

Properties of isolated nerve fibres from alloxanized rats¹

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Rat sciatic nerve fibres do not conduct nerve impulses at a normal rate after the rat has been injected with alloxan (Eliasson, 1964). The dose given to the rat must be large enough to cause clinically detectable diabetes. The conduction rate slows to levels of 30 to 50% of the original within the first 10 days after the injection; possibly this is due to a direct toxic effect of the alloxan on the neural structures. Nerves from rats which have been diabetic for six weeks or longer have less conduction impairment. Sensory and motor fibres are involved at both time intervals, but no change has been noted in the autonomic nerves. The conduction velocity change is not reversed by insulin, but hypophysectomy may result in a return of normal conduction velocity (Mayher, Mimbs, and Allen, 1967).

Different pathological findings found in toxic neuropathies could be used to account for the decrease in the conduction velocity in the alloxanized, diabetic animal. Paranodal demyelination is the lesion seen in chronic alcoholism (Mayer, 1966), lead neuropathy (Lampert and Schochet, 1968), and diphtheric neuropathy (Waksman, Adams, and Mansmann, 1957), whereas acrylamide intoxication results in axonal degeneration (Fullerton and Barnes, 1966). Neither of these findings has been noted in chronic alloxanized animals, but focal Schwann cell damage was observed to occur in acute alloxan intoxication by Preston (1967).

Loss of nerve fibre with a change in fibre spectrum and conduction was observed in isoniazid intoxication by Cavanagh (1967). Data from our animals as seen in Table I did not substantiate any such change in the composition of the nerve for alloxanized animals. It therefore remained to explore the possibility that the conduction velocity changes described in the diabetic animal were due to changes in the insulating properties of the internodal segments and/or the nodal membranes themselves.

METHODS

The animals were killed by a blow on the head. The nerves were removed immediately and placed in a glass-stoppered flask containing Krebs-Ringer bicarbonate solution with 0.1% glucose 37°C. The pH range was from 7.34 to 7.40. The enhanced excitability which is normally seen in the acute preparation diminishes after 10 minutes (Adrian, 1930). The nerves were then taken from the flask and placed on an array of platinum or silver wire electrodes in a small chamber with electric see-through fittings for stimulating and recording leads. Additional thermocouple leads were incorporated and all experiments were performed at $37 \pm 1^\circ\text{C}$ in a constant temperature bath. Control and experimental nerves were compared and selected at random.

Paired electrical shocks, here called conditioning and test shock, were applied to the nerve trunk with the intensity of the stimulating current selected to be two to three times that sufficient to obtain a maximal response. The latency of the response to each shock was measured and the time interval between the two shocks varied from 0 to 5 msec.

The properties of individual nerve fibres were investigated using the collision technique of Tasaki (1955). Single nerve fibres were dissected from the tibial nerve of the rat. A segment of the nerve was freed from perineural tissue under the dissection microscope and all except one of the fibres in the prepared region were cut. Observations were made on 47 of the larger nerve fibres, the diameter of which ranged from 10 to 13 μ . The responsiveness of each fibre was checked by repeated measurement of its ability to carry an electric shock at the same speed.

During the preparation the trunk with the isolated fibre was mounted on a bridge insulator. The fluid bathing the preparation was divided into three pools, separated by air gaps of 0.1 to 0.2 mm (see diagram in Fig. 1). First an internodal segment of the isolated fibre was located in the central pool. After grounding the two lateral pools, electric shocks were applied to both ends of the neural trunk. The time relation between the two shocks was adjusted until they collided in the internodal segment in the central pool. Emerging current from the internode was recorded through a resistor and a cathode follower and amplified. It was further quantitated with a simple RC network (Fig. 1).

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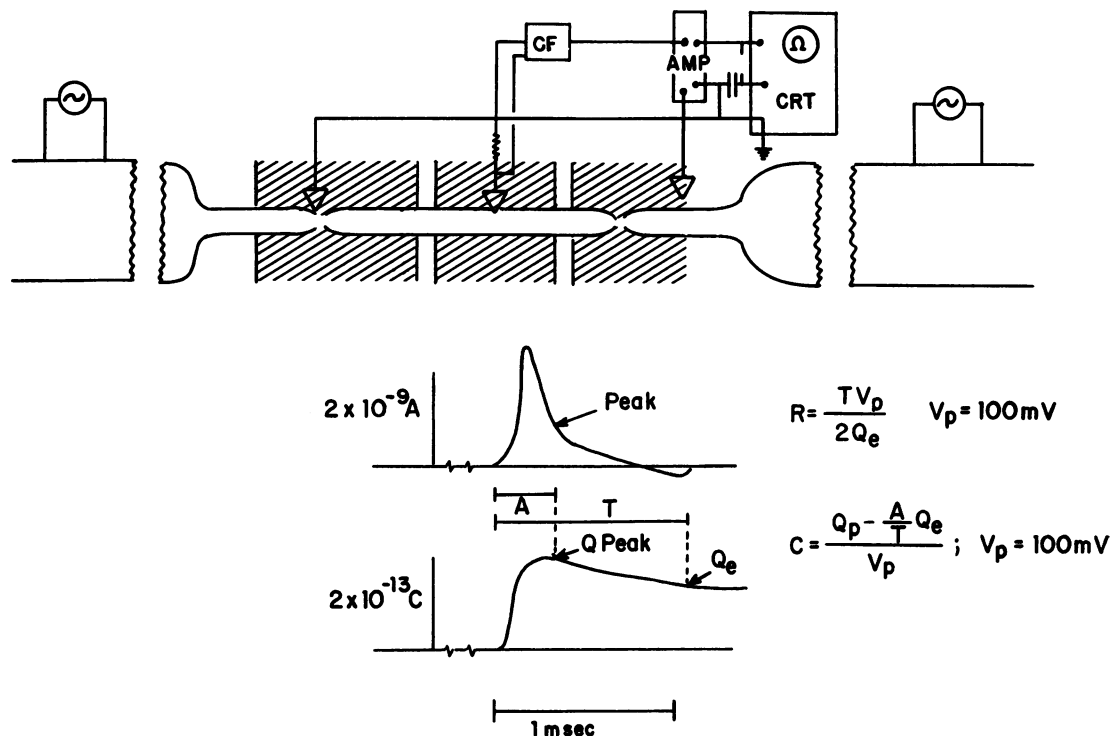


FIG. 1. *Upper half: Diagram of experimental arrangement. The single fibre is immersed in three pools. The two lateral pools are grounded. The control pool contains an internodal segment and the recording electrode is connected via a cathode follower (CF) to an amplifier (AMP) and an oscilloscope (CRT).*

Lower half: Tracing of action potential. Calibrations: 2×10^{-9} A; 2×10^{-13} C.

Peak = Peak of intra-axonal voltage (V_p), here assumed equal to 100 mV; Q_p = Current flow at peak voltage; Q_e = Current flow at end of action potential; A = Time of intra-axonal voltage increase; T = Duration of action potential; R = Resistance; Q = Capacitance.

Measurements of the properties of the nodes of Ranvier were performed after slight displacement of the fibre leaving one node in the central pool and the two adjacent nodes in the lateral pools. Additional 0.5% cocaine Ringer to these permitted isolated measurements of the characteristics of the node in the central portion. Typical tracings of obtained potential variations and current have been incorporated in Figure 1. On the tracings are indicated the calculations which lead to approximate figures for resistance and capacitance of the myelin sheath and node. Mathematical analysis of the data followed the method described by Tasaki (1955). For the purpose of comparison, a peak intra-axonal voltage of 100 mV and an attenuation factor of 0.4 were assumed.

The instruments used included two Grass S-4 stimulators (later a Grass S-8) with SIU4A insulators, Tektronix 122 preamplifier, Tektronix 502 oscilloscope, and a Grass camera.

Preparation of the alloxanized rats was similar to that described earlier (Eliasson, 1966). The nerves

studied were selected from rats in two different groups: (1) those that had gone 10 to 14 days after injection of alloxan and showed evidence of glycosuria and hypoglycaemia; and (2) those which had continued to show evidence of diabetes for six weeks and more after alloxanization.

Transverse sections were prepared and fibre diameters measured on four sciatic nerves. Two of these were from normal rats and two from rats which had been alloxanized for 77 days. The nerves were removed and fixed in osmic acid under light stretch. The major fibres were tabulated in two micron groups (see Table I).

RESULTS

A conditioning supermaximal shock applied to the proximal portion of the excised sciatic nerve of normal rat evokes a compound action potential in the tibial branch. The time interval until the onset of the compound action potential is representative

TABLE I
DISTRIBUTION OF NERVE FIBRE SIZES IN SCIATIC NERVE IN NORMAL AND ALLOXANIZED RATS

Rat	Wt	Total fibres	Absolute fibre numbers						
			3-4 μ	5-6 μ	7-8 μ	9-10 μ	11-12 μ	13-14 μ	14 μ +
Normal	315	5018	744	1961	1361	788	158	4	0
Normal	335	5642	801	2142	1696	812	210	8	1
Diabetic	295	4768	817	1854	1105	799	170	11	2
Diabetic	290	5310	816	2015	1509	740	222	7	1

Fibres less than 3 μ not counted. Rats 140 to 150 days old. Time after alloxanization—77 days.

for the fastest conducting fibres in the A group. A test shock of equal or greater intensity travels with the same rate provided the interval between the test and conditioning shocks exceeds 3.5 msec in the normal rat nerve. As the interval between the two shocks is shortened, the travel time of the second impulse is prolonged until, at intervals of less than 0.6 msec, the absolute refractory period prevents conduction of the second impulse (Fig. 2).

The conditioning shock travels at a slower rate in nerves from acute alloxanized rats. It is even more obvious that a test shock which starts less than

2 msec after the conditioning shock, reaches only one half to one third of the rate of the impulse in the normal rat tibial nerve. The length of the absolute refractory period appears to be the same. Isolated fibre experiments were done in order to determine some of the reasons behind the differences between the two preparations.

Single fibres were dissected as described in the Methods section and their resistive and capacitive properties of nodes and internodes were measured. The results of measurements on internodal segments from control, recently alloxanized, and long-term rats

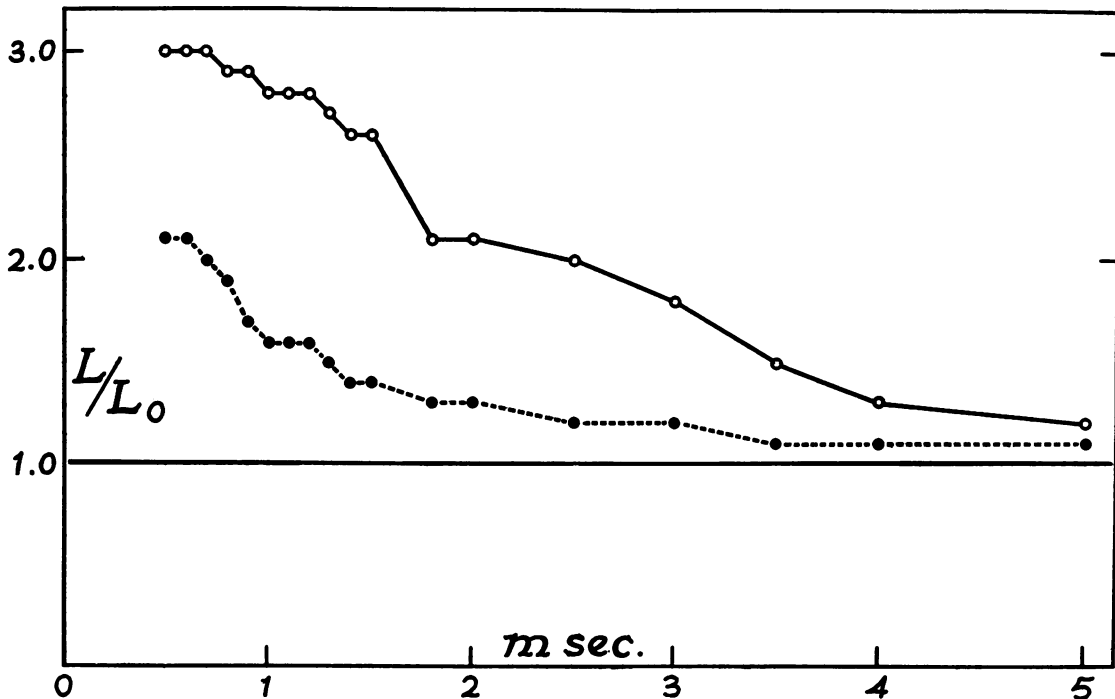


FIG. 2. Changes in test shock-response interval (L) after the application of a conditioning shock. Normal nerve ●-----●. L_0 = interval between conditioning shock and response. Diabetic nerve ○-----○ Abscissa = interval between conditioning shock and test shock.

are seen in Table II. A decrease to less than one-third of the original value of the resistance per unit area was found in the internodal segment of the acutely alloxanized animal. The decrease was not as large if the rats had been alloxan diabetic for six weeks or longer but was still significant. Capacitance per unit area of internode did not change in any consistent fashion after alloxanization. Values obtained for the capacitance and resistance at the nodes of Ranvier are less reliable, due to difficulties in estimating the area of the nodal membrane. The data in Table III imply that no significant change takes place in the physical characteristics of the node after the injection of diabetogenic doses of alloxan.

TABLE II
PHYSICAL CHARACTERISTICS OF INTERNODES

<i>Rat tibial nerve fibres</i>			
Type	Number	Capacitance $F \times \text{cm}^{-2} \times 10^{-9}$	Resistance $\Omega \times \text{cm}^2 \times 10^4$
Normal	13	2.2 ± 0.5	2.0 ± 0.2
Alloxanized 10 days	11	2.6 ± 0.9	0.8 ± 0.2
Alloxanized 42 to 50 days	12	2.7 ± 0.7	1.4 ± 0.4

TABLE III
PHYSICAL CHARACTERISTICS OF NODES

<i>Rat tibial nerve fibres</i>			
Type	Number	Capacitance $F \times \text{cm}^{-2} \times 10^{-6}$	Resistance $\Omega \times \text{cm}^2$
Normal	11	4.2 ± 0.4	12 ± 2
Alloxanized 10 days	9	6.1 ± 0.5	15 ± 4
Alloxanized 44 to 50 days	9	5.4 ± 0.9	8 ± 3

DISCUSSION

The similarity between the fibre size distributions in sciatic nerves from normal and chronic alloxanized rats eliminates degeneration of large fibres as an explanation for the decrease in conduction velocity. As a single explanation, it would seem unlikely because the maximal decrease occurs early after alloxanization. Preston (1967) described changes in nodal length, myelin sheath configuration and nodal width, but none in fibre diameter.

The application of two consecutive shocks to the nerve trunk demonstrates that not only is the

first impulse conducted more slowly in the alloxanized preparation but also that the recovery of the fastest conducting fibres is prolonged. This leaves the impression of damaged, poorly conducting, rather than functionally blocked fibres. Changes in external resistance provided by alteration of composition of the cellular and non-neural elements in the nerve trunk might also be important in the whole nerve preparation.

The values obtained for the single fibres from tibial nerves in uninjected animals were within the range expected from earlier data (Hodgkin, 1964). Errors probably occur due to placement of the fibre, inaccuracies in estimation of diameter of fibre and width of nodes and the assumption of equal attenuation factors and intra-axonal peak potential in the three preparations. Certain additional explanations over and beyond the observed internode resistance change can therefore not be ruled out. This applies particularly to the changes in nodal width which were described by Preston (1967) and the basal membrane thickening demonstrated by Bischoff (1967).

Could the resistance change noted account for the decrease in conduction velocity of the nerve impulse in the alloxanized rat? The conduction velocity is proportional to the space constant and the latter varies with the square root of the specific resistance according to Davson (1964). If this relationship holds, both the shortening of internodes and the resistance decrease would affect the conduction velocity and a value of 40 m/sec or less could be expected for the fastest fibres. This is in good accord with our observations on nerve fibres in the acute stage following alloxanization. Less marked reduction would occur in the chronic state which agrees with the findings by Lovelace (1967).

The nature of the change of the electrical properties of the myelin sheath needs to be explained. There is reason to believe that the physical properties of the myelin sheath in the acute alloxanized animals are not uniform from one node of Ranvier to the next. If the myelin sheath were actually dissected from the node, the introduction of fluid between the myelin layers could account for the change in resistance properties. Segmental demyelination is very effective in changing the relative refractory period as Lehmann (1967) has shown convincingly. Finally, changes in the composition of the myelin sheath with structural alterations due to changes in fatty acid chain length, saturation or branching could alter the specific resistance (Leslie, Chapman, and Hart, 1967). Such changes have been reported by Eliasson (1966), but their quantitative role has not been determined.

SUMMARY AND CONCLUSIONS

The myelinated fibre content of the sciatic nerve from rats with alloxan diabetes is the same and has the same diameter spectrum as the normal rat's nerve.

Two weeks after alloxan injection, the nerves from the diabetic rats contain large fibres with a 60% decrease in membrane resistance and unchanged capacitance of the internodal segments.

Six weeks after introduction of the diabetic state the nerve fibres' internodes still have normal capacitance, but the resistance decrease is only 30%. The nodes of Ranvier have normal physical characteristics within the limits of the method.

The occurrence of 'leaky' myelin in the internodes of the large fibres could explain the decrease in conduction velocity seen in diabetic subjects. Additional studies are needed to rule out paranodal current shorage and the effect of enlarged nodal membranes.

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