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Comparison Between Electrophysiologic and Morphologic Changes in Lead Induced Peripheral Neuropathy in Rats

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Compound nerve action potential (CNAP) of the mixed peripheral nerve is composed of A $\alpha\beta$, A δ , and C potentials. All components of CNAPs in the sciatic nerve were recorded by stimulating the tibial nerve of both control and lead-poisoned rats. Marked decrease of nerve conduction velocity and prolonged duration were found in A $\alpha\beta$ and A δ fibers especially in large myelinated A $\alpha\beta$ fibers. The amplitude decreased in A $\alpha\beta$ potential, but the area did not change. In C potential produced by activation of unmyelinated fibers, nerve conduction velocity slightly decreased, but the amplitude and area did not significantly change. Pathologic correlates revealed prominent segmental demyelination with significant decrease of large myelinated fiber densities. Minimal axonal degeneration of unmyelinated fibers was present. We can conclude that electrophysiologic changes in the lead-poisoned rats correlate with pathologic changes in them.

Key Words: Compound nerve action potential, lead, segmental demyelination

INTRODUCTION

Lead is well known to be a heavy metal which with chronic exposure induces the demyelination of the peripheral nerve in rats (Lampert and Schochet, 1986; Dyck et al., 1977; Ohnishi et al., 1977). But the presence of axonal change is still controversial. In lead induced peripheral neuropathy, some studies have found that compound muscle action potential or the A $\alpha\beta$ component of CNAP correlates with the diameter frequency distribution of myelinated nerve fibers (Fullerton, 1966; Ohnishi et al., 1977). But there have been no studies in which A $\alpha\beta$, A δ and C potentials have been studied separately and correlated with morphometry. Therefore, the aim of this study is to evaluate the changes of all components of the CNAP in peripheral neuropathy induced by lead poisoning and to study the correlation with the pathologic changes found.

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MATERIALS AND METHODS

Sprague-Dawley rats with an initial weight of approximately 250g were divided into control and lead groups. Rats of lead group were fed 50% solution of lead acetate, 0.5-1gm/Kg, every other day for 3 months. Control rats received a matched amount of an identical diet without the lead acetate.

Electrophysiologic study: CNAP was studied in 24 sciatic nerves of 14 control rats and in 28 sciatic nerves of 14 lead rats. All rats in both groups were anesthesized with intraperitoneal ketamin hydrochloride (1-2mg/Kg). After surgical exposure of the sciatic and tibial nerve of the same side, liquid paraffin of 35°C filled the pool that was made with the overlying skin of the exposed nerve for protecting the nerve and maintaining the temperature of the nervous tissue. The arrangement of stimulating and recording electrodes is illustrated in Fig. 1. The tibial nerve was stimulated supramaximally with a square pulse of 0.1 msec du-

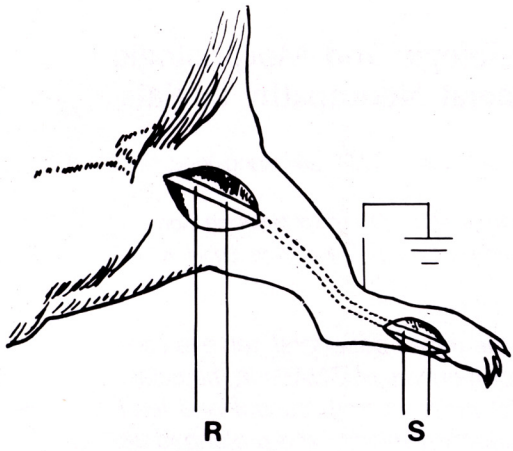

Fig. 1. Schematic diagram of arrangement of recording (R) and stimulating (S) electrodes.

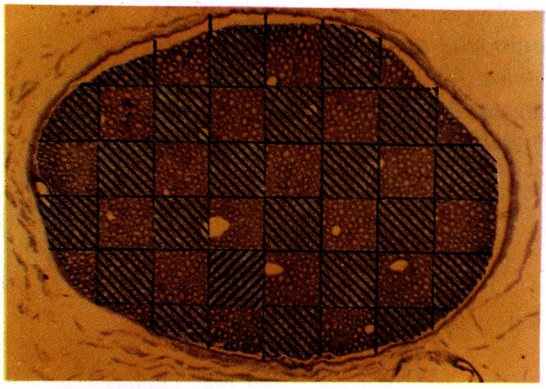

Fig. 2. Schematic view of transeverse sections of tibial nerve. Areas of the hatched line were photographed.

ration, 1mA intensity for A $\alpha\beta$, 0.1 msec duration, 5mA for A δ , and 0.5 msec duration, 10mA for C potential using stainless steel electrodes. Repetition rate was 1/sec for A $\alpha\beta$ and A δ , and 1/4 sec for C potential. The CNAP was recorded biphasically using stainless steel needle electrodes with a hooked tip and amplified and recorded by DISA 1500 EMG equipment. The parameters used in the measurement were onset and peak latencies, peak to peak amplitude, and duration of each component of the potential. Area of the potential was also measured with YAP 1.0 computer programming. Stimulating and recording points were marked and the distance between these two sites was measured during pathologic study by extraction of the nerve.

Pathologic study: This study was performed on 5 tibial nerves of 5 control rats and 12 tibial nerves of 10 lead rats. Soon after the electrophysiologic studies, about 1.5cm lengths of the tibial nerve were obtained following perfusion fixation with 3% formaldehyde and 1% glutaraldehyde mixture in phosphate buffer of pH 7.4. Portion of the nerve was stained with 2% osmium tetroxide and processed for embedding into epoxy and for teased fiber preparations. Transverse epoxy sections (1.0μ in thickness) from tibial nerves were examined with a light microscopy and utilized for morphometry. These were derived on photographic enlargements (×2,000). Photographs were taken as seen in Fig. 2. The diameter of the nerve fiber, and of the axon and myelin thickness were calculated by using YAP 4.0 computer programming.

Thin transverse sections of these nerves were cut and examined with an electron microscope. Another portion of the nerve was fixed and stained with Dalton's solution composed of 5ml of 4% potassium dichromate, 5ml of 3.4% sodium chloride, and 10ml of 2% osmium tetroxide for 24 hours, dehydrated with glycerin, and then teased apart under a dissecting microscope at a magnification of ×10 and ×100.

RESULTS

Electrophysiologic findings

Typical evoked potentials in control and lead rats are shown in Fig. 3. The statistical value of electrophysiologic data for all control and lead rats tested are shown in Table 1. The conduction velocities significantly decreased in lead nerves as compared to control in all components of CNAP ($p < 0.01$), being most prominent in A $\alpha\beta$ potential, especially in initial latency. Duration also prolonged in all components ($p < 0.01$) and dispersion due to pathologic and unequal slowing of conduction in different nerve fibers could sometimes be seen, especially in A $\alpha\beta$ potential. The amplitudes of A $\alpha\beta$ decreased ($p < 0.01$) but did not change in A δ and C potential ($p > 0.05$), whereas the areas of the A δ and C potentials were not significantly different between two groups ($p > 0.05$).

Pathologic findings

Teased fiber studies of most of the poisoned animals showed demyelination of peripheral nerves as shown in Fig. 4A. Recovery occurred with remyelination of part of an internodal segment and an example of this is shown in Fig. 4B. Short segments of thin, presumably restored myelin sheaths were seen between normally myelinated segments.

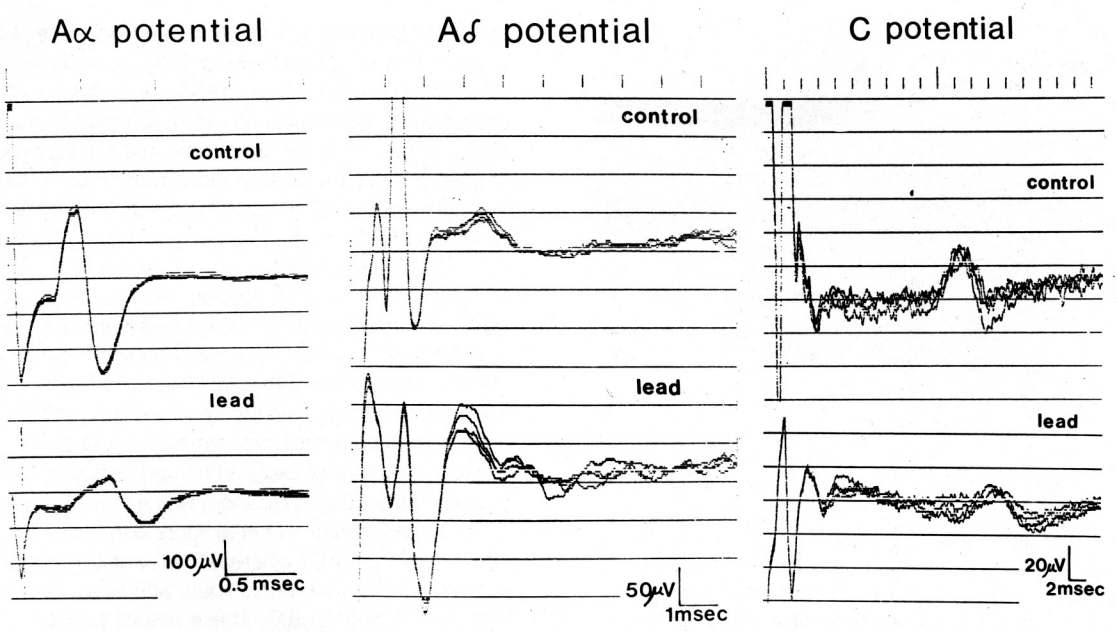

Fig. 3. Compound nerve action potentials in vivo from a control (upper) and lead (lower) rats produced by supramaximal nerve stimulation at distal tibial nerve and recorded in the sciatic nerve.

Table 1. Quantitative and statistical evaluation of compound nerve action potential recorded on the sciatic nerve to superamaximal stimulation of tibial nerve in control and lead-poisoned rats.

		A			A			C		
		Control (N=24)	Lead (N=28)	p value	Control (N=24)	Lead (N=26)	p value	Control (N=24)	Lead (N=28)	p value
NCV, start (m/sec)	Mean	67.2	44.4	p 0.01*	14.7	10.2	p 0.01*	40	30	p 0.01*
	SD	8.1	4.4	(t=12.3)	3.5	2.2	(t=5.3)	0.5	0.5	(t=6.7)
NCV, peak (m/sec)	Mean	34.4	23.9	p 0.01*	12.1	8.5	p 0.01*	3.4	2.5	p 0.01*
	SD	3.6	3.5	(t=10.6)	3.6	1.8	(t=4.5)	0.3	0.4	(t=8.4)
Duration (m/sec)	Median	1.5	2.2	p 0.01**	1.5	1.9	p 0.01**	3.7	4.2	p 0.01**
	Range	0.9-2.0	1.4-3.7		1.0-3.5	1.1-3.9		2.5-4.4	2.3-5.8	
Amplitude (μV)	Median	560	265	p 0.01**	44	40	p 0.05**	41	40	p 0.05**
	Range	200-820	70-660		12-140	10-150		20-120	18-94	
Area	Median	372	308	p 0.05**				113	79	p 0.05**
	Range	103-702	69-903					54-483	41-338	

Electronmicroscopic studies were performed to confirm axonal change in lead induced peripheral neuropathy. In myelinated fibers of lead rats demyelination (splitting of myelin sheath and loosening of individual myelin lamellae) was found, whereas there was no significant axonal change (Fig. 5A). But in unmyelinated fibers axonal degeneration such as decreased density of neurofibriles and mitochondri-

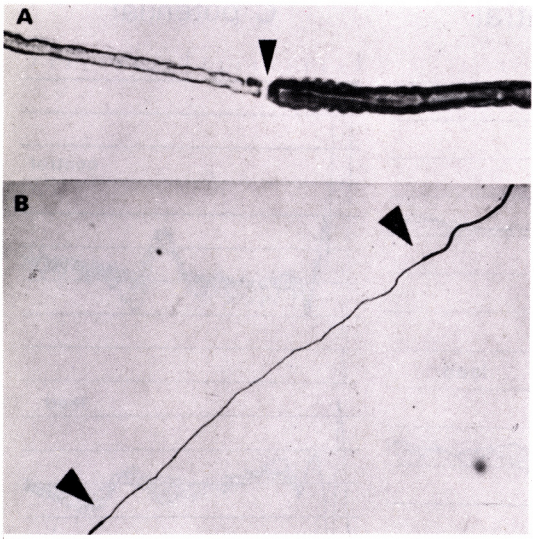

Fig. 4. Teased single fiber from a rat on the lead diet for 3 months. A) Demyelinated segment which connected to normal segment at higher magnification (×100). B) Remyelinated region at lower magnification (×10).

nerves, 78.1% of the total 4,990 nerve fibers in the photograph and in the lead group 7,936 nerve fibers of 12 tibial nerves, 56.8% of 13,898 nerve fibers in the photograph, were measured in morphometric study. Morphometry of myelinated fibers in the tibial nerve of poisoned rats revealed reduced proportion of large diameter fibers and increased proportion of small diameter fibers. In control nerves, the distribution was bimodal, but in lead poisoned nerves, unimodal (Fig. 7A). Changes in myelin thickness were prominent (Fig. 7B) and similar to those in the nerve fiber diameter, whereas there was no significant change in the axon diameter compared with control group (Fig. 7C). To evaluate the selectivity in the changes of myelin thickness, myelin thickness histograms were obtained in different diameter of axon. In nerve fibers with axon diameter larger than 2.0μ, there was a marked reduction in the proportion of nerve fibers with thick myelin thickness (Fig. 8B, C), whereas there was no changes in myelin thickness in nerve fibers with axon diameter less than 1.0μ (Fig. 8A). These results provide evidence that large myelinated fibers are more severely

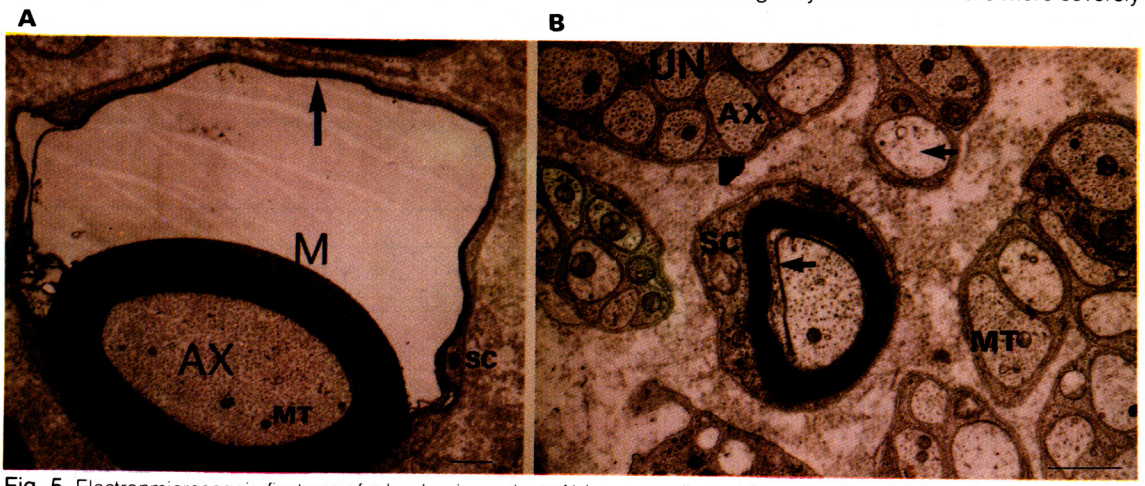

Fig. 5. Electronmicroscopic findings of a lead-poisoned rat. A) Large myelinated fiber showing disintegrating myelin sheath (M) with normally appeared axon (AX) (×8,500). B) Unmyelinated axons (AX) showing the reduction of neurofibriles and swelling of mitochondria (MT) in some of the unmyelinated fibers (UN) (arrows) (×15,000).

al swelling was occasionally found (Fig. 5B).

In almost all rats of the lead group, the transverse section at higher magnification revealed similar findings as seen in Fig. 6. Endoneurial edema was remarkable, and many fibers with abnormally thin myelin relative to the diameter of their axis cylinder were found. Many of the nerve fibers were distorted and irregular and macrophages were occasionally seen in lead poisoned nerves. These irregular shaped nerve fibers were excluded when measuring the diameter. In the control 3,898 nerve fibers of 5 tibial

affectad by lead than those of a small diameter. For further evaluation of the relationship between axon diameter and myelin thickness, regression lines were obtained (Fig. 9). With an increase in axon diameter, the increase in myelin thickness was less prominent in lead nerves than in control.

DISCUSSION

Systematic examination of the CNAP of various nerves of different composition reveals that nerve fibers

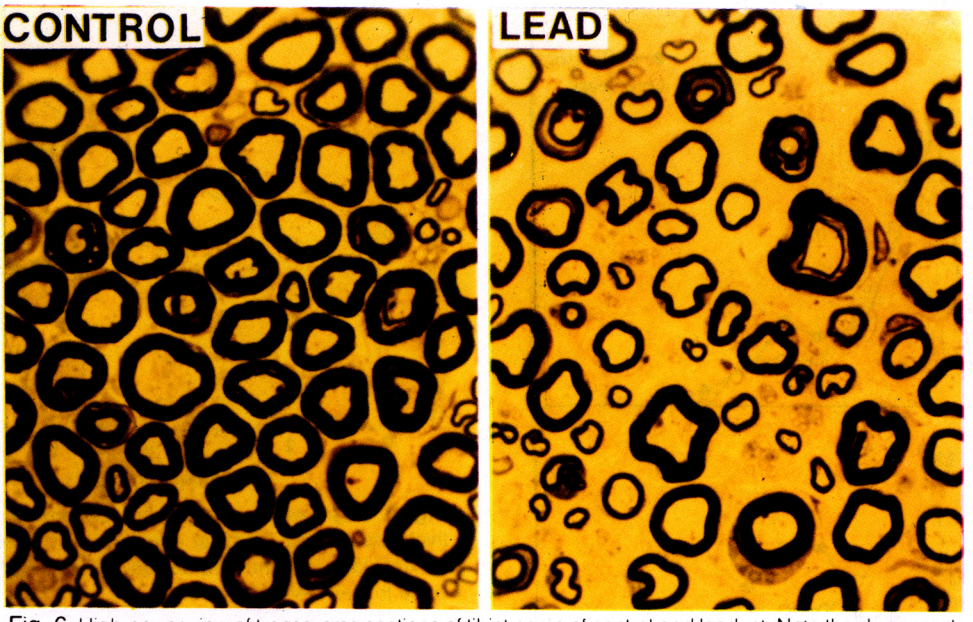

Fig. 6. High power view of transeverse sections of tibial nerve of control and lead rat. Note the decreased density of myelinated fibers in lead nerve. In addition thinnly myelinated fibers relative to the diameter of their axis cylinder are seen (×1,000).

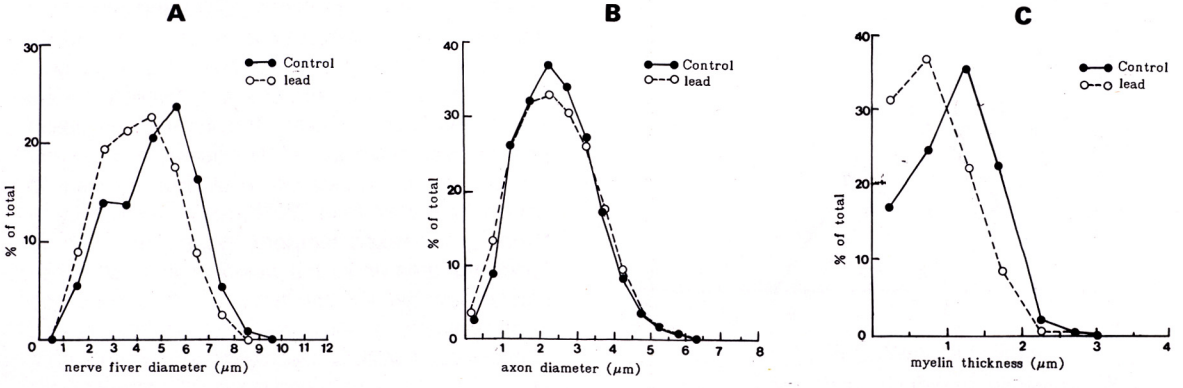

Fig. 7. Myelinated fiber histograms of tibial nerve in control and lead-poisoned rats. The fregnency distribution of nerve fiber diameter (A), myelin thickness (B) and axon diameter (C).

can be classified into various distinctive types (Boyd and Davey, 1986; Li and Bak, 1976; Patton, 1965). Tibial nerve, used in the present study, contains both afferent and, efferent A fibers and C fibers. Therefore CNAP recorded in the sciatic nerve with stimulation of the tibial nerve is composed of A α, A β, A δ and C potentials. In the present study A α and A β merged as A $\alpha\beta$ because of the short distance from the stimulation to the recording electrode. In this work, conduction velocity of A $\alpha\beta$ and A δ potential of control belong to range studied before (Li and Bak, 1976; Patton, 1965). However, the average conduction velocity at the beginning of the C component was 4.0m/sec. This was faster than the previously reported measurement of 2.0m/sec (Gasser, 1950). This discrepancy can be accounted by several reasons. One is technical differences. But the technical differences were the reason, other potentials would also have been influenced and their normal data would also be different from that reported previously. So technical differences seem the most insignificant reason. Another possible cause is the difference of species

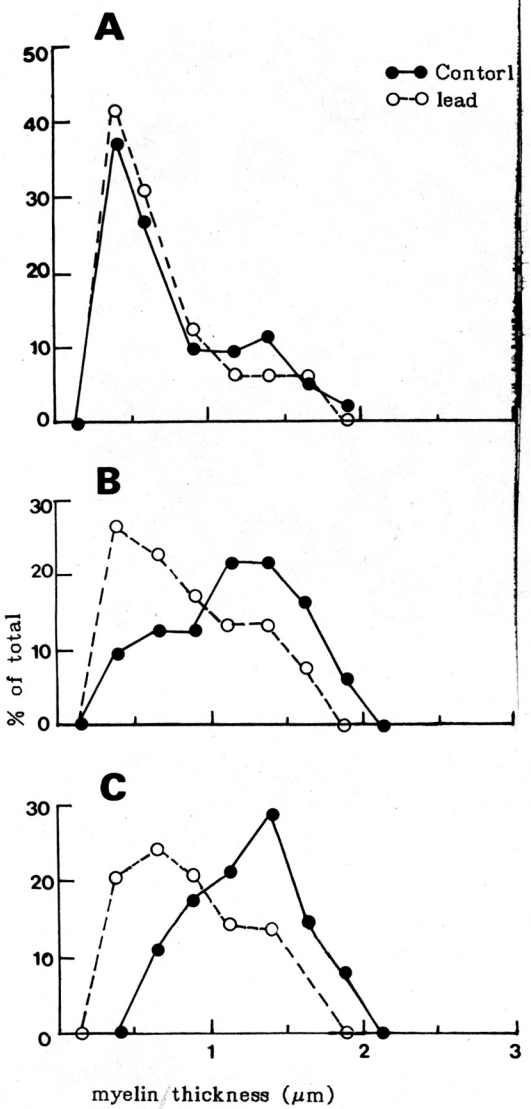

Fig. 8. The frequency distribution of myelin thickness of myelinated nerve fibers with different diameter of axon (A: less than 1μ, B:2.0-2.9μ, C: above 4.0μ). Note obvious reduction of myelin thickness in the nerve fibers with axon diameter above 2μ, whereas no overt change in the nerve fibers with axon diameter less than 1μ.

examined. It is also possible that the potential evoked by the very thinly myelinated fibers mixed with the C potential in respect that very thinly myelinated fibers with conduction velocity less than 12m/sec are similar to C fibers in electrophysiologic characteristics (Painthal, 1967).

Study of muscle and nerve action potentials evoked by supramaximal stimulation of nerve has been clini-

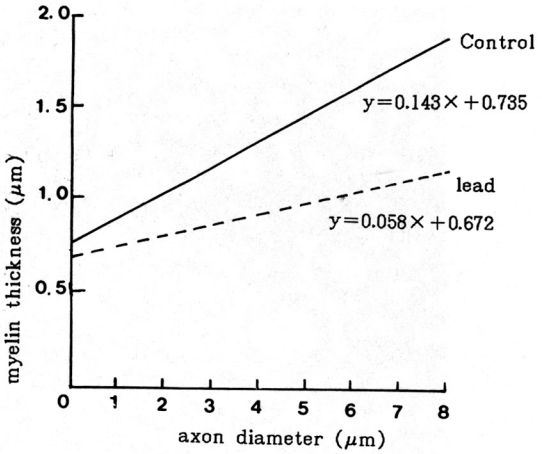

Fig. 9. Regression analysis of the relationship of myelin thickness on axon diameter of myelinated nerve fibers in control (solid line) and lead nerves (dotted line). Note less slope in lead nerves.

cally used for diagnosis and differentiation between demyelination and axonal degeneration. Nerve conduction velocity is closely related to the presence of abscence of myelin, myelin thickness and nerve fiber diameter. Nerve conduction is much faster in myelinated fibers than unmyelinated fibers. Nerve conduction velocity increases as increase of myelin thickness and nerve fiber diameter. Amplitude of an action potential is an expression of fiber density or fiber number per unit cross-sectional area. But in diseased nerves the waveform of CNAP may be sinificantly altered by increased temporal dispersion. In these cases, the area under the waveform is a better indicator of fiber density than the amplitude. Conduction in demyelinated axons is characterized by decrease of conduction velocity. Decreased amplitue by conduction failure or lengthened duration by temporal dispersion may also be found occasionally. With axonal degeneration amplitude of compound action potential is reduced due to loss of nerve fibers. And in cases with loss of the fast conducting fibers some slowing of conduction can also be seen. Conventional nerve conduction study provides information only about A α fibers. But preferential loss of A δ and C fibers may occur in nerves from some kinds of peripheral neuropathy. Dyck et al. (1971) compared changes in all components of CNAP in vitro with changes in the histogram of nerve fibers in patients with various peripheral neuropathies. But there was no statistical evaluation in that study because the number of cases was small and strict comparison was impossible be-

cause the subjects were human. In our work, strict control study was observed and a statistical analysis was made. In addition, for a more detailed comparison of electrophysiologic and pathologic changes, duration and area of the action potentials was measured and a histogram of myelin thicknss and axon diameter was made. In order to make inferences about which population of nerve fibers was affected, histogram of myelin thickness with different diameter of axon was also obtained.

Since the time of Gombault's study (1880) of lead neuropathy in guinea-pig the predominant type of pathology was thought to be a segmental demyelination. But axonal degeneration has been reported in guinea pigs (Fulleron, 1966), rabbits (Shimazono, 1914) and cats (Ferraro, 1932). In considering why different types of fiber degeneration have been found in different studies, several possible explanations includes: differences in severity of the neuropathy, differences in species and age of experimental animals, and differences in sensitivity of methods for detection of fiber pathology. In adult rats chronically intoxicated with lead for more than 3 months as in our study (Dyck et al., 1977; Lampert and Schochet, 1968; Ohnishi et al., 1977; Winderbank et al., 1980) only pure segmental demyelination was reported. But in the present study, although segmental demyelination was prominent, minor axonal degeneration was also found in electronmicroscopic study, which also reflected on the electrophysiologic study. In many experimental studies using rats, electronmicroscopic examination was not performed except in the study by Ohnishi et al. (1977) who did not find axonal degeneration.

There are few studies of he pathophysiologic correlation in lead induced peripheral neuropathy. Fullerton (1966) tested compound muscle action potentials and Ohnishi et al. (1977) tested A α potential of CNAP. Both found decreased nerve conduction velocity compatible with demyelination. There has been no study to evaluate the changes of A δ and C potential in correlation with morphometric changes as we have done. Morphometric results in the present work have shown a decrease in myelin thickness without overt change in axon diameter in lead group compared with control. This means that decreased nerve fiber diameter is due to demyelination, which was also confirmed by teased fiber study and electronmicroscopic study. This pathologic change correlates nerve conduction study and shows that in demyelinative polyneuropathy nerve conduction velocity decreases by more than 30% (Buchthal et al., 1975). The results shown in histogram of myelin thickness in different diameter of axon and correlation between axon diameter and myelin thickness showed peripheral nerve damage induced by lead mainly in large myelinated fibers. These results also corresponded well to electrophysiologic study because there was a marked slowing of nerve conduction in A $\alpha\beta$ potential, but lesser in A δ and C ptentials in that order. These results suggest clinical usability of nerve conduction study in lead induced peripheral neuropathy. In A $\alpha\beta$ potential ampliude decreased and duration lengthened but the area did not change. These findings correlated well with pathological findings that in large myelinated fibers, axonal degeneration was not found even though demyelination was severe. Whereas in C potential nerve conduction velocity decreased, duration lengthened and area slightly decreased but without statistical significance. These results can be explained as minor axonal degeneration of unmyelinated fibers observed in electronmicroscopic study in spite of no overt change in histogram of axon diameter.

In summation, severe demyelination with mild axonal degeneration in peripheral neuropathy of lead poisoned rats was confirmed electrophysiologically and pathologically. We are able to assure that electrophysiologic changes closely correlate with pathologic changes of peripheral nerves. The CNAP study is a good tool to assess the degree of nerve damage and possibly the underlying pathomechanisms.

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