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Nerve Conduction Changes in Experimental Diabetes*

SVEN G. ELIASSON †

(From the Department of Medicine, University of Texas Southwestern Medical School, Dallas, Texas)

The problem of diabetic neuropathy has not been studied experimentally to the same extent as have other manifestations of diabetes mellitus. One reason may be that neurological symptoms have not been apparent in animals that have been made diabetic. Lukens (2) stated in his review of alloxan diabetes that nervous system lesions had not been observed. Pancreatectomized animals have been prepared in large numbers, but no mention has been made of neurological complications. Minor neurological deficit, particularly of the sensory functions, could easily have been overlooked (3).

The data presented below demonstrate a decrease in conduction velocity under *in vitro* conditions in the peripheral nerves from alloxanized rats but only if these rats also become diabetic. Because of the toxic effects of alloxan, a group of pancreatectomized rats was also prepared and showed essentially the same slowing of conduction. Treatment with insulin of the animals before the experiment or addition of insulin *in vitro* did not restore conduction velocity to normal values.

Methods

Sprague-Dawley rats¹ were used. The rats weighed between 300 and 450 g and were over 5 months old. The animals were injected with alloxan monohydrate in citrate buffer in amounts of 40 to 50 mg per kg (4). Litter mates were used as controls. Intravenous injection method was used, and injections were given after 18 hours of starvation. Some of the injected animals did not become diabetic. These animals were used as

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† Present address: Department of Neurology, Washington University School of Medicine, St. Louis, Mo.

¹ Obtained from Holtzman Rat Co., Madison, Wis.

controls of the effect of nondiabetogenic doses of alloxan on nerve conduction.

Blood sugar levels were measured at weekly intervals and on the day of the experiment with the glucose oxidase method. The rats were considered to be diabetic when the fasting blood sugar exceeded 200 mg per 100 ml and glucosuria was present. In the experiments with insulin treatment 24-hour urine volumes were collected daily under toluene for 1 week (up to the day preceding the experiment) from both treatment and control groups. Glucosuria is recorded for each group of rats in grams glucose per 24 hours \pm SD. The weights of the rats were checked before injection of alloxan and at the time of the experiment. To provide control for loss of body weight, a number of animals were starved on water only, and the conduction velocity of nerves from these animals was tested.

Insulin treatment was given according to two different schedules. In one group the insulin treatment was started 3 weeks after the injection of alloxan at a time when the initial hepatic and kidney damage should have disappeared. A dose of 4 U protamine zinc insulin² was selected on the basis of the studies of Steiner, Rauda, and Williams (5).

In a second group of animals the insulin treatment was started on the second day after the injection of alloxan. Those animals were used in which a strongly positive test for urine sugar was obtained at that time. The treatment was then continued with 4 U per day for 40 days.

Pancreatectomy was performed essentially according to the technique described by Scow (6) but leaving some pancreatic tissue around the bile duct. The animals that developed diabetes behaved clinically in accordance with the preparation described by Treadwell and Roe (7). The intestinal absorption difficulties were avoided, and the animals could be kept alive for 30 to 40 days without insulin. By minor variation in operative technique other rats were obtained that showed signs of a more severe and promptly developing diabetes and that could be kept alive only through the application of the Scow (6) scheme for postoperative care. These animals had to be maintained on insulin for the remainder of the experimental period and were given regular and protamine zinc insulin as needed.

The rats were fasted 18 hours before the experiment except for pancreatectomized animals on insulin and their controls. The animals were then stunned by a

² Protamine, zinc, and Iletin, Eli Lilly and Co., Indianapolis, Ind.

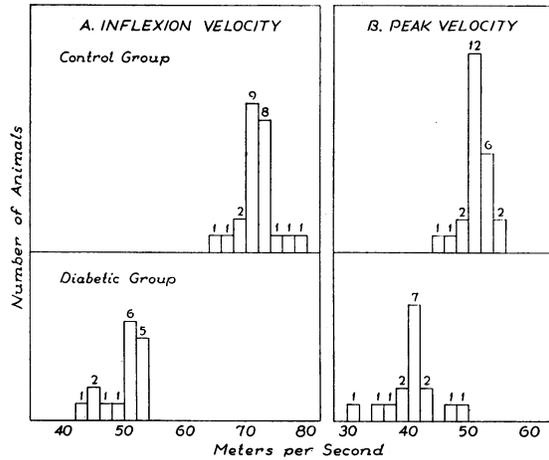


FIG. 1. INFLEXION AND PEAK VELOCITIES IN SCIATIC NERVE FROM NORMAL AND DIABETIC RATS.

blow on the head and decapitated. The sciatic nerve from the sciatic notch to the gastrocnemius tendon was removed and soaked 10 minutes in Ringer-Locke buffer to eliminate spontaneous firing (8). The nerves were then placed in a moist chamber on bridges made from silver chloride-silver electrodes or platinum wire electrodes. Temperature was kept constant by immersing the chamber in a constant temperature bath. Electrical stimulation was obtained from Grass S-4 stimulators with stimulus isolators. The stimulation intensity was kept 3 to 5 times the value necessary for maximal response. The evoked potentials were amplified and recorded with Tectronix equipment. Conduction times were measured from photographs after magnification. After the experiment the nerves were removed, and the distance from stimulating to recording electrodes was measured. Conduction velocity is given as meters per second \pm standard deviation.

In experiments designed to study the changes in sensory versus motor nerve fibers, a preparation was obtained by rapid dissection of a dorsal and ventral root together with the sciatic nerve. The continuous structure from the ventral and dorsal root to the tibial nerve was then placed on a Y-shaped bridge so that stimulation of the ventral root and recording from the tibial nerve, as well as stimulation of the tibial nerve and recording from the dorsal root, was possible (see Figure 2).

In all experiments the corresponding sciatic nerve from a normal noninjected animal was treated in the same fashion as the diabetic nerve. The remaining diabetic sciatic nerve and the remaining normal nerve were used for neurochemical studies as described elsewhere (9).

Results

The tibial nerve from which the recordings were obtained contains a number of large, myelinated fibers. The conduction velocity in the fast-

est conducting tibial nerve fibers activated on supramaximal stimulation of the sciatic nerve at the sciatic notch will be referred to as inflexion velocity (10). It was found to be 70 ± 4 m per second in normal rats (24 animals). Peak velocity is defined as the conduction velocity of the largest uniform group of nerve fibers and is calculated on basis of the time elapsed from the onset of the stimulus until the nerve action potential reaches its maximal amplitude. It is 52 ± 3 m per second in normal rat sciatic nerve. The range of variation is shown in the top panels of Figure 1. The end point of the nerve action potential was often not sharply defined and was therefore not used. Only minor variations were seen in the amplitude of the nerve action potential.

A decrease in conduction velocity was observed in the diabetic animals. The data from 16 diabetic rats are presented in the lower panel of Figure 1. The animals had been injected with alloxan 2 weeks before the conduction experiments. The reduction in conduction velocity is most marked in the rapidly conducting fibers with a drop to 52 ± 1 m per second (16 animals). Peak velocity also slowed and was calculated at 41 ± 2 m per second.

Alloxanized diabetic animals initially undergo a fair amount of weight loss. An experimental group was set up to study the *in vitro* conduction velocities in sciatic nerves after starvation with unlimited water intake. The rate of weight loss in the animals corresponded to that of alloxanized diabetic animals. Another group was composed of animals that had been injected with alloxan but had had only a transient episode of hyperglycemia

TABLE I
Nerve conduction velocities*

Preparation	Number of animals	Inflexion velocity <i>m/sec</i> \pm <i>SD</i>	Peak velocity <i>m/sec</i> \pm <i>SD</i>
Normal	10	71 ± 3	53 ± 2
Starved	9	74 ± 4	55 ± 3
Alloxanized Nondiabetic	10	70 ± 2	53 ± 3
Alloxanized Diabetic	7	54 ± 1	40 ± 3
Alloxanized Diabetic +insulin†	7	52 ± 3	40 ± 2

* The velocities were measured *in vitro* 14 days after preparation of animal.

† Insulin treatment with 4 U protamine zinc insulin beginning 2 days after alloxan injection.

TABLE II
Glucosuria and conduction velocity in alloxan diabetic rats with and without insulin treatment

	Number of animals	Dose insulin* U/day	Duration of treatment	Glucosuria† g/24 hrs ± SD	Conduction velocity m/sec ± SD
Control	6	0	0	0.02 ± 0.01	74 ± 2
Diabetic No treatment	8	0	0	8.1 ± 1.0	51 ± 3
Diabetic Treatment from day 21	6	4	21	0.30 ± 0.06	54 ± 4
Diabetic Treatment from day 2	5	4	40	0.19 ± 0.06	53 ± 3

* Protamine zinc insulin.

† Calculated from 24-hour urine collections for 7 days before determination of conduction velocity.

or none at all. The results after an experimental period of 14 days are given in Table I together with normal controls and alloxanized, diabetic animals of the same sex and age. The fifth group listed in Table I consists of animals that had been made diabetic with alloxan and treated with insulin from the second day after alloxanization as indicated in the Methods section. The only two groups with reduction in conduction velocity were the diabetic. Treatment with insulin had no effect on the reduced conduction velocity. Addition of regular insulin in a concentration of 0.1 to 1 U per ml to the *in vitro* bath was likewise ineffectual.

Three groups of diabetic rats and a control group were studied after 6 weeks in order to establish the adequacy of the insulin treatment (Table II). Urine was collected from all four groups daily for 1 week before the determination of the conduction velocity. Two of the diabetic groups were treated with insulin. One of the groups was started on insulin on the second day after alloxan injection. The other group was started 3 weeks after injection at a time when the diabetes was well established. The insulin treatment reduced the amount of glucose in the urine sharply but had no effect on the development or magnitude of the conduction defect.

Some animals appeared severely ill during the first week after injection of alloxan. The presence of diabetic ketoacidosis was suspected and confirmed by laboratory studies. A decrease in inflexion velocity was noted already after 5 or 6 days in all diabetic animals. Because of the known

occurrence of lesions of kidney and liver and the disturbances of electrolyte and acid-base balance that take place in the early stages, data obtained before 10 days were considered less unequivocal and were not tabulated. In no animal, however, could any correlation be demonstrated between the appearance and amount of ketoacidosis and conduction velocity.

Inasmuch as electrical stimulation at the sciatic notch would not distinguish between sensory and motor fibers, these two types were studied separately. The ventral root was stimulated with recording taking place at the distal portion of the tibial nerve. This was compared to stimulation of the tibial nerve and recording from the dorsal root. Figure 2 shows the results obtained in six experiments. The decrease in conduction velocity

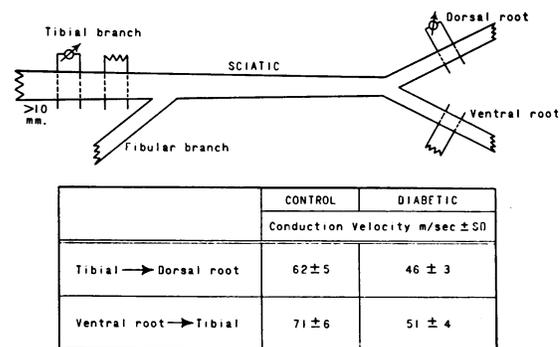


FIG. 2. COMPARISON BETWEEN CONDUCTION VELOCITY IN SENSORY AND MOTOR FIBERS FROM NORMAL AND DIABETIC NERVES. The schema indicates the placement of electrodes, one stimulating electrode being on the ventral root, one recording electrode on the dorsal root, and one of each on the tibial branch.

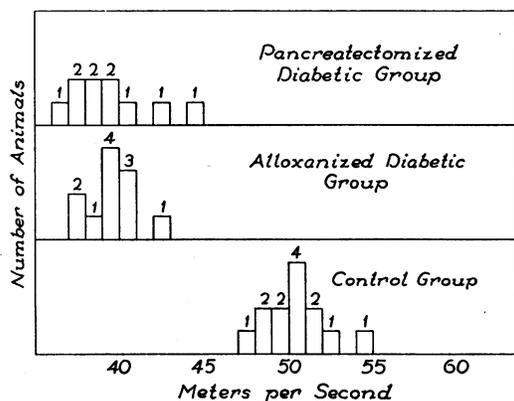


FIG. 3. PEAK CONDUCTION VELOCITY DISTRIBUTION IN TWO TYPES OF DIABETIC ANIMALS VERSUS CONTROL ANIMALS.

in the largest sensory and motor fibers from six animals is 26% and 28%, respectively (Figure 2). The results thus obtained corresponded to the slowing observed in whole peripheral nerve.

It has been stated (11) that autonomic nerves are frequently involved in diabetic neuropathy. Autonomic fibers in the vagus of the diabetic rat were stimulated at the level of the diaphragm in order to avoid exciting the much larger, myelinated fibers from the atria, lungs, and larynx present at higher levels. Studies on eight preparations from alloxanized diabetic rats and an equal number for normal animals revealed the presence of two groups of small fibers. One of these groups conducted impulses at a rate of 3.4 ± 0.3 m per second in the diabetic and 3.3 ± 0.2 in the normal rat. The other group conducted slowly (0.7 to 0.9 m per second), and exact measurements were not possible because of the small size of the nerve action potential. Both normal and diabetic animals had vagal nerve fibers conducting in this range, and no differences could be made out.

Rats made diabetic through subtotal pancreatectomy were studied in order to see if this type of experimental diabetes would be associated with a reduction in conduction velocity. One group of animals was sacrificed on day 32 after operation. These were animals that had developed diabetes on day 4 after operation and had not required insulin. The results are shown in Figure 3 together with alloxan diabetic animals and controls of the same age. The velocity reduction in the pancreatectomized group and in the injected group is approximately the same with more scatter

in the data from the surgical group. The duration of the diabetic state in both groups was the same or 26 days. Only peak velocities are given in the figure, but the reduction in the inflexion velocities was similar.

The time course of the observed reduction in peak conduction velocity was examined in both alloxanized and pancreatectomized groups that had not been treated with insulin. In order to avoid the period of generalized toxic effect of alloxan, only the values at 10 days and later are charted. Already at 10 days a clear reduction in peak conduction velocity can be observed (Figure 4). A slowly progressive decrease in conduction velocity takes place over 2 months in the alloxanized group. A continued slowing of conduction velocity is also seen between days 10 and 30 in the pancreatectomized rats.

Additional studies were made on a group of rats with more extensive removal of the pancreas. These animals had to be maintained on insulin from the first postoperative day and were limited to a food intake of not more than 10 g per day with 12 U of insulin. Normal and alloxanized diabetic animals were given the same amount of food; the latter group was given 4 U of protamine zinc insulin (see Methods). The experiment was terminated after 3 weeks. Weight changes and blood and urine sugars as well as inflexion and peak conduction velocities for the three groups are given in Table III. The addition of exogenous insulin, which made it possible for the pancreatectomized rats to maintain a modest weight gain and controlled the amount of glucosuria, was without effect on conduction velocity of the nerve just as

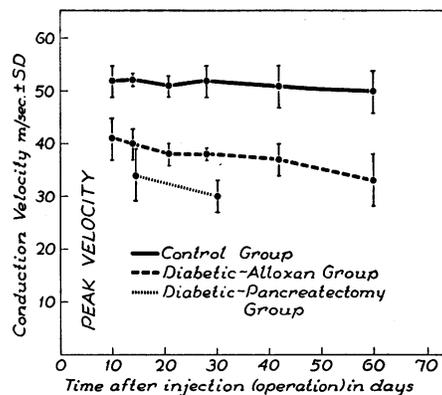


FIG. 4. CHANGES IN PEAK CONDUCTION VELOCITY WITH TIME AFTER INDUCTION OF DIABETES.

TABLE III
Weight change, blood sugar, glucosuria, and sciatic nerve conduction velocities in insulin-treated diabetic rats and controls over a 3-week period

Preparation*	Body weight		Blood sugar†	24-hour urine sugar†	Conduction velocity	
	Initial	Gain			Inflexion	Peak
	g		mg/100 ml	g/100 g	m/sec ± SD	
Pancreatectomized (6) 12 U insulin/day	320	10	164	0.13	49 ± 6	38 ± 4
Alloxanized (6) 4 U insulin/day	335	18	144	0.08	55 ± 4	42 ± 3
Control (9)	305	32	103	0.02	73 ± 2	54 ± 1

* Numbers in parentheses refer to the number of animals in the group.

† Measured twice a week and on day of experiment; value given is the highest observed.

it was ineffective in overcoming the nerve lesion in alloxan-induced diabetes.

Discussion

Alloxan-induced diabetes is secondary to destruction of beta cells in the pancreas (12). The destruction is a result of the toxic action of alloxan, but the exact mechanism remains unknown. The effect of the injected alloxan can be nullified by rapid inactivation in the blood stream, and this occurs in some animals in spite of the buffering system used at the injection. The conduction velocity defect was observed only in animals in which the effectiveness of alloxan was evidenced by the presence of a persistently elevated blood sugar, increased urine output, and glucosuria. The possibility that the nerve conduction defect was due to direct action of alloxan on the nerve conduction mechanisms was in part eliminated by the finding of normal conduction velocities in animals that had not become diabetic upon injection of equivalent amounts of alloxan per unit body weight. The fact that nerves from pancreatectomized diabetic rats have a decrease of conduction velocity comparable to that of the alloxanized rats is further evidence that the conduction deficit in the alloxanized animal is not an expression of pharmacological neurotoxicity of alloxan but rather the secondary consequence of a diabetic stage.

Biochemical studies of peripheral nerves have indicated that alloxan diabetes is associated with an impaired incorporation of acetate and glucose into nerve lipids (9, 13, 14). Eventually this could affect the size or properties of the myelin sheath, or both. The difference between nerves

from normal and diabetic rats in the conduction of nerve impulses is noted 5 to 6 days after alloxan injection, which is comparable to the biochemical defects in lipogenesis noted by Field and Adams (13) after 48 hours and by Eliasson and Hughes (9) after 1 week. The relationship between fiber diameter and conduction velocity is not exact enough to permit any conclusions as to the effect of small variations in diameter (15). The slowing was uniform along the axon from the roots to the distal tibial nerve, and therefore neither increased branching nor degeneration with subsequent regeneration could be invoked as explanation for the decreased conduction velocity.

It seems clear from the experiments that a diabetic state induced by alloxan or pancreatectomy results in a slowing of conduction velocity. Without wishing to imply that the neural lesion of human diabetics is the same as that observed in these studies, we point out that slowing of motor fiber conduction was noted in almost all of the 103 unselected diabetic patients by Mulder, Lambert, Bastron, and Sprague (16) and in sensory nerve fibers in diabetics by Liberson (17). A considerable number of similar observations in man are now available (18-22). The nature of the factors involved in addition to carbohydrate metabolism could not be ascertained. Mayer (22) brings up the possibility of a widening of the nodes of Ranvier as an explanation for the slowing of conduction. This mechanism was originally proposed by Denny-Brown and Brenner (23, 24) to explain slowing after focal ischemia. Such a widening could also be the result of a local decrease in turnover of myelin lipids.

The lack of effect of insulin *in vitro* on the conduction velocity could easily be attributed to a

permeability effect or to an inability of insulin to reverse instantaneously the changes wrought by a prolonged diabetic state. The lack of effect on the conduction defect of *in vitro* treatment with insulin is similar to the lack of effect of exogenous insulin on lipogenesis noted by Field and Adams (13). Shichiri (14), however, could reverse the depression of lipid synthesis from acetate and glucose caused by alloxan diabetes with exogenous insulin treatment. It is interesting to note the clinical parallel in the varying response of many cases of human diabetic neuropathy to insulin treatment.

Summary

1) Conduction velocity in rat nerves was evaluated after alloxan injection or pancreatectomy.

2) When a diabetic state was induced, a reduction of conduction velocity of approximately 30% was noted in both sensory and motor fibers of the sciatic nerve.

3) No slowing was observed in vagus nerve fibers presumably with autonomic function.

4) Nondiabetic alloxanized animals and starved rats showed no reduction in sciatic nerve conduction rates.

5) Insulin treatment of the diabetic rats or addition of insulin to the *in vitro* preparation did not affect the reduced conduction velocity.

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