# WALLERIAN DEGENRATION OF NERVE FIBERS AFTER REPEATED TRAUMA TO NERVE TRUNKS

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The study of the pattern of Wallerian degeneration of nerve fibers arising after division of or injury to nerve trunks is of essential importance to the understanding of the general course of the repair processes taking place in the injured nerve. Many authors [2, 3, 4, 6, 16] have repeatedly drawn attention to the importance of the degenerating peripheral segment of the nerve in its subsequent regeneration. The views of these authors may be summarized by saying that without the preliminary degeneration of the nerve elements there can be no successful regeneration, and that regeneration is possible only in the presence of a degenerated peripheral segment.

As the researches of many authors have shown, the rate of progress of dystrophic processes in the peripheral segment of an injured nerve is not constant, but is subjected to many variations depending on the character of the nerve and the functional importance of its component nerve fibers [9, 10], the species and age of the animal [11, 14], the conditions of nutrition [12] and so on. In cold-blooded animals the time of year and the temperature of the external environment have a definite influence on the velocity of this process. Differences in the rate of progress of the degenerative process in various parts of the peripheral segment of the nerve must also be emphasized [7, 8, 13, 14]. Destruction of the medullary sheaths and the neurofibrillary structures arises first of all in the terminal divisions of the nerve fibers and in the nerve endings, from whence the process spreads to the center, i.e., to the site of injury. There are reports in the literature that the velocity of the progress of Wallerian degeneration may be severely retarded by repeated injury to the regenerating nerve [1].

In view of the practical and theoretical importance of this problem, we decided to study the influence of repeated transection of a nerve on the process of Wallerian degeneration, using both morphological and physiological methods of investigation for this purpose.

## METHOD

Experiments were conducted on 12 rabbits. The operation consisted of division of the tibial part of the sciatic nerve with fine pointed scissors at or above midthigh level, at the level of the tendon of the obturator internus muscle. The peroneal nerve was not injured, but acted as a bridge which prevented the separation of the ends of the divided tibial nerve. Form 2 to 2 $\frac{1}{2}$ months later the same nerve was again divided, this time 2 cm above the first scar in six rabbits and 2 cm below the site of the first operation in the other six rabbits. The animals were sacrificed 6, 8, 14, 20, 30 and 50 days after the second operation. The excitability of the divided nerve was first determined in all the animals. As controls, observations were made on the tibial nerve divided once only in 12 rabbits sacrificed at the same times as the experimental animals. Furthermore, in a proportion of the experimental animals the left tibial nerve was divided at the same level at the same time as the second operation was performed on the right tibial nerve. This nerve was also used for control purposes.

The sciatic nerves were fixed in 20% neutral formalin and then investigated throughout the entire length.

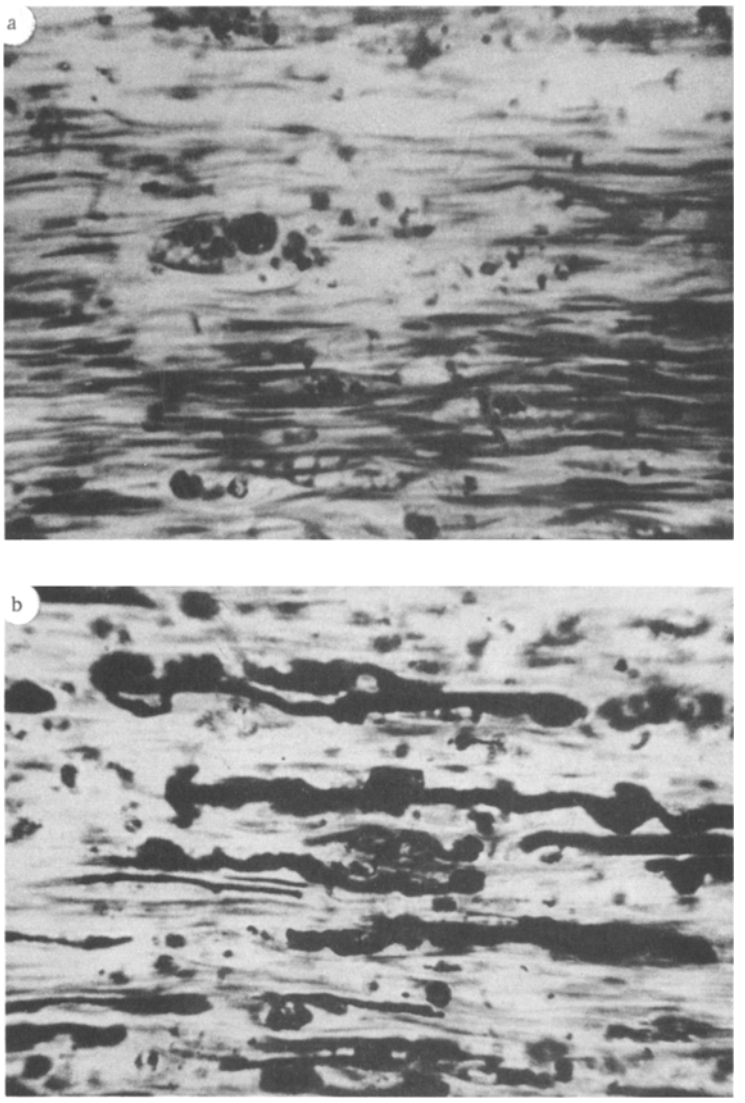

Fig. 1. Wallerian degeneration after repeated division (above the site of the initial defect) of a regenerated nerve: a) Distal segment (below the region of the first scar) on the 14th day after operation. The degenerated nerve fibers have been converted into thin bands of Schwann cells, almost free from products of disintegration; b) proximal segment of the nerve (above the site of the first scar) on the 14th day after operation. Large fragments of axis cylinders are seen. Rabbit No. 275. Silver impregnation. Objective 100×, immersion; ocular 2 ×.

Frozen sections were impregnated with silver by the Bielschowsky-Gros method and counterstained with hematoxylin. Some sections of each nerve stained by Spielmeyer's method, with Sudan III and with Sudan black.

## RESULTS

The study of Wallerian degeneration after repeated trauma to the regenerated nerve revealed serval distinctive features in the course of this process by comparison with degeneration of the nerve fibers after a single division of the nerve. The distinctions consisted mainly of differences in the rate of the process of disintegration

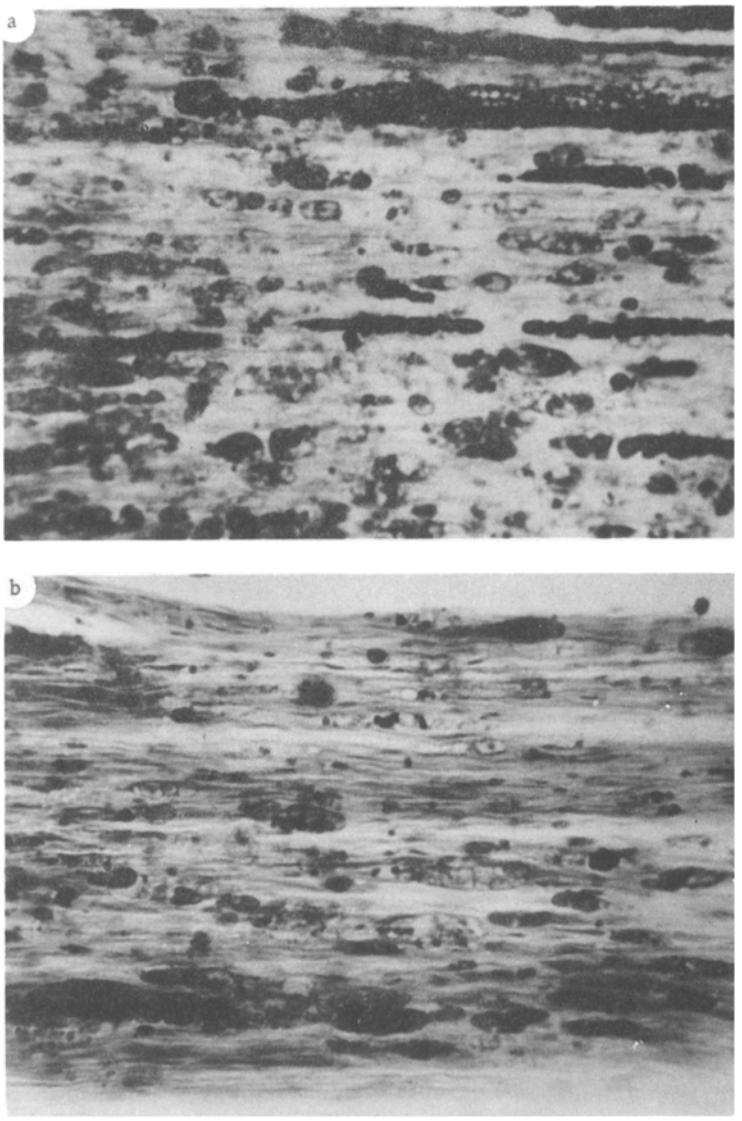

Fig. 2. Degenerative changes in nerve fibers after repeated trauma of the regenerated nerve (division above the site of the original defect) a) Proximal segment of the nerve (above the first scar). In places fragments of medullary sheaths and ovoids, preserving their cellular structure, may be seen; b) distal segment of the nerve (below the first scar). Empty Schwann sheaths can be seen. Stained by Spielmeyer's method. Rabbit No. 374. Objective 45×, ocular 2×.

of the nerve structures and of the resorption of the resulting products. This was most obvious in the case of application of the second injury above the site of the first scar. In these cases the nerve fibers undergoing degeneration had a complex and apparently dual structure. Above the first scar the fibers were essentially normal in their caliber and structure. At the level of the first scar they continued as regenerated fibers which had grown through the scar and the entire peripheral segment of the nerve. During the 2-2½ months elapsing since the first operation, these fibers had not attained their normal structure. Their axis cylinders and medullary sheaths were thinner than usual. After redivision of the nerve above the site of the first scar, these and other areas of the nerve fibers underwent degeneration. However, the tempo of this process showed significant differences between the normal and the regenerated parts of the nerve fiber.

On the 6th-8th day after operation, in the segment of the nerve lying above the first scar (i.e., in the interval

between the second and the first scars), fragmentation and disintegration of the axis cylinders and medullary sheaths took place. The fragments of the axis cylinders swelled, lost their fibrillary structure and often were curiously curved. When stained by Spielmeyer's method an enormous number of ovoids and of elongated fragments of medullary sheaths, covered with connecting bands, were observed. Most of these preserved their cellular structure. Only here and there did the disintegrating medullary sheaths show signs of impending fatty degeneration. This pattern of degeneration corresponded completely to that found at the same times in the control animals.

A quite different picture was found in the segment of the nerve lying below the region of the first scar, where the young nerve fibers, newly formed after the first trauma, were subjected to degeneration. On the eighth day after operation, neither long fragments of the myelin sheaths nor typical ovoids were seen here. By this time they had undergone complete disintegration. The products of disintegration of the axis cylinders had almost entirely disappeared. The degenerated nerve fibers were converted into thin bands of Schwann cells, along the course of which small fusiform swellings were present here and there, containing granular disintegration products. When stained with Sudan III the degenerated nerve fibers showed signs of severe fatty degeneration. Fat droplets were found both in the protoplasm of the Schwann cells and in the region of the fusiform swellings, and also in the protoplasm of the macrophages scattered in the stroma of the nerve.

Tests of the excitability of the nerve showed that on the sixth day after the second division of the nerve stimulation with an induction current (above and below the scar) caused no contraction of the muscles, which agreed completely with the morphological picture of degeneration of the nerve revealed at this period of the experiment.

These differences in the tempo of Wallerian degeneration and resorption of the products of disintegration after the first and second division of nerve fibers were also maintained on the 14th day after operation. Irrespective of the level of the second division of the nerve, in its distal area (situated below the level of both scars) processes of resorption of the products of disintegration predominated. In impregnated sections nearly empty Schwann sheaths of denervated nerve fibers could be seen, in the vacuoles of which only occasionally were small black lumps and grains of disintegration visible (Fig. 1a). When stained by Spielmeyer's method the typical ovoids were absent or present in very small numbers. Lightly stained swellings could be seen along the course of the Schwann cell bands, containing pale granules of disintegration. When stained with Sudan black or Sudan III, numerous small droplets of fat were seen in the protoplasm of the Schwann cells and in the macrophages. The study of the process of degeneration in the part of the nerve situated above the first scar (in cases in which the second disivion was carried out ab above the first scar) or in the peripheral segment of the nerve of the control animal showed the presence of numerous, quite large fragments of axis cylinders and medullary sheaths, together with small lumps and granules of disintegrated matter (Fig. 1b). Only in places were areas of empty Schwann sheaths visible, free from disintegration products over a certain distance. When stained with Sudan III or Sudan black, part of the disintegrating medullated fibers showed the picture of severe fatty degeneration, whereas others, consisting of fragments of medullary sheaths, preserving their cellular structure, did not yet give a positive reaction for fat. Thus above the first scar and in the control nerve the process of disintegration of the medullary sheaths 14 days after operation was still far from complete, and the processes of resorption of the disintegration products were at the beginning of their development.

Twenty days after the second division of the nerve above the site of the first scar the processes of Wallerian degeneration had advanced considerably. In the sections impregnated with silver, the differences between the pictures of degeneration of the nerve fibers above and below the first scar were largely obliterated. In both places the appearance was mainly of empty Schwann sheaths, with scattered elongated nuclei. Only occasionally were separate fragments of axis cylinders seen. The process of degeneration of the neurofibrillary structures in the peripheral segment of the nerve in the control animal was in approximately the same state. So far as the medullary sheaths are concerned, their degeneration took place more slowly than that of the axis cylinders; this meant that on the 20th day after operation a difference could be detected in the tempo of this process after division of the nerve twice or once only. When stained by Spielmeyer's method, the segment of the nerve situated above the first scar, and also the peripheral segment of the control nerve still showed the presence of a fairly large number of ovoids and long fragments of myelin sheaths with a well marked cellular structure (Fig. 2a). At this time, below the first scar, where the regenerated nerve fibers undergoing degeneration, disintegration of the myelin sheaths had advanced much further in its development. Typical ovoids and fragments of myelin sheaths were absent here. The degenerated nerve fibers had the appearance of empty Schwann cell bands, along the course

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of which translucent vacuoles and swellings could be seen in places (Fig. 2b).

These differences in the pattern of degeneration of the nerve fibers after repeated or single injury to the nerve subsequently became less and less pronounced, and the degenerated nerve fibers in both cases acquired a similar appearance, being converted into bands of Schwann cells, rich in fat droplets. Resorption of the fat took a long time, and even one month after the second division of the nerve the protoplasm of the Schwann cells contained large numbers of fat droplets.

These investigations showed that the process of Wallerian degeneration after repeated division of a nerve proceeds far more rapidly than after a single division of the nerve. This is shown by the more rapid disintegration of the axis cylinders and medullary sheaths into separate fragments and ovoids and by the more rapid formation of fat from the disintegration products of myelin. The same may be said regarding the resorption of the disintegration products, especially of the fragments of the axis cylinders, which takes place much more rapidly than usual after a second injury. Consequently the degenerating nerve fibers are converted comparatively early into "empty" bands of Schwann cells, containing large numbers of fat droplets. This corresponds to the rapid loss of excitability of the degenerated nerve. This picture of degeneration of the nerve fibers in the distal segment of the nerve takes place in every case, irrespective of where the second injury is applied – below the first scar (i.e., within the limits of the regenerated fibers) or above the site of the first injury (i.e., within the limits of the apparently normal nerve). In the latter case, i.e., after the second division of the nerve above the first scar, the nerve fibers subjected to trauma are complex in structure.

Each fiber consists of two different parts: the normal fiber and its continuation – the fiber which has regenerated after the first division. From a comparison of the pattern of degeneration of the nerve fibers above and below the first scar, it is easy to be convinced that in the first case it is quite indistinguishable from the degeneration of the nerve fibers of the control nerve, whereas the disintegration of the regenerated nerve fibers is effected at a much quicker tempo. In this respect the regenerated nerve fibers are very reminiscent in their properties of the nerve fibers of young animals, degeneration of which takes place much more rapidly than in adult animals [11]. This suggests that regenerated nerve fibers possess less resistance and that their reactivity is increased.

The quicker tempo of disintegration of the regenerated nerve fibers after division is to some extent associated with their structural features. Even two or three months after trauma the regenerated nerve fibers, their axis cylinders and myelin sheaths are far short of their normal size and structure. Their caliber is thinner than usual. In the degeneration of these fibers, less products of disintegration are therefore formed and they are resorbed more quickly. Such a purely mechanical explanation can hardly apply to the phenomena of fatty degeneration of the myelin. The fat appears earlier in the process of disintegration of the myelin sheaths of these fibers than it does in Wallerian degeneration of normal nerve fibers, and this cannot be explained purely by the smaller caliber of the regenerated fibers. It is necessary here to consider the increased enzymic activity of the Schwann glia of these fibers, bringing about a more rapid conversion of myelin into fat. Such a hypothesis is in full agreement with the results of I. F. Ivanov's work [5, 6], in which the important role of the Schwann glia in the process of Wallerian degeneration was asserted some time ago.

## SUMMARY

The process of Wallerian degeneration and resorption of disintegration products after repeated section of the nerve is much more rapid than following a single nerve trauma. The rate of degeneration and disintegration of regenerated nerve fibers after their division is more rapid in all cases, irrespective of the level at which the second trauma is inflicted – above or below the first scar. By their properties, regenerated nerve fibers greatly resemble the nerve fibers of young animals, degeneration of which after trauma is more rapid than in adult animals. This points to a lower resistance of the regenerated nerve fibers and their increased reactivity.

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