THE NON-CENTRIFUGAL DEGENERATION OF SEVERED PERIPHERAL NERVE

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INTRODUCTION

Neumann in 1868 proposed the idea that severed peripheral nerve degenerates centrifugally; i.e., the degenerative processes start at the cut end and progress slowly towards the periphery. This view, opposing the conception of simultaneous degeneration advanced by Lent in 1856, started a controversy as yet unsettled. Most of the arguments on both sides have been based on histological evidence—only relatively recently has there been any physiological experimentation on the subject. The early physiological studies were concerned chiefly with the time of disappearance of indirect excitability of the muscles innervated by cut nerves (Bethe, 1903; Courrier, 1926).

Apostolaki and Deriaud (1925) found no change in chronaxie following section until the “complete degeneration” of the nerve (i.e., loss of indirect excitability of the muscle). This occurred by 9-10 days in frogs kept at 12-15°C. The nerve’s rheobase increased by the fourth day after the cut, but there was no appreciable change in gastrocnemius chronaxie for 20-30 days. Titeca (1932, 1935) confirmed this for frogs kept at 22°C. (no change in chronaxie for 8 days). He also described an early “fatiguability,” manifested as an increasing threshold with repeated stimulation. This was found to progress slowly centrifugally and was correlated by him with Parker’s observations on centrifugal degeneration of motor fibers of the frog sciatic (Parker, 1933) and of myelin sheaths of the lateral line nerves of catfish (Parker and Paine, 1934). On the other hand, Titeca reported that action potentials disappeared simultaneously all along the degenerating frog nerve. Koch (1925) had earlier shown a uniform loss of resting potential throughout degenerating mammalian nerve.

More recent experiments on rats and frogs (Holobut and Jalowy, 1936) again showed no change in chronaxie until loss of indirect excitability, and the uniform loss of action potential along a cut nerve (indirect excitability disappeared at 6-9 days at a temperature of 10-12°C.; Holobut, 1937).

In view of the discrepancy between the action potential findings, which indicate simultaneous degeneration of frog nerve, and the evidence for centrifugal degeneration obtained from twitch heights, histology, and fatiguability, it seemed desirable to study several of these attributes on the same preparations.

METHOD

Winter and spring frogs (Rana pipiens and R. sylvatica) were kept in a large tank, with a constant flow of water sufficient to maintain a layer half a centimeter deep. Only healthy animals were used. Under light ether anesthesia, the skin on the back was cleaned.
with alcohol, an incision, about 1 cm. long, made between the urostyle and the ilium, the underlying muscles separated with fine-pointed forceps, and the sciatic plexus revealed. Avoiding adjacent blood vessels, the left sciatic trunk was picked up on a pair of curved forceps and sectioned with sharp scissors just below the anastomosis of the spinal nerves. The cut ends of the nerve were allowed to slip back, the muscles drawn together, and the lining of the dorsal lymph sac and the skin were closed, usually together, with one or two silk sutures. In most cases, healing occurred rapidly: infected animals and those which developed red leg were discarded.

In the first series, frogs were kept at a temperature between 18°C. and 20°C.; in the second, between 12°C. and 15°C. For these longer experiments, the frogs were forced-fed with small bits of chopped meat. Only those animals vigorous at the time of examination were used.

From 1 to 20 days after operation, a frog was pithed and immobilized, and both sciatic nerves dissected free without injury to the blood vessels of the leg. In all experiments, the left (operated) and right (control) nerves were treated alike as far as possible. For the kymograph records the usual gastrocnemius-muscle-lever set-up was used except that the femoral end of the muscle was left intact so that its circulation remained unimpaired and the bone was clamped in situ. In a number of experiments records were taken simultaneously from the gastrocnemius and from the peroneal group of muscles. The same length lever arms were used with both right and left sides to permit a roughly quantitative comparison.

The nerve was stimulated using bipolar metal electrodes 1 mm. apart from a Harvard inductorium with 6 volts in the primary and with the secondary, to start with, at 13 cms. and an angle of 5° from vertical. In no case was it necessary, in order to get maximal responses, to have the coil horizontal at less than 9 cms. The electrodes were placed at three positions: H, 1–2 cms. below the cut end (to avoid the area of traumatic degeneration), M, just before the bifurcation of the sciatic to form tibia and peroneal nerves, and, K, at the knee. Each region was tested in order with progressively stronger stimuli, from subthreshold to slightly supramaximal. To minimize as much as possible the inevitable fatigue (which is more pronounced in the operated nerve), the whole preparation was repeatedly washed with Ringer’s solution. This procedure eliminated bizarre results found in preliminary experiments, such as a period of inexcitability between two periods of good excitability.

After muscle responses were recorded, the nerves were removed and their action potentials studied with the cathode-ray oscillograph (Gerard, Marshall, and Saul, 1936). The trace was sometimes photographed, more often measured on the screen. A standing wave was obtained by using a commutator interrupter in the primary of the inductorium. The central end of the nerve was placed on stimulating electrodes and a pair of silver lead electrodes, 1 cm. apart, moved along the nerve. Change of spike height with distance was compared for normal and sectioned nerves.

In a few cases, with conduction on the verge of failing, extensive kymograph records were omitted and only tests for the presence of indirect excitability carried out before removing the nerve. Other frogs from the same batch and in the same operative period were then used for the contraction height measurements. This was necessitated by the excessive fatiguability of such nerves.

In all cases, following these tests, the nerves were examined histologically.* A few were washed, teased, and immersed in 1:10,000 neutral red, according to the method of Cove1 and O’Leary (1932). The majority were fixed in 10 per cent formal in 0.7 per cent NaCl or in H₂O, teased, and stained with a modified Herxheimer stain by the following procedure:

1. Cover slide upon which nerve is teased with Sudan III (Romeis, 1929) for ten minutes
2. Wash rapidly (distilled water) until wash water is almost clear
3. Flood with 1% aqueous haematoxylin for five minutes
4. Wash off excess stain with distilled water
5. Flood with 1 per cent ferric chloride for 1–5 minutes, depending on intensity of stain desired
6. Wash well with tap water
7. Mount in glycerine jelly, after complete teasing of nerve.

* The Marchi stain was not used because it is held (Cajal, 1928) not to show accurately the myelin configuration in the early stages of degeneration.
The whole process is carried out on the slide: with practice the nerve does not come off. Final teasing with steel needles or quills under a strong dissecting microscope clearly exposes individual nerve fibers for comparatively long distances (1–2 cms.). The myelin products appear orange-red, the nucleus and cytoplasm deep blue, and, if washing was adequate, the preparation lasts at least two months.

Camera lucida drawings were made of significant preparations.

In all, over 75 frogs were examined 2–20 days after nerve section, and of these about a third in 12–16 days, the period supposed to show progressive degeneration most clearly. To eliminate the possibility of missing any progressive degeneration because of too rapid degeneration at 18°–20°C., a second series of frogs was run at 12°–15°C. Except for a prolonged time scale in the latter case, the results in the two series were the same. In addition, a series of 8 rats was run to confirm the simultaneous degeneration of mammalian (monkey and cat) sciatic nerves described by Heinbecker, Bishop, and O’Leary (1932).

Results

When adequate stimuli are applied to various points along the sciatic nerve, the gastrocnemius contracts to the same degree for all electrode positions. The contrary findings of Parker, indicating centrifugal degeneration, seem due to the use of stimuli which were partly submaximal for one of three reasons.

The motor fibers do not run together as a bundle in the center of the sciatic nerve, but rather pass from a loose accumulation of fibers on the dorsal side of the nerve in the proximal stretch to a denser group on the medial-ventral side more distally (Kurkowsky, 1935). With the secondary coil at 13 cms.
and 5° from the vertical, a stimulus is delivered which is not strong enough to excite all the motor fibers unless the electrodes are placed directly on them. It is clear that stimuli which are adequate in one region of the nerve may not be so in another. Further, the eccentric course of the motor fibers makes any twisting of the nerve upon the electrodes of extreme importance. Finally, still with submaximal stimuli, confusing results are obtained when the fiber distribution is altered by branches leaving the main trunk between different regions of stimulation.

These points are illustrated by results on a normal nerve. With stimuli of the strength indicated, the contraction was less with electrodes on the hip region of the nerve than when they were at the knee, while with stronger stimuli the responses from both regions were equal (Fig. 1). This is the result Parker (1933) obtained on a degenerating sciatic nerve. In these experiments,

<table>
<thead>
<tr>
<th>Stimulus strength</th>
<th>Hip</th>
<th>Middle</th>
<th>Knee</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Over¹</td>
<td>Under</td>
<td>Over</td>
</tr>
<tr>
<td>Weak (13 cm., 70°)</td>
<td>(14)</td>
<td>52</td>
<td>65</td>
</tr>
<tr>
<td>Medium (13 cm., 0°)</td>
<td>33</td>
<td>90</td>
<td>83</td>
</tr>
<tr>
<td>Strong (9.5 cm.)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

¹ Over indicates electrodes were held on top of nerve; under indicates electrodes were held under nerve: in both cases, without regard for true dorso-ventrality of the nerve trunk.
² Numbers give contraction height in per cent maximal. Those in parentheses indicate proportionate values interpolated from duplicate experiment.

also, with weak stimuli peculiar responses can be obtained from various nerve levels (and with twisting of the nerve), whereas with adequate stimuli, the responses from all levels of the nerve are the same (Table 1). There is especial danger of using inadequate stimuli on a degenerating nerve because there is a progressive increase in threshold from day to day. (This is independent of the decreased response to a maximal stimulus as more and more fibers become fully inactive.) But when adequate stimuli are used, muscle twitches of constant height result from stimulation at any level of the nerve. Further, the rise in threshold, increase in fatiguability, and falling off of contraction all progress with the duration of degeneration simultaneously along the entire cut nerve.

Current spread from a less excitable central region to a more excitable peripheral one is ruled out by the following experiment: with continued stimulation at H, the muscle response falls to zero due to local nerve fatigue. Stimuli applied at K are still fully effective at this time. With rest, H stimuli recover their full action. Clearly no significant stimulus spread could occur.
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Complete loss of indirect excitability occurred about the thirteenth day at 18°–20°C.; about the seventeenth day at 12°–15°C. (Table 2), in the frogs. In the rats (male albino), loss of indirect excitability took place between 50 and 70 hours after operation.

The peculiarities in motor fiber distribution do not significantly influence the oscillograph records. It was found that action potentials declined with time and disappeared simultaneously at all points along the degenerating nerve, as has been clearly shown also by Titeca (1935).

With adequate stimuli, then, and regard for anatomical peculiarities of the nerves, physiological tests show that functional degeneration occurs simultaneously throughout a cut, degenerating, peripheral nerve.

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tbody>
<tr>
<td>Loss of indirect excitability of gastrocnemius with increasing period of degeneration of cut sciatic</td>
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</table>

<table>
<thead>
<tr>
<th>Temperature</th>
<th>18°–20°C.</th>
<th>12°–15°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of frogs</td>
<td>41</td>
<td>19</td>
</tr>
<tr>
<td>Days since cut</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–8</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
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<td>3</td>
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<tr>
<td>16</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>17–20</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

+ Indicates visible response to stimulation.
- Indicates lack of visible response to stimulation, starting with weak stimuli, and ending with coil distance of 6 cms.

The histological findings also agree, for, as Ranson, Cajal, and others have emphasized, when the region of traumatic degeneration near the plane of section of a peripheral nerve is eliminated from consideration, degeneration of the myelin sheaths proceeds in all parts of the nerve at once. An incomplete histological examination of degenerating nerve, however, can be very misleading. In different fibers at one level, nodes may vary from normal to completely degenerate; but in one individual fiber, though the condition is not the same at all levels, the variation from place to place is much less than that from fiber to fiber at one level. Fig. 2 was taken from a nerve cut four days previously, hence too early for observing progressive degeneration. It is obvious that the nodes of the three figured axones are different—varying from the essentially normal condition in fiber c to the moderate degeneration of fiber a, shown by the widened node, clearly visible axis cylinder, and beginning myelin globule formation. In like manner, the internodes at the level of the Schwann cell nucleus are respectively normal or fragmented. The signs of degeneration were equally present throughout a 9 mm. length of fiber a (teased out), and absent throughout a similar length of c.
FIG. 2. FNS 4. Left sciatic cut 4 days previously. T = 18°-20°C. At this level, the condition of the nodes is not the same in individual fibers: in fiber a, the nodes are approximately the same throughout 8 mm.—there was no gradation towards the more normal node of fiber c. Fiber b represents a somewhat intermediate stage.

Drawings made with camera lucida on Zeiss microscope, from preparations stained with Sudan III-haematoxylin. In all cases, magnification is 800 X.

FIG. 3. FOSC 1. Left sciatic cut 13 days previously. T = 18°-20°C. a is 4 cms. from cut end; b is 5.3 mm. from a on the same fiber; c is intermediate between the two. It is usually held that the smaller the myelin segments, the more advanced the degeneration. This, then, could not be centrifugal progressive degeneration. Essentially the same condition was found 8.4 to 9 mm. from a.
A nerve degenerating at 18°-20°C. for thirteen days, still conducting sufficiently to cause muscle contraction, was examined for a cm. length. 4 cms. from the cut end, as Fig. 3 shows, there is still relative uniformity of degeneration, at a stage much more advanced than that shown in Fig. 2. In no case were fibers found with great fragmentation at one end, and normal appearing segments at the other. It should be emphasized that isolated fields containing different fibers give erroneous impressions unless sufficiently large numbers of fibers are counted. Heinbecker, Bishop, and O'Leary (1932), who made adequate counts, pointed out (p. 7): "... the occurrence of undoubted degenerative changes in a variable number of fibers observed in histologic sections is not a true index of the state of the nerve as a whole." This was not considered by Parker and Paine (1934) in their work on the lateral line nerves. The necessity for observing large numbers of fibers on a statistical basis was obviated in this study by teasing fibers and groups of fibers so that they could be followed for comparatively long distances. 300-400 fibers were teased from the frog nerves cut 12-16 days previously, and in no case was progressive degeneration observed: on the contrary, similar degenerative changes were found at widely separated regions of the same fiber.

**DISCUSSION**

Loss of indirect excitability might be due to a failure at the end plates or to breaks in the functional continuity of the motor axones. If the motor end plates degenerate prematurely so that even when axones still conduct, block occurs at the neuro-myal junction (Tello, 1907; Titeca, 1935; Holobut and Jałowy, 1936), then no conclusive statements regarding progressive degeneration in nerve can be drawn from muscle twitch experiments. It would be impossible to obtain twitches from stimulation of one nerve region and not from others, as claimed by Parker. If, however, the motor axones degenerate first, or simultaneously with the end plates, then the simultaneous loss of indirect excitability along the nerve trunk is positive evidence of a non-progressive degeneration. Only if axones degenerate first and progressively as described by Bethe (slow progression from the cut end, starting immediately after the injury) or by Parker (progressive degeneration found only between 12 and 16 days after injury) could a centrifugal loss of indirect excitability result. Since both requirements are refuted by the facts presented, this may be excluded.

It is clear that a definitive answer to the question of the progress of degeneration in nerve must rest on analyses within the nerve itself to exclude the end-plate complication. Action potential studies, of nerve function, have been unanimously against progressive degeneration; and histological ones, of nerve structure, predominantly so.

One hypothesis as to degeneration posits that some chemical substance, arising in or near the nucleus, is transmitted to the rest of the nerve fiber, and that it is the lack of this compound (or compounds) which causes degeneration. The "chemical theory" is compatible with either simultaneous or pro-
gressive degeneration, depending on the subsidiary assumptions made; and although Parker and Paine (1934) used it as support for their observation of progressive degeneration in catfish nerve, Bethe (1903), long an adherent of the progressive degeneration theory, rejected the chemical theory for, to his mind, it left no alternative but simultaneous degeneration. This theory was first proposed by Goldscheider in 1894. It has recently been supported and amplified, as against a theory requiring constant passage of trophic impulses, by Cook and Gerard (1931) and Gerard (1932), who observed that increased activity of severed mammalian nerve caused a more rapid degeneration; and similar results were obtained on frog nerve in vitro by Abrams and Gerard (1933). Torrey’s (1935) observations on the temperature coefficient of degeneration in frog nerve also supports such an hypothesis. Plausible means for the sufficiently rapid spread of chemicals along nerve fibers have been suggested (Gerard, 1932; Abrams and Gerard, 1933; Parker and Paine, 1934). The nature of the essential substance has been speculated about (Marinesco, 1930, and others), and Minea (1932) has reported a slowing of degenerative processes in rabbit nerve by injections of lecithin and antilipase sera. Certainly the axone, with its independent blood supply, furnishes an admirable test object for studying substitutes for the nuclear influence.

**SUMMARY**

The cut left sciatic nerves of leopard and wood frogs kept at 18°–20°C lose the capacity to transmit impulses to their attached gastrocnemii 13–14 days after section. This loss, measured by muscle twitch height, occurs simultaneously throughout the length of the peripheral stump. At 12°–15°, the physiological degeneration is not complete until the seventeenth day after the cut.

Action potentials from these nerves decline and disappear simultaneously throughout the length of the nerve as degeneration progresses.

Histological examination of teased out fibers of degenerating nerves with a modified Sudan III-haematoxylin stain, shows no linearly progressive myelin degeneration.

Discrepant findings by others can be accounted for in terms of the use of stimuli which were inadequate in view of the presence of branches and of the irregular distribution of motor fibers in the frog sciotic, and to failure to eliminate the area of traumatic degeneration at the cut end of the nerve.

These results have been confirmed in rats, where the loss of indirect excitability occurs from 50–70 hours after section. This conforms with the results of Heinbecker, Bishop, and O’Leary on various nerves in cats and monkeys.

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REFERENCES


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