximally than it can dispose of distally. The axon thus outgrows its tubular sheath and the various configurations of ballooning, telescoping, beading and coiling are the results of the accommodation between the expanding content and its resistant container. It is highly significant that the blocked column shows the signs of its expansion not over its full length, but confined to its far end and grading off proximally. This fact proves that we are not dealing with growth alone. For the excess pressure resulting from the stuffing of additional axoplasm into the nerve fiber would build up from the end where the material
is added, that is from the central end, and therefore would widen primarily the base of the column. If the column were relatively fluid, its gain in width would be evenly distributed. In neither case, would the proximal portion retain its proportions and only the far end show the effects of pressure, as they do in reality. This objection holds whether we ascribe the central pressure to growth or to an increased osmotic "turgor" due to the dissociation of nucleo-proteins, as sketched by Young ('44a). Static pressure from a central source of whatever origin is physically unfit to explain the location and shape of damming. To explain the observations, we must postulate a dynamic pressure mechanism which moves the column along (see chapter 3).

Such a dynamic pressure could most readily be conceived of as a peristaltic wave, progressing proximo-distally either in the membrane of the nerve fiber or perhaps in the neuro-fibrillar chains themselves. Alternating contraction and relaxation in the proper sequence would massage the axonal substance distad. Indications of axonal peristalsis of the requisite kind have been observed in young growing axons (Lewis, '45), but nothing is known about corresponding processes in mature fibers. Perhaps there is a direct connection between the postulated "axomotile" mechanism and the forces effecting the steady centrifugal propulsion of the endoneurial fluid between the nerve fibers, described previously (Weiss, Wang, Taylor and Edds, '45). The fact that the rate of the interaxonal flow is about 25 times faster than that of the intra-axonal convection (cca. 1 mm per hour against cca. 1 mm per day), could be ascribed to the great difference in the respective viscosities. Many observations, as for instance, tandem damming (chapters 10, 11) and the minor piling at the nodes (Chapter 3D), indicate that whatever the conveyor mechanism be, it is present in every fraction of the peripheral course of the fiber. The energy needed for the motile apparatus is almost certainly derived from local metabolic processes. Just what parts of the axonal system are actually being conveyed, requires further definition.
The primary object is evidently the relatively coherent matrix of the axon, which has some degree of form stability. This does not imply absolute rigidity, and in fact, our hypothetical interpretation of the consumption of the moving mass for local protein replacement could not be reconciled with the assumption that the axonal framework retains its full integrity as it descends. On the contrary, its stability must be considered as of statistical nature, with mechanical links being dissolved and reformed continually.

The extent to which this semirigid framework also acts as a vehicle for interstitial liquid, remains to be determined. Some independent mobility of the fluid relative to the matrix, especially in the capillary spaces along the neurofibrils, is quite probable, and is clearly indicated by the local extrusion of liquid from the compressed framework in the phenomenon of "beading" (chapter 3) as well as by the differential condensation of the fibrillar constituents of balloons after release from constriction (chapter 11). This fluid medium could also serve for the diffusion of dissolved substances and for the possible transport of materials by electrophoresis and interfacial spreading. The contention of a descending movement of the axon system as a whole, thus, is no more at variance with the established fact that neurotrop viruses or drugs can ascend in neurons, than the flow of a river excludes upstream navigation. While a peristaltic mechanism would adequately explain all aspects of damming, the possibility remains open that some wholly different and unknown principle is involved, as the directive forces of growth in general are still rather obscure. That impulse conduction can have no part in the axonal convection, has been explained in the text (chapter 3E).

An important feature of the damming process that can serve as a clue to its nature, is its linear gradient. As described in the text, the spacing between certain regular landmarks along the fiber, such beads and nodes, decreases in linear order as the distance from the constriction decreases (figs. 13, 16, 25). If we regard the change in spacing as an
expression of actual shortening, it follows that a standard length of axon suffers a lengthwise compression that is the greater the closer the segment lies to the constriction. This in turn, means that the longitudinal compressive force diminishes as a function of distance. On the assumption that the propulsive force is constant, the data, therefore, imply that the resistance to proximo-distal convection declines at a linear rate as one proceeds backwards from the constriction. This decline is readily explained by the fact that force is spent in the work of deformation (lateral distension of the sheath, telescoping, detachment between axon and sheath, etc.) so that the resistance met with by successively proximal segments is bound to diminish in proportion to the length of the stretch already deformed. While the linear characteristics of the over-all pattern of damming are thus plausibly accounted for, the apparent inversion of the pattern within each individual internode (chapter 3, fig. 17) remains to be explained. It could be related to some additional resistance to, and delay of, transfer at the nodes, coupled with a rebound effect, but we have no really satisfactory solution to offer. At the same time, as outlined in the text, the nodal polarity resulting from this effect has been a convenient and reliable index of the direction of axonal convection.

In summary, we arrive at the following concept of the growth of a nerve fiber. The perikaryon reproduces new axoplasm at a rate determined by constitutional properties of the cell and external conditions (e.g., nutrient supply, func-

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9 The essential features of damming can be reproduced in a simple model of the following characteristics. One yard of rubber tubing, pulled over a snugly fitting, slightly longer metal rod, is placed in a wider rubber tube. All surfaces are wetted. The outer tube is to represent the neurilemmal tube, the inner assembly the axon. Lengthwise movement of the metal core carries the inner tube with it by friction, thus imitating the translatory mechanism of the axon. A perforated cork with a bore slightly wider than the rod, but too narrow to let the inner tube pass, represents the bottleneck. By this resistance, the inner tube is gradually stripped back from the moving core and thrown into folds, which by deforming the outer tube, gives rise to configurations closely resembling those of dammed axons. The resulting bulges and folds show the same linear gradient of spacing that was observed in the axon.
tional activity, peripheral connections, etc.; see below). The nerve fiber constitutes an outlet into which the new axoplasm is siphoned by an "axomotile" mechanism. The width of the fiber is normally so adjusted that it permits unobstructed passage of axoplasm at a commensurate rate. The moving axoplasm is then consumed in the metabolic activity of the fiber. An axon could thus be vaguely compared to the wick of a kerosene lamp.

Further elucidation of the axoplasmic convection can be expected from precise determinations of its rate and fate. If it is true, as proposed in the text, that katabolized axoplasm cannot be resynthesized from local peripheral resources, but must be replenished by the moving supply column, then the rate of the movement should be found to be different in fibers of different lengths, being the faster the longer the fiber. On the other hand, no appreciable difference is to be expected between fibers of different calibers, but of similar lengths. In general, rate of protoplasmic reproduction, size of perikaryon, fiber diameter, rate of convection, and axoplasmic consumption are properties that are interdependent and, in the normal fiber, mutually adjusted.

Constriction does not affect the continuous growth process, but interferes with the distribution of the grown material by reducing the cross section of its channel. For the traffic from the wider into the narrower section to continue unimpeaded, would require that the velocity of the movement through the narrower zone be proportionately increased, as would actually be the case in an ideal liquid system (Bernoulli's principle). If the consistency and form stability of the moving system resist such local acceleration, the volume passed down at any given moment will be smaller than the volume received from above, and damming will result, comparable to a traffic jam at a communication bottleneck. Moreover, all parts lying "downstream" will receive correspondingly smaller volumes per unit of time, entailing a permanently reduced caliber for the whole distal portion.
The factors determining the rate of protoplasmic reproduction in the cell body remain to be explored. Some of the differences among different types of nerve cells date back to the early embryonic phase and are strictly of prefunctional origin. Evidence is increasing that these are later subject to modifications as a result of trophic and functional interrelations with other nerve cells and non-nervous tissues. To judge from its effects on the sizes of cell, nucleus, nucleolus and Nissl bodies, as well as on more direct criteria of protein metabolism, the functional load of a nerve cell is a factor in determining rate of protoplasmic reproduction (Hydén, '43; Hamberger and Hydén, '45; Vogt, '47). That trophic interactions with the terminal tissues act similarly, is proved by the changes which neurons undergo after they have been disconnected from their functional periphery (see Bodian, '47). Though often ascribed to the trauma of injury, these changes are more properly accounted for by the peripheral loss. Severed nerve fibers which have been prevented from reestablishing peripheral connections show a marked loss of size (Weiss and Taylor, '44a; Weiss, Edds and Cavanaugh, '45; Sanders and Young, '45). The primary reduction occurs presumably in the cell body, for it has been noted that after permanent peripheral disconnection, cell size declines (Howe and Bodian, '41; Cavanaugh, '48). Although Bodian ('47) views this shrinkage of the cell body as an adaptation to the decreased residual volume of the neuron after axon amputation, the fact that the same atrophy occurs in neurons with axons of full normal length but lacking effective peripheral connections, such as sensory axons ending on muscle fibers (Weiss and Edds, '45), proves that it must be attributed to the loss of a positive trophic interaction with the terminal tissue. Just how the state of the peripheral end of a fiber is reflected back on the cell body, is unknown, but striking evidence of very specific ascending communication from periphery to center, other than impulse conduction, has been obtained previously (Weiss, '36).
The concept of nerve growth derived from our observations inserts concrete meaning into some of the more formal theories of earlier authors. Held ('09) postulated a "vis a tergo" and Cajal ('28) a "formative turgor," as the driving forces of nerve growth; both, of course, referring exclusively to the "outgrowth" phase. For the mature fiber, on the other hand, there have been contentions of a proximo-distal flow of substance in axons (though not of axons), as mentioned earlier in the text (Cook and Gerard, '31; Parker and Paine, '34). One easily recognizes now that all of these statements contain an element of truth, but that none of them portrays the real situation either correctly or completely. Conversely, there are numerous data and observations scattered through the older literature, especially of neuropathology, which, at the time, had to remain unexplained or conjectural, but now fall into places and add confirmatory evidence to our concept. Since this is not the proper occasion to review this material, only a few illustrative examples will be mentioned; one group pertaining to the distal size deficit of regenerated fibers, the other to proximal damming.

In ordinary nerve regeneration experiments, the regenerated fibers usually remain somewhat below their full normal size (Greenman, '13; Gutmann and Sanders, '43). Several factors have been identified as causing this deficit; chiefly, (a) the shrinkage of distal tubes denervated for some length of time (Holmes and Young, '42; Sanders and Young, '44), (b) shunting of fibers into tubes of non-matching size (Nageotte and Guyon, '18; Hammond and Hinsey, '45; Simpson and Young, '45), and (c) lack of proper terminal connections (Weiss, Edds and Cavanaugh, '45; Sanders and Young, '45). The fact that a large fiber forced into a small distal tube fails to attain normal dimensions, is attributable partly to the limited distensibility of the tube (see chapter 11), and partly to the fact that such cross unions usually are functionally incongruous, hence entail atrophy of the type (c). However, a small fiber entering a large tube does not expand either, although it would have plenty of space to do so
(Nageotte and Guyon, '18; Simpson and Young, '45; Hammond and Hinsey, '45). This fact finds now a ready explanation in terms of our concept, which implies that no fiber can become appreciably wider in any of its more distal portions than is the narrowest part of its stem; the narrow part acting much as the "bottleneck" in our experiments.

To these 3 factors limiting the size of regenerated fibers, we can now add a fourth, namely, (d) the encroachment on regenerating nerve fibers by fibrous connective tissue at the point of union or intraneurally. By pressing against the nerve fibers, such connective tissue creates the same conditions from the inside as does constriction of the whole nerve from the outside, and fibers distally to the affected area are bound to remain thin. A spectacular example of this phenomenon has recently been described in monkey nerves regenerated over bridged gaps (Matson, Alexander and Weiss, '48), but the fact can also be verified by comparing the diameters of regenerated fibers attained after transection and suture with those attained after mere crushing (Gutmann and Sanders, '43). Since the scar tissue at a suture line is usually much denser than the collagen deposits at the site of a crush, nerve fibers are exposed to much more strangling in the former condition than in the latter, hence, will retain smaller distal diameters. The deficit of regenerated fiber size is, therefore, slight after crushing (Greenman, '13; Guyon, '18), but greater after transection (Sanders and Young, '44; Hammond and Hinsey, '45). Pictures of markedly reduced fiber size in regeneration following chemical injury (Duncan and Jarvis, '43) are virtually proof of residual fibrosis at the point of injection.

Records of what we would now identify as damming, can likewise be found in earlier literature. Most of these are incidental and the true nature of the phenomenon has escaped the observers. The terminal balloons and plaques in arrested regenerated fibers ("arrested balls," "gigantic clubs," Cajal, '28; "boules terminales," Dustin, '17) are plainly manifestations of damming. So are the varicosities and spirals in such
blocked fibers, depicted, but left unexplained, in many of the earlier works on nerve regeneration. In this connection, detailed analysis of the so-called spirals of Perroncito should prove particularly instructive. It is noteworthy that Cajal ('28, figs. 116, 117) had actually observed and illustrated the phenomenon of beading in the fibers of ligated nerves proximally to the constriction, but completely misinterpreted its character and origin. He regards the widenings in the "chains of ball," as he calls them, as residual marks of successive stations on the way of free "growth cones" of fibers regenerating in spurts. In his version (p. 296), "it appears as though the fibers travelled by stages, impulsive efforts alternating with more or less prolonged resting periods. There is here a static-dynamic rhythm, whose irregular periodicity is objectively seen in the string of balls. All the incidents along the path, all the conflicts occurring in the struggle for space, remain as though photographed in these long strings of beads." Evidently, Cajal was unaware that the beads form in the continuity, rather than at the end, of a fiber, and even in uninterrupted primary fibers (chapter 9).

Damming can be expected proximally to any point at which the density of the tissue surrounding the nerve fibers increases. In our extensive work on nerve regeneration, we have observed countless instances of damming at the transition to nerve scars, islands of intraneural fibrosis, fibrotic nerve grafts, and at angulation points. Incidental pictures of dammed fibers can be recognized in many an illustration in the neurological literature (e.g., Dustin, '17, fig. 24; Oppenheimer, 41, fig. 1, and comment on p. 585 of Oppenheimer, '42), and it would be rewarding to gather all this scattered and unexploited evidence under the unifying guidance of our concept of nerve growth.

The evidence that cell growth does not occur throughout the cytoplasm, but exclusively within, or in the vicinity of, the cell nucleus, has of course fundamental biological implications. A discussion of this problem in the light of the results described in this paper has been presented elsewhere (Weiss,
Our tentative conclusion, that the manufacture of all cell protein is confined to the nuclear territory, finds strong support in the work of Caspersson and his school (see Hydén, '43; Thorell, '47), which has adduced an impressive amount of cytochemical evidence in favor of such a concept. Two wholly different lines of approach have thus led to essentially the same conclusions, which is all the more gratifying, as owing to wartime conditions, neither knew about the other. Any attempt, however, to generalize the case of the neuron, must take into account that while the fundamental mechanism of growth is undoubtedly the same in all cells, not all cells are necessarily in a state of perpetual replacement growth (e.g., erythrocytes; cornifying epidermis, and other short-lived cells), and furthermore, that the peculiar mechanism for the convection of the protoplasmic replacements found in nerve fibers, may be a special adaptation of the latter to their overextended supply lines and need not be present in other types of cells.

**SUMMARY**

Evidence is presented for the following concept of the growth of a nerve fiber. (A) Growth, in the sense of production of new protoplasm, occurs solely at the base of the fiber in the nucleated part of the cell body. (B) the column of axoplasm is maintained in constant proximo-distal motion. (C) Growth and centrifugal convection of axoplasm are not confined to the period of active elongation and enlargement, but continue in the mature fiber which has reached a stationary condition. (D) The perpetual growth of the neuron presumably serves to replace katabolized protoplasmic systems, especially proteins, which cannot be synthesized in the peripheral cytoplasm.

These conclusions are based on over 300 experiments with constricted nerves, mostly of rats, as follows.

1. Nerve fibers which have regenerated through a constricted zone show (a) a permanent deficit of width distally to the constriction; (b) a permanent surplus of axoplasm dammed up immediately proximally to the constriction in a
variety of configurations (ballooning, telescoping, beading, coiling), referred to hereafter as "damming."

2. The damming effect grades off proximal in linear order. In conjunction with the morphological analysis of damming figures, this indicates that the phenomenon is due to the increased resistance offered to the movement of the axoplasm by the reduced cross section of its channel.

3. The intensity of damming increases with time.

4. The intensity of damming is directly related to the degree of reduction suffered by the fiber diameter in the constricted zone. Accordingly, it varies with the amount of constriction, and the size and depth of the fiber.

5. The functional capabilities of dammed fibers are not grossly impaired.

6. The configurations of damming are not artifacts, as they can be clearly seen in the living fiber.

7. The configurations of damming are not residues of deformations produced by the act of constriction itself.

8. Damming occurs irrespective of whether the regenerated fibers have already passed or have not yet entered the level of constriction, when the latter is applied.

9. Damming likewise occurs in the continuity of uninterrupted mature fibers, proximally to points of reduced cross section.

10. Damming can occur in more than 1 place in the same fiber, e.g., if tandem constrictions are placed on the nerve.

11. Release of constricted fibers by removal of the constricting agent is followed by a downward movement of the dammed up surplus of axoplasm, widening the formerly constricted and the equally narrow distal part of the fiber.

12. The rate of this descent after release has been determined as of the order of 1 mm per day, and there is reason to believe that this is the rate of the normal process of axoplasmic convection in the studied nerves.

13. This rate has been found to be of the same order as the requisite rate of protein replacement to be postulated on the
assumption that protein breakdown can be estimated from the known values of ammonia production of nerve.

14. If these findings apply to other cell types as well, one would have to conclude that the reproduction of cytoplasm, and perhaps of all basic protein systems, is strictly confined to the territory of the nucleus and cannot be accomplished in the more peripheral cytoplasm itself.

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