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IMPULSE CONDUCTION VELOCITY OF EXCISED PERIPHERAL NERVE FROM LEAD-FED RATS AND DOGS

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In 1964 Eliasson demonstrated reduced impulse conduction velocities in excised peripheral nerves from depancreatized rats, and from rats with experimental alloxan diabetes[1]. This demonstration suggested that conduction velocity of excised nerve might serve as a physiological indicator of peripheral nerve involvement resulting from exposure to industrial chemicals. This suggestion is supported by "in situ" conduction velocity studies on experimental animals: slowed conduction velocity in peripheral nerve has been demonstrated to accompany allergic polyneuritis in guinea pigs[2], diptheric polyneuritis in guinea pigs[2] and cats[3], lead poisoning in guinea pigs[4], and chronic acrylamide poisoning in rats[5]. Clinical studies on humans also lend support: reduced conduction velocity along the ulnar rerve in 10 women with severe polyneuropathy associated with triorthocresyl phosphate poisoning[6], and "borderline" slowing in two patients with lead neuropathy and in five patients with neuropathy subsequent to cutaneous absorption of an industrial solvent (85% trichloroethylene, 10% ethylene dichloride, 5% tritoluol phosphate)[7].

Two-year lead feeding studies on rats and dogs in progress in this laboratory[8], provided the opportunity to determine the impulse conduction velocities of peripheral nerves available from these animals at the time of sacrifice. The preparations so available could not be obtained, for the most part, under the controlled conditions usually considered necessary for physiological studies on excised nerves[1], and the procedure of

nerve dissection with subsequent recording of its action potentials had to be adapted to conform to the tight schedule and requirements of the personnel in the autopsy room. Nevertheless, it was considered important to determine whether such preparations would provide nerves which are suitable for the study of impulse conduction velocity, and whether dietary lead, in doses ranging from 10-500 ppm for 1½-2 years (rats, dogs), or 5000 ppm for 6 to 13 months (rats), would have a demonstrable effect on the impulse conduction velocity of these nerves.

METHODS

The Nerves (Tables I and II).

The nerves were obtained from 262 rats and 33 dogs: a sciatic nerve with its tibialis branch from each rat and a tibialis nerve from each dog. Two hundred and twenty-four of the rats had received 0 to 500 ppm of lead, by weight, in their diets for 18 or 24 months. At sacrifice, these rats were chloroformed. The other 38 rats had received 0 or 5000 ppm lead in their diets for 6 or 13 months. At sacrifice, these rats were decapitated. The dogs had 0 to 500 ppm lead in their diets for 24 months; at sacrifice they were electrocuted.

The left hind leg of the animal was obtained as soon as possible after death, and the nerve removed and mounted on electrodes in a warm and moist shelter. Due to factors beyond our control, the interval between death and mounting of the nerve was undesirably long and variable, especially for the nerves from the chloroformed rats (for these rats: average: 28 minutes, range: 19 to 47 minutes). During the dissection, the nerve was

protected against drying by covering exposed portions with gauze soaked with a commercial lactated Ringer's solution (Cutter's, for injection) or by application of a silicone preparation (360 medical fluid, viscosity grade 50 CSTKS, courtesy of the Medical Products Division, Dow-Corning Corp.). The nerve was then mounted on the electrode set so that its central end (sciatic nerve, rat) was in contact with the stimulating electrodes and its peripheral end (tibialis nerve, rat and dog), with the recording electrodes. As judged by the stabilization of the conduction velocities, rat nerves adjusted to the shelter environment in 5 minutes, and the dog nerves in 10 minutes.

The Nerve Shelter.

The 36 x 18-inch shelter had a framework of galvanized steel, a back wall of 1/4-inch Teflon® sheet (to provide support for air inlets), and side and front walls of 0.003-inch Mylar® sheeting. A sloped roof provided drainage for water condensing on its inner surface. Within, the shelter was divided into two sections: one with a permanent opening in front for mounting the nerves on the electrodes, the "outer" chamber, the other "inner" chamber for sheltering the nerve while recording its action potentials. Each chamber received an ample, and adjustable, supply of warm moist air from a constant temperature room. When the temperature within this room was maintained at 43.06°C (109.5°F) dry bulb and 40.28°C (104.5°F) wet bulb, R.H. 87%, the dry bulb temperature a few inches (centimeters) above the nerve in the "inner" chamber was 37.06° ± 0.04°C (98.7° ± 0.7°F).

The Electrodes.

The two electrode sets, a small one for rat nerves and a large one for dog nerves, were of similar construction. Each set consisted of a holder made from 1/4-inch Teflon® sheet which supported a horizontal row of equally spaced and parallel platinum wires, Figure . The small set, which had the wires (electrodes) spaced at 5 mm intervals, provided a maximum conduction distance of 4 centimeters. The large set, with electrodes spaced at 10 mm intervals ("dummy" electrodes in between gave added support to the nerve and minimized sagging), provided a maximum conduction distance of 18.5 centimeters. Actual conduction distances were regularly checked with a vernier caliper. Placed forward in the shelter, the back of the holder protected the nerve from direct contact with the flow of warm air entering through the back wall.

Stimulating and Recording Equipment.

A Grass® S4G square wave stimulator and SIU 478 A isolation unit were used for stimulation of the nerves from rats sacrificed after 6 and 18 months of lead feeding (Table I). These units were later replaced by a Tektronix® unit which consisted of a pair of Type 161 pulse generators, each driven by a Type 162 wave-form generator. An Argonaut® LIT 069 isolation transformer was used with this Tektronix® unit.

Tektronix® equipment was used for recording the action potentials of all nerves. The potentials were amplified by a differential amplifier, Type 2A63 or Type 3A3 Dual Trace, and fed into a Type 561A cathode ray oscilloscope. Sweep speed was controlled by a Type 2B67 time base which

provides a range of sweep speeds from 1 microsecond to 5 seconds per screen division. The action potentials were photographed with a Tektronix® C12 camera unit, using Polaroid® Type 410 black and white land film. Measurements were made directly from the photographs.

Measurement of Impulse Conduction Velocity.

The measurements used by Eliasson[1] were adopted: 1) the time interval between the shock artifact and the beginning of the upstroke of the compound action potential from which the "inflection" velocity can be calculated, i.e. the velocity of the impulses along the fastest conducting filters in the tibialis nerve, and 2) the time interval between the shock artifact and the peak elevation of the action potential, for calculation of the "peak" velocity, i.e. the velocity of the largest group of similar fibers.

RESULTS

Although procedures varied from one sacrifice period to another, Tables I and II, all preparations of a given sacrifice period were treated as nearly alike as possible. Comparisons between treatment groups of the same sacrifice period are valid, but differences in velocity observed for nerves of different sacrifice periods may reflect procedural changes.

Method of Sacrifice.

1. Decapitation. Following decapitation, the rat legs were obtained from the autopsy room and the excised nerves mounted in the "inner" chamber of the shelter within 14 $\pm$ 2 minutes (average $\pm$ 1 standard deviation). These nerves were, on the whole, the best preparations obtained. The inflection velocities of the nerves from the control rats (Group I) averaged

70 to 73 M/sec., which values overlap those of 71 and 74 M/sec. reported by Eliasson for nerves from normal and starved decapitated rats[1]. In contrast, peak velocities averaged 44 to 45 M/sec., which values are distinctly lower than the values of 53 and 55 M/sec. reported by Eliasson[1].

2. Chloroform. The nerves obtained from the chloroformed rats were less satisfactory than those obtained from the decapitated animals. The inflection velocities of the nerves from the controls (Groups I and IA) averaged 76 to 79 M/sec., and peak velocities, 43 to 46 M/sec. Although these values compare favorably with those of the nerves from the decapitated rats, the larger standard deviations (S.D.) indicate larger within group variances in velocity. The distinctly reduced velocities of the nerves obtained during the 24-month sacrifice may reflect the debilitated condition of these very old and moribund rats.

3. Electrocution. Unfortunately, technical difficulties with the stimulator (later resolved) prevented the measurement of the conduction velocities of the nerves from the male control dogs (Group I). However, the average values (all treatment groups) of 88 to 95 M/sec. for inflection velocity, and of 51 to 53 M/sec. for peak velocity, with standard deviations only slightly larger than those for the nerves from the decapitated rats, are indicative of acceptable preparations.

Procedural Variations.

1. Stimulator. As expected, replacement of the Grass® Stimulator by the Tektronix® unit did not alter conduction velocities as demonstrated by the data obtained on the nerves of the decapitated rats during the two sacrifice periods, Table I.

2. Silicone preparation versus Ringer's solution. The conduction velocities of the nerves protected by the silicone preparation tend to be lower than those of the nerves protected by the Ringer's solution. When substituted for seawater as the immersion fluid, mineral oil has been demonstrated to sharply reduce the impulse conduction along a single nerve fiber from a crab. It is not unlikely that the silicone preparation substituting for Ringer's solution, may have had a similar effect on the many fibers contained in a nerve trunk such as the tibialis nerve. Although Krebs-Ringer's solution is to be preferred for experimental work on nerves, the commercial Ringer's solution used during the present experiments appeared to be satisfactory for the relatively short intervals (15 to 20 minutes) available for dissecting each nerve and recording its action potentials.

Effect of Age on the Rat Preparations.

Characteristically, large and obliterating masses of perineural adipose tissue had to be removed before the nerves could be located in the legs from the chloroformed rats in the 18 and 24 month sacrifices. This additional manipulation added to the danger of injury to the nerve and so, at least in part, may account for the relatively large within group variances in conduction velocity. The distinctly reduced conduction velocities of the nerves obtained during the 24-month sacrifice is probably a phenomenon of aging, for in humans, a decrease in conduction velocity with increasing age has been reported .

Effect of Lead on Impulse Conduction Velocity.

Comparison of the conduction velocities of the nerves from the lead-treated animals with those of the nerves from their respective controls does not reveal a decrease in velocity which may be attributed to the lead. The conclusion is confirmed by the results of analysis of variance performed on the data obtained during each sacrifice. With the exception of a significant "treatment" effect on the inflection velocities of the nerves from the male dogs, differences between treatment groups of the same sacrifice period are insignificant, $p > 0.05$. Without control data for the male dogs, the "treatment" effect on inflection velocity is difficult to interpret. Application of the principle of "least significant difference" to the data demonstrated that the inflection velocities of the nerves from the males in Groups III, IV, and V do not differ significantly. In contrast, the inflection velocities of the Group II nerves are significantly lower than those of Groups III and IV, but not, however, than those of Group V. These differences in velocity cannot be attributed to differences in shelter temperature, or to differences in the length of time between electrocution of the dog and the mounting of his nerve within the "inner" chamber of the shelter.

TABLE I

IMPULSE CONDUCTION VELOCITY: RATS

Group	Lead Added to Diet ppm	N	Average Conduction Velocity + Standard Deviation			
			Inflection velocity: M/Sec.		Peak Velocity: M/Sec.	
			Males	Females	Males	Females
Killed by Decapitation						
After 6 months treatment. Silicone preparation. Stimulator: Grass® S4G						
I	0	9	70±5.1	None	44±1.5	None
II	5000	10	69±3.2	None	44±1.8	None
After 13 months treatment. Silicone preparation. Stimulator: Tektronix® unit						
I	0	9	73±4.8	None	45±2.3	None
II	5000	10	70±5.0	None	44±3.2	None
Killed by Chloroform						
After 18 months treatment. Ringer's solution. Stimulator: Grass® S4G						
I	0	6M 6F	79±7.3	78± 6.8	46±3.6	45±2.1
IA	0	6M 6F	76±7.5	77± 4.3	43±1.1	44±2.7
II	10	6M 6F	71±9.8	71±14.0	43±6.4	44±3.9
III	50	6M 6F	80±4.7	74± 8.4	41±6.0	44±3.4
IV	100	6M 6F	81±9.7	76± 7.4	47±3.8	44±1.8
V	500	6M 6F	77±7.6	78± 5.5	42±1.7	44±2.6
After 24 months treatment. Silicone preparation. Stimulator: Tektronix® unit						
I	0	12M 16F	65±13.7	65± 5.3	37±4.9	38±4.1
IA	0	13M 12F	67± 5.8	63± 6.0	38±5.6	36±5.5
II	10	13M 16F	65± 7.3	64± 4.3	38±3.6	38±3.1
III	50	12M 16F	65± 4.6	64± 4.8	36±4.4	39±5.1
IV	100	13M†17F	64± 3.9	64± 9.0	37±4.8	37±4.2
V	500	0M*12F	None	63± 4.9	None	37±4.1

†inflection velocity could not be measured in one photograph

*Group V males sacrificed earlier

TABLE II

IMPULSE CONDUCTION VELOCITY: DOGS

Group	Lead Added to Diet ppm	N	Average Conduction Velocity ± Standard Deviation	
			Inflection: M/Sec. Both Sexes*	Peak: M/Sec. Both Sexes*
After 24 months treatment. Ringer's Solution. Stimulator: Tektronix® unit				
I	0	0M† 4F	91 ± 2.9	53 ± 1.3
II	10	4M 4F	88 ± 5.0	51 ± 2.8
III	50	4M 4F	94 ± 5.5	52 ± 2.7
IV	100	4M 4F	95 ± 6.0	53 ± 2.3
V	500	3M‡ 2F‡	92 ± 1.1	51 ± 2.2

* Variance analysis: differences between sexes insignificant, $p > 0.05$.

† Technical problems prevented measurement of conduction velocities of the nerves from the four males.

‡ 1 male and 1 female sacrificed after 12 months treatment; 1 female sacrificed for reasons not related to test.