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ENDONEURIAL EDEMA IN CONSTRICTED NERVE

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THIRTEEN FIGURES

This is a report of certain observations on intraneural edemas, made in the course of nerve splicing experiments, which indicate that seepage of fluid goes on continually in the endoneurial spaces. At the same time, these observations point the way to a practical method of collecting the intraneural fluid in amounts large enough for biochemical analysis. Inasmuch as this fluid constitutes the medium in which the nerve fibers normally live and in which, as we shall discuss below, they seem to grow best during regeneration, information about its source, composition and behavior is desirable for both theoretical and practical reasons.

The observations reported here were made in nerves which had been severed and reunited by means of segments of artery, the so-called sleeve-splicing method described previously (Weiss, '41 b, '43). In such cases the proximal nerve stump reacts differently depending on the size of the arterial sleeve. If the arterial lumen is as wide as the nerve in its interior, the proximal stump will show no more than the usual traumatic reaction of a transected nerve, which means ascending degeneration for a few millimeters from the cut, followed by the regenerative outgrowth of new axons from the level at which this process is arrested. If, however, the artery is of smaller

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diameter, so that it had to be forced over the nerve end by distension, the subsequent constriction of the sleeve produces local compression of the nerve of various degrees, and this, in turn, affects the condition of the proximal stump much more profoundly than does mere transection. Even arteries which are apparently sufficiently wide at rest to receive the nerve in their interior may later produce constriction owing to the gradual contraction of their muscular wall. This contracture becomes particularly evident under the influence of adrenalin. Adrenalin applied to an arterial sleeve produces heavy constriction of the latter followed by block of impulse conduction, and frequently morphological degeneration, of the compressed nerve fibers (Weiss and Davis, '43). Arteries of only slightly insufficient width produce effects of much less severity. In all cases of constriction, however, whether moderate or extreme, a fairly large amount of fluid was found to accumulate in the endoneural spaces at the central side of the bottleneck. It is on this endoneural edema that we shall focus our attention in the present report. Another associated phenomenon, the damming up of axonal substance proximal to the constriction, will be dealt with in a later paper.

MATERIALS AND METHOD

Edemas following constriction or other obstruction of the nerve have thus far been observed in sixteen chicken and fourteen rat nerves between 1 and 17 weeks after the operation. Five chickens had edematous records on nerves of both wings, while the rats were always operated on unilaterally. The chickens were operated on at an age of between 5 and 7 months; the rats at an age of between 2 and 10 months. As the operation was described in detail in an earlier publication (Weiss, '43), only the main steps may be repeated here. The segment of artery to be used as splicing sleeve is slipped over a specially designed forceps-like instrument. The cut end of the proximal stump of the nerve is then grasped between the two prongs of this instrument and the artery is pulled from the instrument over the seized nerve end. The cut end of the distal stump is

then introduced into the open end of the artery by means of a so-called spreader. For our present purpose, it is important to remember that when it is stripped from the splicing forceps, the artery is sufficiently distended to slide easily over the nerve stump; likewise, the cylindrical shape of the splicing instrument precludes crushing of the nerve during manipulation. Therefore, none of the effects observed in the preparations can be ascribed to damage sustained in the operation. This is further confirmed by the fact that only those nerve splices showed edematous involvement of the proximal stump in which the artery had subsequently contracted, while no effects were noted in cases in which the nerve had remained uncompressed, although both types had been manipulated in exactly the same way.

In some cases, the central and peripheral stumps of the severed nerve were simply united; while in other cases, a fragment of the peripheral nerve was spliced to the proximal stump as a graft. In the latter cases the graft ended freely with an open end. Of the plain nerve reunions some were done with close apposition of the nerve ends while in other cases, a gap was left between the nerve ends in the sleeve. This gap was either left open or filled with blood plasma. The latter procedure stimulated the formation of fibrous tissue in the gap.

All histological studies were made after fixation in Bouin's fluid and silver impregnation according to Bodian, frequently supplemented with a Mallory triple Azan stain. Measurements of nerve cross sections were made by planimetry of camera lucida drawings, while the diameters of longitudinally sectioned nerves were determined by measuring the greatest width appearing in the serial sections.

PROXIMAL EDEMAS

Gross inspection of nerves spliced with a tightly fitting arterial sleeve reveals swellings of the nerve both at the proximal and at the distal end of the sleeve. Of these, the proximal one is much more conspicuous while the distal one may be

completely absent. As we shall demonstrate presently, the two swellings are of different nature, only the proximal one being caused by edema. Figure 1 reproduces a nerve which has already been described on an earlier occasion in connection with studies on the physiological effects of nerve compression (Weiss and Davis, '43). It may serve here as an introductory example, though it does not represent a severed and reunited nerve, but a specimen in which the sleeve of artery was simply pulled over a proximal nerve stump bracelet-fashion. In the

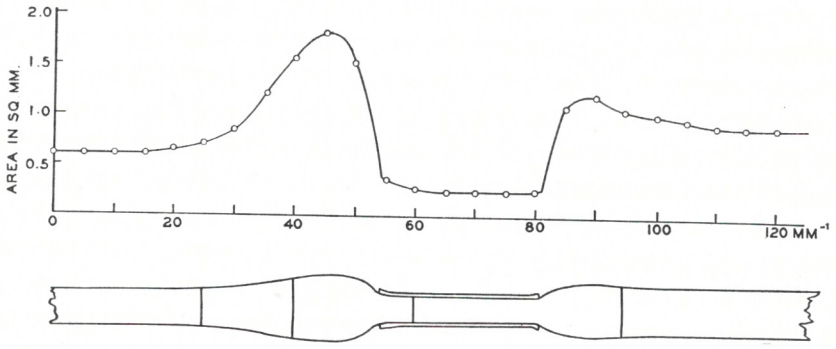

Fig. 1 Edematous tibial nerve (rat R50) constricted by undersized arterial sleeve, 10 days after operation. Lower diagram: Profile of nerve in correct proportions. Upper graph: Cross-sectional area at 0.5 mm. intervals, plotted over length of nerve. Proximo-distal direction from left to right.

lower part of the figure the nerve is reproduced in true proportions, as reconstructed from measurements of cross sections. The proximo-distal direction is from left to right. The upper part of the figure gives the values of cross sectional areas measured at intervals of 0.5 mm. each, plotted over the length of the nerve. One notices that the diameter of the nerve begins to swell more than 3 mm. proximal to the sleeve, first gently, then rather steeply, reaching a maximum of about three times its original size 1 mm. proximal to the sleeve. It then declines abruptly to the narrow dimensions of the bottleneck. At the emergence from the sleeve, at mark 80, the diameter increases again with a slight hump. The fact that the whole distal portion of the nerve is larger than the

proximal part to the left is due to Wallerian degeneration. The compression has produced degeneration of most of the fibers distal to the constricted area, and since freshly degenerating nerve fibers are swollen, the distal nerve as a whole has thickened. The proximal swelling, on the other hand, is entirely due to the accumulation of interstitial fluid between the nerve fibers (fig. 4 in Weiss and Davis, '43).

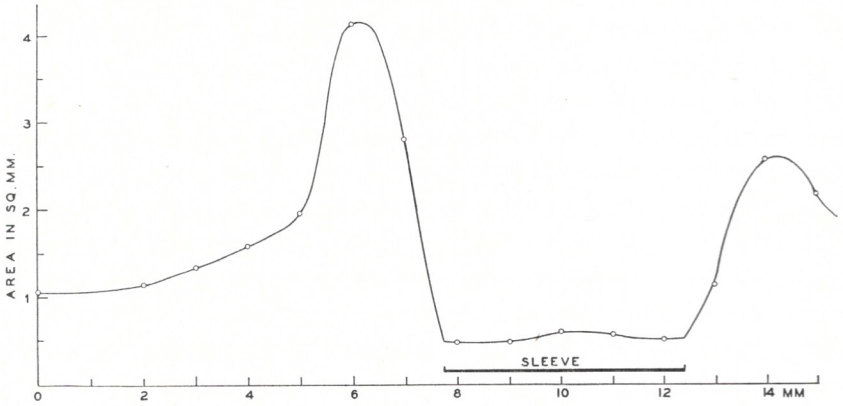

Fig 2 Variation of cross-sectional area of edematous sciatic nerve (rat R7; 200 g.) severed and spliced with sleeve of carotid artery (donor: 360 g.), 5 weeks after operation. Oscillographic tests of this case have demonstrated conductivity of regenerated fibers through the sleeve. Proximo-distal direction from left to right.

While this nerve was fixed only 10 days after the operation, specimens preserved after much longer intervals show essentially the same situation. In the graph, figure 2, cross sectional area of a sleeve-spliced nerve 5 weeks after the operation is plotted over the length of the nerve. The nerve within the sleeve has been compressed to about one-half of its normal size at more proximal levels. Proximal to the sleeve, there is a bulbous edema, the beginnings of which can be traced about 7 mm. upwards in the nerve and which, at its maximum width just in front of the sleeve, distends the nerve to about four times its original size. These two examples are representative for the rest.

Figure 3 represents in summary fashion the measurements obtained in all sixteen chicken and fourteen rat specimens. Diameters were measured at three levels as indicated: at a far proximal level of the nerve (P); at the level of the edema at its maximum width (E); and at the level where the sleeve is narrowest (S). The graph reveals several facts. Firstly, it

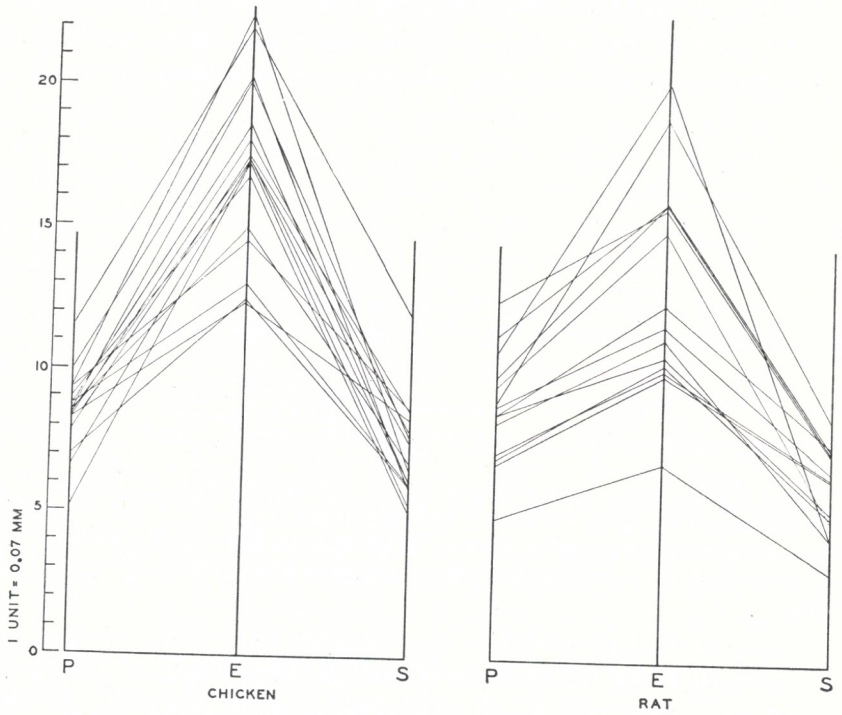

Fig. 3 Size of edemas of thirty nerves with constrictions. Ordinates: Nerve diameter. P, proximal normal part of nerve; E, edema at greatest width; S, sleeve at narrowest point.

can be seen that the chicken, by and large, shows greater accumulation of edematous fluid than the rat. Secondly, in the rat, the diameter of the nerve inside of the sleeve (S) is usually well below that of the proximal stump (P) (figs. 1 and 2), while in the chicken, the difference is much less pronounced although the edema, nevertheless, is well marked. In some cases, the diameter of the nerve in the sleeve is even larger

than that of the proximal nerve, but this is due to a secondary widening of the sleeve long after the proximal edema has become established. Thirdly, while there seem to be two distinct classes, one with mild edema and one with considerable edema, there is no proportionality between size of the edema and size of the nerve. The difference between the diameters at the level of the swelling (E) and in the unaffected portion of the nerves (at P) is approximately the same in small and large nerves. This would mean that the amount of accumulated liquid is relatively independent of the size of the nerve. Nevertheless, in order to give a general idea of the order of magnitude of the swelling, we have calculated the average ratio of cross sectional area at the widest point of the edema over cross sectional area of the unaffected proximal nerve. The values are 4 and 2.3 for the chicken and the rat, respectively.

Once it has appeared, the edema does not seem to change appreciably in size. This conclusion has been reached from a study of the size of the edema in relation to its age. Figure 4 shows a record of all thirty observed cases in which the size of the edema was plotted against the post-operative age of the specimen. White circles represent rat, and black circles chicken cases. The largest diameter of each edema was chosen as representative of its size. Ratios of edema diameter over proximal nerve diameter were plotted as ordinates. If we consider the rat nerves first, it can be seen that animals studied as early as 1 week, or as late as 17 weeks, after the operation show edemas of approximately the same extent, with the cases preserved at intermediate times falling in line. The chicken cases are distributed over a shorter period, but likewise cluster around a nearly steady average value. The five pairs of chicken data connected by bars represent values obtained from nerves of opposite wings of the same individual. In these cases, the nerves of both sides had been severed and reunited with certain asymmetries of procedure; for instance, apposition of nerve ends on one side, intra-arterial gap on the other; or empty gap on one side, gap filled with a blood clot on the other; etc. Even so it can be seen that paired values for op-

posite nerves of the same individual lie within a close range. Whatever asymmetries were observed between the two sides can presumably be attributed to the asymmetrical condition of the operated nerves (see below).

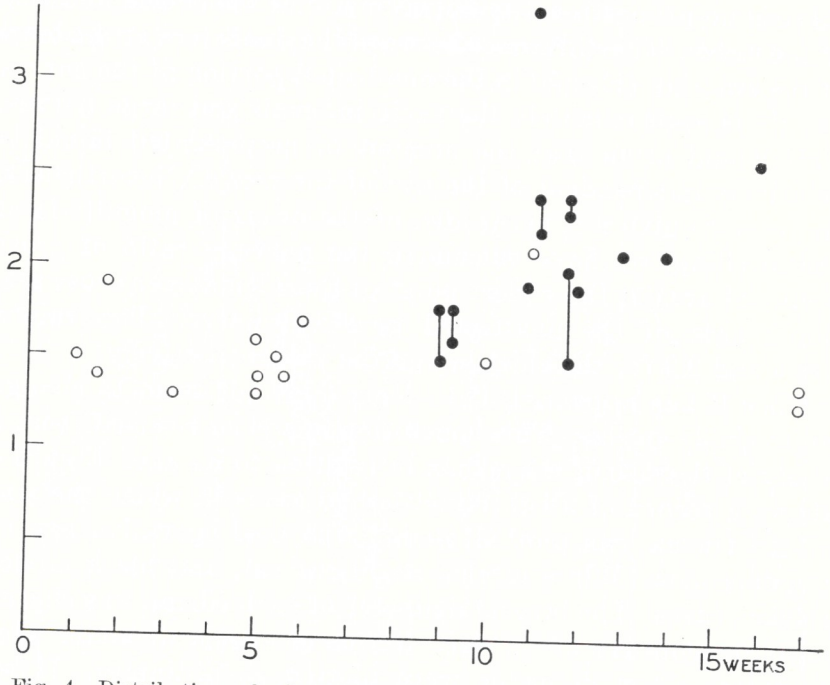

Fig. 4 Distribution of edemas in respect to postoperative age. Ordinates: Ratios of diameter of edema over diameter of proximal nerve ($\frac{E}{P}$ from fig. 3). ○ rat; ● chick. Vertical bars connect paired experiments on symmetrical nerves of same animals.

We may conclude, therefore, that the edema is established in its full size within a week of the appearance of the constriction, and the bulbous shape of the nerve produced by the edema will be maintained without appreciable change, either increase or diminution, for at least the 4-month period over which our observations extend.

The observations thus far reported may be summarized as follows. Constriction of a nerve by an arterial sleeve, used either as a jacket over an intact portion of a nerve or as a

splice over reunited ends of nerve stumps, causes the accumulation of edematous fluid in that part of the nerve trunk which lies immediately proximal to the constriction. The liquid always accumulates at the near side of the sleeve and reaches its maximum extent immediately prior to the entrance of the nerve into the bottleneck. The swelling tapers off in the proximal direction. Once established, it remains in evidence for several months. The location, distribution and size of these edemas point to the existence in the endoneurial spaces of a steady centrifugal seepage of fluid which, when peripherally obstructed by nerve compression, becomes dammed up and distends the nerve locally. A further discussion of this will be presented below.

HISTOLOGICAL OBSERVATIONS

Cross sections through the normal and edematous regions of a nerve during the early stages of constriction have been presented in a preceding paper (Weiss and Davis, '43). A later stage is illustrated here in figure 5. The picture shows cross sections through the unaffected proximal part (A), the edematous region (B), and the constricted sleeve region (C) of a sciatic nerve spliced 5½ weeks previously. The swollen region of this nerve is distinguished from the normal region by the wide open clefts which have appeared between the various nerve bundles, and the dissociation of the nerve fibers within each bundle. Since normal rat nerve trunks show no very distinct fasciculation, we have no way of telling whether or not the cleavage planes along which the edematous nerve breaks up into distinct fascicles correspond to the peripheral grouping of the fibers. Even between the individual fibers, however, the endoneurial meshes have become greatly distended, leading to wide separation of the nerve fibers from one another.

Of special interest is the fact that the edematous substance filling the widened spaces shows up in the fixed preparations as a precipitate impregnated with silver. In figure 5B, this granular mass is particularly well defined in the larger spaces.

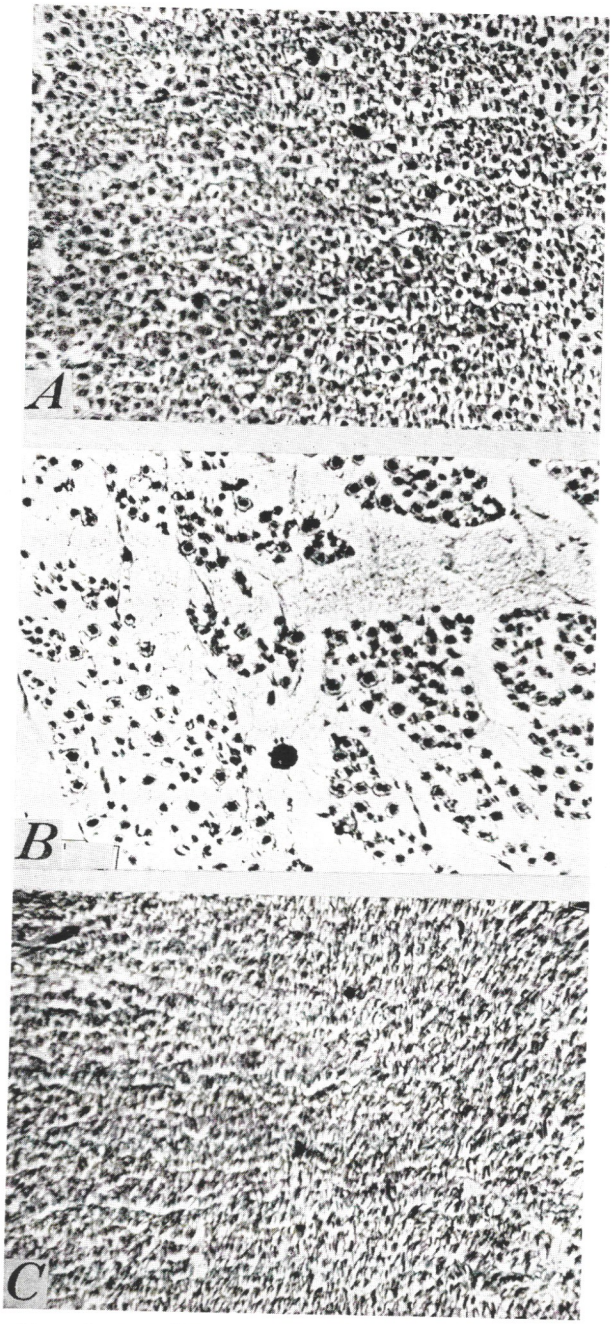

Fig. 5 Packing of nerve fibers in three different regions of constricted edematous rat nerve, sleeve-spliced 5½ weeks previously. x 250. A, proximal unaffected part; B, edematous region; C, sleeve (fiber course slightly oblique).

Under high magnification, it resolves itself into a very fine reticulum, of the sort familiar to the histologist in precipitates of fiber proteins treated with silver. Therefore, judging from the histological evidence, the endoneurial edematous fluid contains coagulable protein.

Figure 5C, a section through the compressed segment distal to the edema, shows that in this region the endoneurial spaces are largely obliterated. Whatever small spaces may have

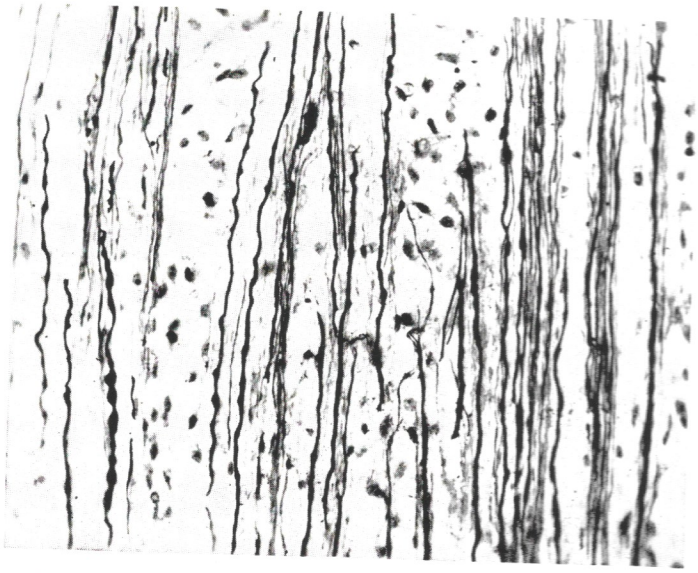

Fig. 6 Nerve fibers in edematous bulb of sleeve-spliced chicken nerve C13R, 66 days after the operation. x 230.

persisted, are obviously inadequate for the complete drainage of the vast amount of liquid present in the edematous region.

Figure 6 is from a longitudinal section through the edematous region of a constricted chicken nerve (radial), 2 months after the operation, and illustrates the separation among individual nerve fibers or small nerve fiber groups produced by the imbibition of the endoneurial tissue with the edematous fluid.

While during the first week after its appearance, the edema does not seem to contain a significantly larger number of cells than could be expected to have been preexisting in the endoneurium, later on the cell content in the liquid-filled meshes gradually increases. Most of the new nuclei are round to ovoid, with the smooth contour of fibrocytes, and are settled along fine fibers which begin to span the originally liquid

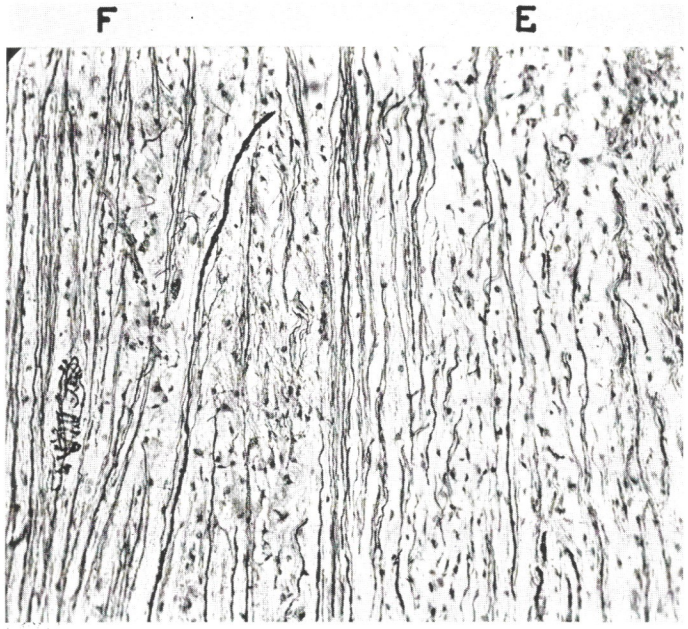

Fig. 7 Edematous region of sleeve-spliced chicken nerve C12L, 97 days after operation. x 120. The picture shows transition from edematous condition (E) in the interior to fibrotic condition (F) near the surface of the bulbous swelling. Note spiral apparatus of Perroncito formed by an occasional regenerating fiber encountering growth resistance in the fibrotic area.

spaces. Within a few months, this spongy tissue becomes more consolidated, primarily in the peripheral portions of the bulb (fig. 7). The nuclei assume more irregular outlines, some look atrophic and some show signs of pyknosis. As the intercellular matrix becomes denser, the cell population in it seems to decline. The formation of this first loose and later

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dense connective tissue begins at the most distal pole of the swelling, i.e., immediately in front of the entrance to the sleeve. From here it may gradually extend proximad, but even so, it always remains confined to the periphery of the bulb, while in the center wide liquid spaces persist. The fact that the central portion of the edema can resist fibrous transformation for several months suggests that the edematous fluid contains an antifibrotic principle, which partly explains its superior properties as a medium for nerve regeneration (see below).

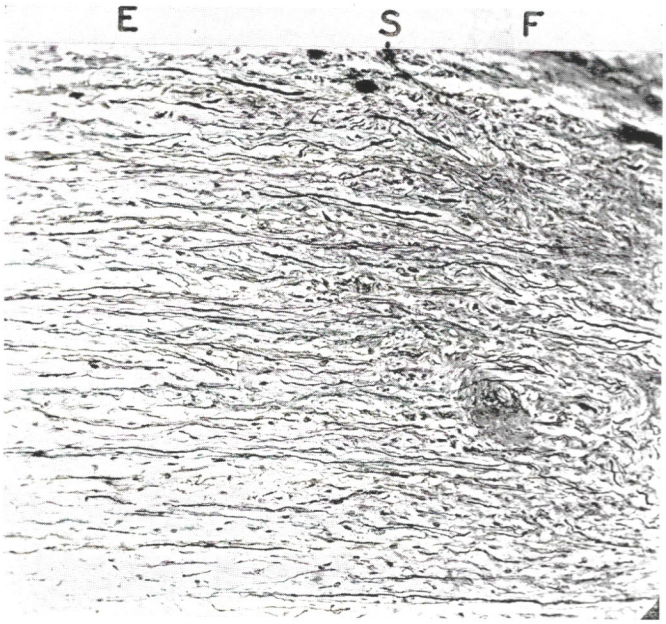

Fig. 8 Transition from proximal edematous (E) to distal fibrotic (F) zone of sleeve-spliced nerve (chicken C18R), 90 days after operation. x 120. Entrance to sleeve at S. See also figure 11.

Figure 8 presents a typical example of this transformation. The proximo-distal direction of the nerve is from left to right in the picture. The wide endoneurial spaces on the left can be seen to narrow toward the right and to terminate in a dense, more darkly stained zone, marked F, which has become fibro-

tic. In many cases, the process of condensation continues until a type of sclerosis is reached in which the interstitial tissue has become very dense, more homogeneous and very sparsely settled with cells. Nerve fibers passing through these fibrotic or sclerotic regions are forced into a contorted and meandering course, a condition which in severe cases may perhaps lead to physiological pressure block.

The progressive fibrosis of the distal end of the edematous zone may be correlated with an observation commonly made in early edemas. These show granular tissue debris accumulating near the constriction. This granulated matter may be assumed to consist of degeneration products liberated in the course of ascending traumatic degeneration of the proximal nerve stump. Its localized accumulation just proximal to the level of constriction can be easily accounted for if we assume that the edema is produced by the partial damming up of an interstitial liquid seeping distad. If this fluid carries debris and, upon coming up against the constriction, continues to seep on, even though at a reduced rate, into the compressed region, it is likely that all larger particles suspended in it would be retained at the entrance to the sleeve by the sieve-like action of the suddenly reduced endoneurial channels. In a second phase, the presence of this debris would then stimulate connective tissue formation. While the precise sequence of steps has not been determined, the analogy with inflammatory processes makes this interpretation fairly plausible.

A comparison of figures 9 and 10 with figure 8 reveals some of the story. Figure 9 reproduces an early stage in the formation of the edema, 8 days after arterial splicing of a rat tibial nerve, and figure 10 shows part of figure 9 at higher magnification. One recognizes clearly the zone of the proximal stump to which traumatic fiber modifications have ascended: the axis cylinders are swollen and vacuolated in the manner characteristic of reversible pseudodegeneration as described by Cajal ('28). Edema is already present (E), but at the level we are considering, the endoneurial meshes are filled with a granular debris of obviously local origin, which makes this

zone stand out in the silver preparation as a darker band across the nerve (B). Gradually, however, this interstitial matter will be displaced in a peripheral direction, but it will move no farther than the entrance of the sleeve, where it will pile up. At this more peripheral level, then, the fibrotic transformation illustrated in figure 8 will occur.

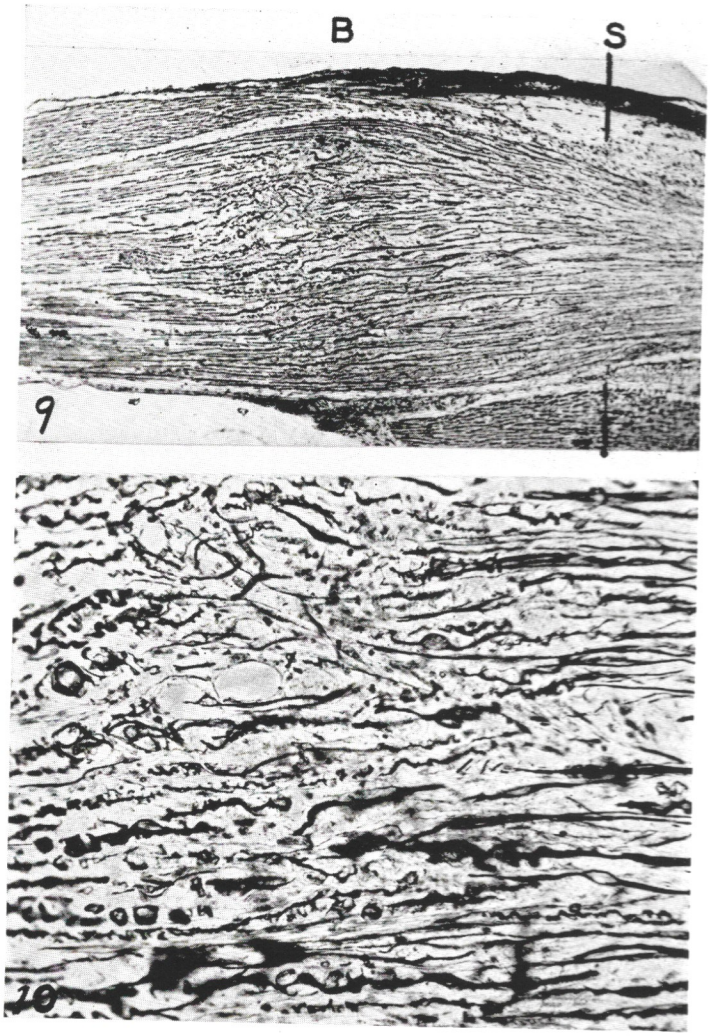

Fig. 9 Irritative phenomena in proximal stump of sleeve-spliced nerve (rat R92) 8 days after operation. x48. Proximal end of sleeve at S.

Fig. 10 Detail of axon vacuolization from zone B of figure 9. x 205.

The formation of a fibrous, and sometimes sclerotic, zone at the distal end of the edema affords an explanation of why edemas persist even in cases in which the arterial constriction is only temporary. If, as we contend, the edema is caused by occlusion of the intraneural spaces, the nature of the occluding agent is immaterial. Compression by the arterial sleeve would initiate the process by narrowing the endoneurial spaces, thus causing the primary edema to form. Then debris would collect and provoke the formation of a connective tissue partition in its place, which, henceforth, would act as a secondary plug of the endoneurial spaces, perpetuating the edema even when the grip of the arterial sleeve on the nerve relaxes, as in some cases it does (compare fig. 3). On the other hand, if we were to assume that the constriction of the sleeve had completely stopped, rather than merely diminished, liquid transfer along the nerve, the accumulation of interstitial debris at the most distal level of the edema could not be explained. We would then find the debris scattered throughout the edematous region much as flotsam in a stagnant pool. In other words, we may consider the fact that the debris always accumulates at this particular level, as a definite indication that the centrifugal seepage in the endoneurial spaces has not been completely arrested but only impeded. This conclusion is fully borne out by observations on secondary and tertiary edemas arising in the more distal course of a nerve.

SECONDARY EDEMAS

The nerve illustrated in figure 11 is a case in point. It is the radial nerve of a chicken, sleeve-spliced to its own peripheral stump with a plasma-filled gap left between the ends, 3 months after the operation. The proximo-distal direction of the nerve is from left to right. The level of the proximal end of the splicing sleeve is marked; it happens to run obliquely. Just proximally (to the left) of this level, one recognizes the heavily fibrotic zone (F_1) of which we have just spoken. Adjoining this zone at the left are the wide spaces of the primary edema (E_1). However, distally (to the right) of the fibrous

zone, inside of the sleeve, we see the structure of the nerve loosen up again, and although this is the portion which was originally compressed, it now contains wide clear spaces. At the far right of the picture, the nerve resumes a denser texture, caused by the fibrous transformation of the plasma clot originally included in the sleeve (F_2). Between the two fibrous zones there is a typical endoneurial edema (E_2). These secondary edemas have been observed in nine nerves in which a

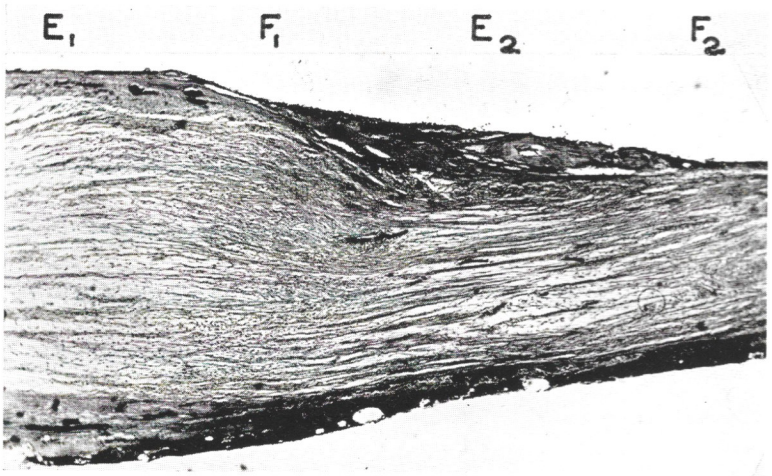

Fig. 11 Secondary edema in sleeve-spliced nerve (chicken C18R; fig. 8) in which plasma clot placed between nerve ends had become fibrotic. Ninety days after operation. x 40. Proximo-distal direction from left to right. Primary (constriction) edema (E_1) with its distal fibrotic zone (F_1) lies proximal to sleeve while secondary edema (E_2) formed behind fibrotic plug F_2, lies inside of sleeve.

secondary zone of peripheral fibrosis had set up another obstruction to endoneurial seepage. They are never found in well spliced nerves with directly apposed ends, which is in line with the observation that fibrosis does not occur in such nerves.

Like the constriction edemas, the secondary edemas can always be referred to a barrier lying immediately distal to them. To be sure, in our present case, the proximal fibrous partition (F_1) has an edema lying to either side. Yet, its relationship to the distal edema is purely positional, and in no way causal.

Not a single case has come to observation in which there would have been such an edema on the distal side of an obstruction, unless there was still farther distally another plug. Moreover, closer inspection of the secondary edema shows that it gravitates, as it were, towards the distal plug; i.e., there appears more liquid, and the spaces carrying it grow wider, towards the distal barrier. Another edema may appear on the distal side of the second plug if the nerve contains still a third obstruction. A nerve may, therefore, contain a number of barriers in its course, each one causing its own "upstream" edema, comparable to a waterway with a succession of sluices.

Under these circumstances, it is not surprising to find cases in which edemas have formed near a fibrotic plug inside the sleeve even in the absence of a constriction edema farther proximally. Such edemas are in the same position and of the same origin as the ones we have described above as "secondary." They appear in cases in which the splicing artery was wide enough not to cause constriction, but in which prospective obstructions had been placed in the way of the nerve. There is a definite correspondence between the filling of the sleeve and the degree of subsequent connective tissue formation inside of it. As mentioned before, with nerve ends closely apposed, there is practically no fibrosis. There may be some, and sometimes even a considerable amount if a gap is left open between the nerve ends. There is even more if the gap was filled with Ringer's solution, and still more if it was filled with blood plasma. In experiments in which opposite nerves were spliced, one according to one, and the other according to another of these procedures, the correlation between the amount of intraneural fibrosis and proximal edema became quite evident. Thus, in two chickens, C8 and C10, the stumps of the severed radial nerve of one side were spliced end-to-end, while on the opposite side a gap of about 7 mm. was left open between the nerve stumps inside the artery. In these cases, no edema formed in the directly spliced nerve, while conspicuous edemas were present in the nerves with the gaps, which

had developed some islands of connective tissue. In case C16, in which both nerves were spliced with a gap left between the ends, but in which one nerve gap was filled with a blood plasma clot, while the other was left open, the former developed some edema while the latter failed to do so. Likewise, in a pair of nerves in which one was capped with an empty sleeve while the other was capped with an artery filled with Ringer's solution, the latter, after becoming invaded by dense fibrous tissue, provoked an edema while the former, filled with loose tissue, did not.

This last case leads to the consideration of edemas which form in proximal nerve stumps left unconnected peripherally; nerves of amputation stumps belong in this class, and so do, in our experiments, nerve grafts which had been spliced to a proximal nerve stump without being connected peripherally. The peripheral open cut surface became covered by a cap of dense scar tissue such as usually forms over open nerve ends. Again, as a result of this fibrous occlusion of the endoneurial spaces, edematous fluid accumulates right under the fibrous cap, and bulbous terminal edemas arise (fig. 12). The terminal swelling, commonly referred to as bulbous neuroma, consists partly of accumulated interstitial fluid. However, just as was found in the primary edemas, some fibrous replacement of the edematous spaces will gradually set in from the periphery and transform the soft into a more rigid bulb.

What all these secondary and tertiary edemas demonstrate is that none of the observed obstructions — except possibly the terminal one, for which it cannot be decided — shut the more peripheral parts of the nerve completely off from their supply of interstitial fluid; while, at the same time, the strict localization of the edema to the proximal side of any one of the multiple obstructions is evidence that the edematous fluid comes from a proximal source and moves distad. If the edema were of local origin, there would be no reason why it should not form at both sides of the supposedly irritated region. Secondary edemas are particularly instructive evidence

against a supposedly irritative origin of intraneural edemas in that they arise only much later than the primary edemas, at a time when all traumatic effects have long since subsided.

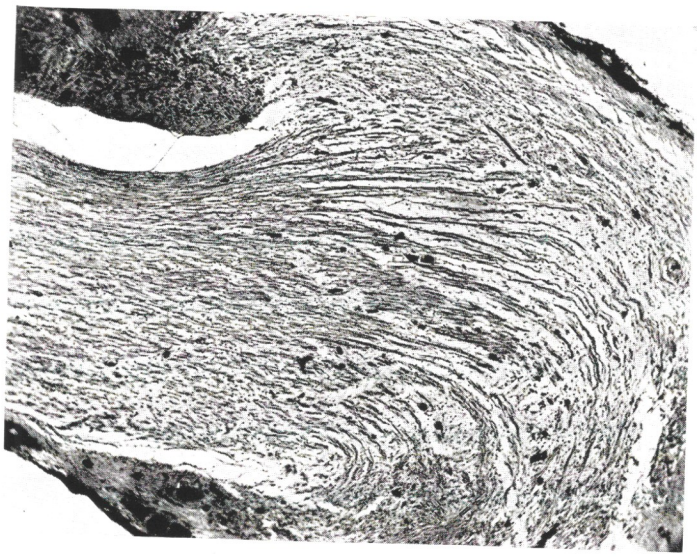

Fig. 12 Terminal neuroma with edema at the free end of a nerve graft which had been sleeve-spliced to the stump of the radial nerve (chicken C8L) 81 days previously. x 48. This nerve contained also a primary edema proximal to the sleeve and a secondary edema within the sleeve.

VASCULARIZATION

The intraneural vascularization in the rat is relatively poor. For instance, measurements of the combined cross sections of the larger blood vessels in the proximal trunk of the sample nerve of figure 1 yield a value of only 1/130 of the total cross section of the nerve. Sixty per cent of these vessels, moreover, lie at the circumference of the nerve directly under the epineurium. While no detailed study has been made of the changes which these vessels undergo in the course of nerve splicing and nerve regeneration, some pertinent facts are revealed by a survey of the series of cross sections of our preparations. These show a definite decrease in the number as well as the diameter of the recognizable intraneural vessels inside the sleeve, par-

ticularly in the regenerated part of the nerve. Moreover, whatever vessels are evident in the sleeve, are mostly situated at the periphery of the nerve. The edematous region proximal to the sleeve shows possibly a slight increase in the number of blood vessels and possibly also dilation in some of them. A similar dilation and increase are found in the peripheral parts of the nerve distal to the sleeve, where degeneration had originally occurred and might be explained as a response to the degenerative phenomena going on in their territory. However, while there is probably an initial deficit in the vascularity of the sleeve region, this is soon made up for by the establishment of collateral vascularization through the sheath. Within a few days after the operation, the wall of the arterial sleeve is richly vascularized from the epineurial blood vessels of the nerve trunk, and if we take the vascularization of the nerve as a whole, we realize that its interruption and disequilibrium is only a transitory event of short duration. This is an important consideration in connection with the problem of the origin of the edema.

DISCUSSION

From the reported observations, the following facts have emerged. Constriction of a nerve results in edema in that part of the nerve bordering on the proximal end of the compressed zone. The presence of a fibrotic zone inside of a nerve likewise leads to edema, localized at the proximal side of the obstruction. Similarly, a terminal edema may arise behind a terminal fibrous occlusion of the cut end of a proximal nerve stump. The common denominator of these three edema-provoking conditions is neither operative trauma nor chronic irritation, nor vascular stasis, but solely the partial or complete obliteration of the endoneurial spaces through either constriction or plugging. The occlusion, depending on its severity, either reduces or arrests any traffic of liquids that might otherwise have proceeded in the blocked channels. And since the edematous fluid accumulates with striking regularity at the proximal side of the barrier, the prevailing direction of

such traffic must have been centrifugal. This would lead us to the assumption of some pressure in the endoneurial spaces of as yet undefined origin, but with a clearly centrifugal resultant. This conclusion seems cogent no matter what may be the source of the fluid itself. The latter may be a regular constituent of the nerve or it may arise only under the conditions of the experiment. In either case, its "upstream" accumulation presents a problem of its own.

However, before committing ourselves definitely to the blocking of endoneurial channels as an explanation of these edemas, we must consider some alternative interpretations.

There is the possibility of a vascular origin of the edema — transudation from occluded vessels as in common edemas; more specifically, from arterial vessels, because of the confinement of the exudate to the proximal side of a constriction. The facts, however, seem to disprove this assumption. First of all, the vascular stasis following the operation is of very brief duration. As described above, collateral vascularization through the sleeve is established within a few days. Stroebe (1893) reports restitution of circulation through a compressed and crushed nerve segment of several millimeters within 48 hours. Blood pressure equilibrium is thus restored to the nerve as a whole before the edema has even reached its full extent. Moreover, nerves which have simply been cut and either left severed or adequately reunited without constriction develop no edemas, although their vascular system has suffered the same initial interference as that of the edematous nerves. In appraising the situation, one must never lose sight of the fact that the nerve edemas in our cases have persisted for many months, long outlasting any vascular instability that may have existed in the beginning. However, perhaps the most convincing evidence comes from the secondary and terminal edemas, which form without even transitory constriction of vessels and in which, in fact, the vascularity beyond the edema is more abundant than it is in the edematous zone itself. The fibrotic nuclei in the nerve, behind which edemas are commonly found, are well vascularized and offer no obstruction to

blood flow. And the same applies to the fibrous scar over a free nerve end, which, nevertheless, may become the seat of a terminal edema.

Discounting the thesis of a vascular origin of the edema, we turn to the possibility that the nerve fibers themselves may have exuded the edematous fluid as a result of temporary trauma or permanent irritation. The problem poses three separate questions: First, do nerve fibers exude fluid? Second, if so, do they do so normally or only under pathological irritation? Finally, could this fact account for persistent edemas of the observed localization? For all we know, nerve fibers may very well discharge some liquid. But this in itself would not explain the locally confined edemas; for if fluid oozes from the fibers at all levels, one would have to postulate some additional factor responsible for the local accumulation of ubiquitous exudate — which would bring us back to the concept of endoneurial flow. The alternative would be that the fibers discharge fluid only at the levels where we later find the edemas.

Let us consider this latter possibility of a local formation and local confinement of the edemas more concretely, taking the sleeve-constricted nerves first. There is Wallerian degeneration in the nerve below, and some ascending traumatic degeneration in the nerve above, the constriction. Degenerating nerve fiber segments contain liquid vacuoles and swell considerably, but from the fact that the packing of the resulting Schwann tubes (Buengner's cords) remains compact (compare fig. 4D in Weiss and Davis, '43), we may infer that no excessive amount of this liquid leaves the tubes. The peripheral stump is swollen (figs. 1, 2), but not edematous. The ascending changes of the proximal stump, on the other hand, are of a somewhat different nature. Here, many axis cylinders go merely through a process of varicose swelling without ever disintegrating. These symptoms, described in detail by Cajal ('28) and observed by Speidel ('35) in living fibers after irritation, are exemplified in figures 9 and 10. Conceivably, these ascending traumatic lesions may involve changes in the

permeability of the fiber sheaths which would let some of the axonal fluid escape into the interstices. In this connection an observation of Stroebe (1893) on rabbit nerves that had been pinched through with a clamp seems worth citing (p. 195; translated from the original German): "The whole central stump . . . shows a diffuse pale blue tint in Anilin Blue, clouding all histological details; this diffuse stain is obviously due to the infiltration of the stump with a protein-containing serous fluid, which must be regarded as a product of the traumatic lesion." Stroebe saw some of this infiltration also in the peripheral stump near the injury. Might we not, then, be confronted here with an incipient edema? According to Stroebe's further description, no. For since, according to his report (p. 200), these infiltrations disappear on the third day, they can hardly be identical with the persisting edemas of our cases. Moreover, the absence of edema in merely transected or transected and well spliced nerves, in which the initial trauma was fully as great as in the edematous cases, as well as the late appearance of secondary and terminal edemas in the absence of all trauma, seem to eliminate trauma as a possible source of our edemas.

Since we have been stressing the persistence of the edema as an argument against its vascular or traumatic origin, it should be reiterated that the edema really is permanent (within the duration of our experiments). This statement does not run counter to our observation reported above, that firm connective tissue gradually invades the edematous spaces, since the latter process remains confined to the peripheral layers of the edematous bulb, while the core remains liquid (figs. 8, 11).

For the outlined reasons, it seems indicated to abandon the concept of a local origin of the edematous fluid in favor of the assumption of its ubiquitous presence in the nerve with merely preferential accumulation in physically predisposed locations. According to this view, the edema would be but the normal fluid content of the endoneurial spaces, dammed up by

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an obstruction. This still leaves the questions of the source of this fluid and of the mode of its distribution unanswered.

There are three possible sources. Firstly, it may be cerebrospinal fluid draining from the subdural space directly into the endoneurial spaces. Secondly, it may be a secretion of the nerve fibers themselves. Thirdly, it may be a mixture of both. There appear to be no anatomical barriers that would prevent cerebrospinal fluid from spreading from the subdural space along the surfaces of the nerve fibers into the peripheral nerves way beyond their intraspinal course, for which such communication has been established, even though the amount of this capillary drainage may be negligible as compared to the volume escaping into the venous sinuses and lympathics (Weed, '22). Experimentally, the passing of substances injected into the peripheral nerve up through the interstices and on into the subdural space has been demonstrated (review in Speransky, '35). The fact that the excess pressure used to force the injected mass into the nerve may reduce the physiological pertinence of such experiments, does not detract from their value in demonstrating the existence of a pathway between subdural and endoneurial spaces. Consequently, it is at least possible that the liquid base of the endoneurial edemas may be derived from cerebro-spinal fluid. In view of the apparently high protein content of the edemas, in contrast to the known low protein content of the cerebro-spinal fluid, the admixture to the latter of some peripheral product would seem likely. This addition may come from the nerve fibers. Possibly even the whole of the endoneurial fluid is exuded from the nerve fibers. But since there are practically no facts known on which to base speculation, we prefer to leave these questions open.

Turning now to the second problem, that of the distribution of the endoneurial fluid, one fact seems to emerge with certainty: the fluid cannot be stagnant, but must be in centrifugal motion. Only thus can we account for the characteristic position and shape of the edematous swellings. The existence of such motion raises the question of the motive force. Under

normal conditions, the fluid is presumably resorbed from the peripheral channels at the same rate at which it is fed into their proximal ends. Any reduction in the width of the channels, e.g., by constriction or fibrosis, leads to proximal damming up, which means that the fluid continues to be fed into the lines although the rate of its peripheral disposal is reduced. This fact explains the "upstream" position of the edema, but not its shape. For equalization of pressure in the dammed up pool would tend to widen the "upstream" channels uniformly rather than just in the region immediately before the obstruction. A pear-shaped widening of the kind seen in our cases could arise on purely hydrodynamic principles, but only in currents of such high velocity that it can have no bearing on our problem, which concerns seepage rather than streaming. Only a constant force pressing the fluid steadily distad can explain why the accumulated edema does not gradually flow back into the rest of the nerve. In view of the large total inner surface of the endoneurial meshwork, the resistance to flow in it must be considerable, and, therefore, the postulated driving force must be correspondingly high. The hydrostatic pressure of the cerebro-spinal fluid (order of magnitude: 100 mm. water; Weed '22), for instance, would be insufficient.

We need some mechanism that would squeeze or massage, as it were, the endoneurial fluid down its channels. In this connection the pulse wave deserves consideration. If we visualize the nerve with its sheath as a closed, relatively incompressible system, the increase in blood volume in the epi- and perineurial arteries during the systolic phase of the pulse would necessarily displace an equivalent amount of extravascular fluid. During the diastolic phase, this fluid could return. Now, if the systolic and the diastolic waves were symmetrical, a regular tide, even though only of small scope, would result. However, the asymmetry of the pulse, with the systolic pressure wave developing faster than the diastolic decompression wave, may yield a resultant pressure, and consequently shift, of the surrounding liquid in the direction of the fast phase, that is, peripherad.

The physical possibility of such an effect can be demonstrated by means of a simple model (fig. 13). The point to be tested is whether a rhythmic succession of asymmetrical compression-decompression waves passing down an elastic tube can produce translatory movement of liquid in a second tube in lateral contact, but without communication, with the first if both are enclosed in a common sheath. In the model, the outer sheath

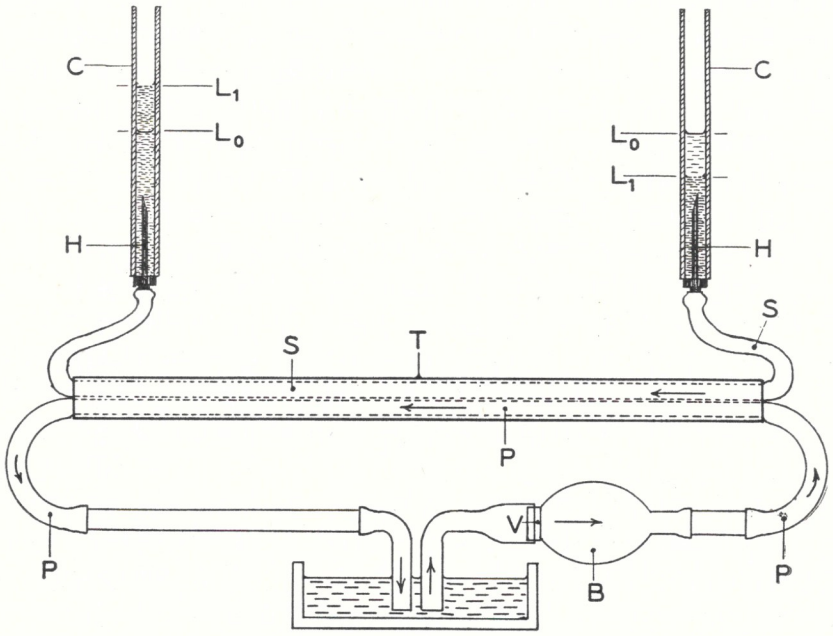

Fig. 13 Model of hypothetic effect of the pulse wave on the shift of fluid in the adjoining nerve spaces. Explanation in text.

of the nerve is represented by a glass tube (T), through which are drawn two rubber tubes (P and S) to serve as conductors for the primary, or pumping, circuit and the secondary, or indicator, circuit. The former is to represent the perineural arteries carrying synchronized pulse waves, while the latter represents the aggregate endoneural space. Both were pulled through the glass tube under stretch so that they partly compress each other. Any dilation of the primary tube will thus

encroach upon the secondary tube. The primary circuit is filled with water by means of a plumber's bulb (B) with an intake valve (V) controlling the direction of flow. Compression of the bulb drives a water column in the direction of the arrow and thus imitates a systolic pulse. Upon release, the bulb fills itself through the valve, reproducing the diastolic phase. The indicator circuit (S) is wholly symmetrical and communicates with two terminal glass tubes (C) through hypodermic needles (H) of identical gauge. When in equilibrium, the water levels (L_o) are the same in both glass tubes.

When gentle pressure is applied to the bulb, the primary tube (P) is dilated, and the secondary tube (S) correspondingly compressed, which registers as a rise of the water levels in both indicator vessels (C). If, however, the bulb is compressed quickly, the rise of the water level is greater in the distal than in the proximal vessel. Evidently, there has been a shift of water in the secondary system in the direction of the primary current, the latter exerting a pumping action on the adjacent channel. While the effect of a single pulse is small, rhythmic repetition at suitable intervals soon leads to a conspicuous difference between the two water levels (L_1), provided the rate of backflow (determined by the width of the hypodermic needles H) is slow enough to permit each successive pressure difference to build up on top of a residue of the preceding ones. Reversal of the flow in the primary circuit (by inserting the pump in the left branch), of course, reverses the effect in the secondary system. This test of reversibility is important in order to eliminate errors due to possible intrinsic asymmetries within the secondary system itself. If the two end tubes were plastic and closed up at the resting level L_o, the excess water in the distal one (corresponding to the water column L_o–L_1) would obviously cause local inflation; in other words, reproduce the edemas of our experiments. Without obstruction, in the living object, the excess would simply drain off into the periphery instead of flowing back, as it does in our model owing to gravity.

The model presents merely a physical possibility. How close it comes to physiological reality, is another matter which can be decided only by further experiments. Even though the individual perineurial vessels are relatively small (p. 510), their synchronized pulsation would summate their effects. Moreover, the cumulative action of incessant pulsation over long periods of time would tend to amplify the imperceptible effects of the single beats.

In conclusion, our contention is that the endoneurial spaces are filled with a liquid which is constantly propelled, even though at a slow rate, in a proximo-distal direction. The vascular pulse is suggested as a means of such translatory motion. Obstruction of the channels of this flow would impede its peripheral drainage and lead to local damming up in the form of bulbous edemas. That even then a certain amount of the fluid continues to seep on beyond the obstruction, is evidenced by the secondary and tertiary edemas forming before blocks situated farther distally. This explains why debris accumulates at the near site of a compression: the liquid continues downward, while particles too large to pass are retained as by a strainer.

Its prevailingly centrifugal course would make the endoneurial fluid a poor vehicle for centripetal transport of particulate matter in the nerve. This gives added, though indirect, support to the theory that such afferent transport, especially of neurotropic viruses, proceeds within, rather than between, the nerve fibers (Howe and Bodian, '42; also review of earlier literature).

The endoneurial fluid seems to play a peculiar role in nerve regeneration. Growing nerve sprouts as well as sheath cells extend only along interfaces, and there are increasing signs that the nature of the interface affects the rate and direction of the advance (Weiss, '41 a). It now appears that impregnation with endoneurial exudate makes a tissue more pervious for sheath cells and nerve sprouts. The presence of this medium in the gap between severed nerve stumps seems to create

exceedingly favorable conditions for regeneration. When two nerve ends are spliced with a hermetically sealing sleeve of artery (Weiss, 41 b, '43), the endoneurial fluid apparently exudes into the space between the nerve ends and forms a bridge which can be easily traversed by the regenerating sprouts without branching and in perfectly straight orientation. At the same time it suppresses the formation of fibrous connective tissue. The high liquid content of the matrix in which regeneration takes place in such regions is in striking contrast to the dense fibrosity of ordinary nerve scars. When a nerve is simply cut, this medium presumably seeps from the wound into the surroundings and is dissipated. However, if the nerve ends are sheathed in by a sleeve, the fluid collects in the gap where it exerts its beneficial influence on the regenerating sprouts. Its coagulation seems to establish interfaces peculiarly suited for sheath cell and nerve fiber application; it may also, by coating existing surfaces, serve as a binding between the nerve fibers and their non-neural surroundings. Whether it has, in addition, nutrient or metabolic significance, is an open question.

These observations have recently received strong support from tissue culture studies (as yet unpublished). When spinal ganglia of chick embryos are cultured along the surface between a cover glass and an inorganic liquid medium, one often notices a substance seep from the ganglion and spread along the solid-liquid interface. When fixed and impregnated with silver, it forms a very fine reticulate precipitate of much the same appearance as was described above for the edema. From its staining reaction, we may again infer its protein nature, and this time we can definitely identify it as coming from the nerve culture. Later, when nerve fibers grow out, they seem to hold themselves within this film and rarely proceed beyond its borders, and even when they do, it is not certain that they are not coated with a submicroscopic layer of this material. These experiments seem to indicate, therefore, that nerve fibers in "liquid-medium" tissue culture move preferentially, if not exclusively, within an exudate of their own making. Al-

though the identity of this substance with the one recovered from the endoneurial spaces in the form of edema remains to be demonstrated, the similarity of behavior and histological reactivity is suggestive.

In practical respects, the significance of the endoneurial fluid in nerve regeneration raises the question of whether what we have described for the nerves of the chicken and the rat, applies to human nerves as well. Dustin ('17) has given incidental mention to edemas observed in human nerves constricted by scar tissue. Judging from his description, the resemblance to our animal cases is close. He states (p. 127; translated from the original French): ". . . The laminated sheaths are distended by edematous fluid which after fixation and impregnation can be recognized as a fine albuminous precipitate. This edema dissociates the constituent bundles of the nerve trunk; it first infiltrates into the meshes of the connective tissue of the nerve, later penetrates between the fibers themselves which it soon separates from one another." By way of interpretation, he proposes (p. 128) that "the lymphatic circulation of the nerve is independent of the general lymphatic circulation," and "the lymphatic circulation of the nerve seems to be in close connection with that of the cerebrospinal fluid." Following Sabin's ('16) precept, it would seem inadvisable to designate the nerve fluid as "lymph," but otherwise, Dustin's remarks seem highly pertinent.

SUMMARY

Chronic constriction of a nerve by an arterial sleeve produces persistent edemas in the endoneurial spaces just proximal to the compressed zone. This phenomenon has been studied histologically in thirty rat and chicken nerves preserved from 1 to 17 weeks after the operation. Similar edemas form at the proximal side of any kind of obstruction within a nerve (e.g., a fibrotic plug in the course of the nerve or a fibrous cap over the blind end of a proximal nerve stump). These edemas are neither of vascular nor of irritative origin. Evidence has been presented to show that they result from the damming up of

fluid normally present in the endoneurial spaces and seeping distad. The pulse wave may hypothetically be assumed to furnish the propulsive force for this seepage. The endoneurial fluid seems to have special significance in nerve regeneration, inasmuch as it forms a superior growth medium for the outgrowing sheath cells and nerve sprouts and also has antifibrotic properties.

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