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Effects of Lead Acetate on Guinea Pig—Cochear Microphonics, Action Potential, and Motor Nerve Conduction Velocity

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Segmental demyelination and axonal degeneration of motor nerves induced by lead exposure is well known in man (Ursan and Suciu 1965; Catton et al. 1970) and animals (Fullerton 1965; Knecht et al. 1979; Ohnishi and Dyck 1981). The effect of lead acetate exposure to man may involve the cranial nerves, since vertigo and sensory neuronal deafness have been reported among lead workers (Cirlo and Ottoboni 1956; Goździk-Żołnierkiewicz and Moszynski 1969). However, there are only few report concerning the dose-effects of lead acetate both to the peripheral nerve and the cranial VIII nerve with measurement of blood lead concentration. We investigated that the effects of lead acetate to the cochlea and the VIII nerve using CM (cochlear microphonics) and AP (action potential) of the guinea pigs. The effects of lead acetate to the sciatic nerve were measured by MCV of the sciatic nerve with measurement of blood lead concentration.

MATERIALS and METHODS

Sixty naive male albino Hartley guinea pigs, 9 weeks of age, were used. The room temperature was 23-27°C and maintained on 12-h light-dark cycle (LD rhythm, L; 7:00 A.M. to 7:00 P.M., D; 7:00 P.M. to 7:00 A.M.). Animals were fed a diet (CLEA Co, CG-3), ad. libitum. Animals were randomly assigned to 4 dose groups/week: 0 mg (control), 10 mg (Exp. 1), 15 mg (Exp. 2) and 20 mg lead acetate (Exp. 3). A 1.0 % water solution of lead acetate was administered by i.p. injection once a week for 5 consecutive weeks.

Sodium pentobarbital (0.4 ml/kg; 50 mg in 1 ml) was injected into the abdominal cavity of the guinea pigs. After sodium pentobarbital was given i.p., the nerve in the right hind limb were surgically exposed. Bipolar stimulating electrodes consisted of stainless steel.

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The proximal and distal stimulating electrode were attached to the sciatic nerve in the upper thigh and the posterior tibial nerve in the ankle, respectively. The potentials were amplified with a Nihon Kohden AVB-10 and displayed on a conventional X-Y recorder. The nerve length between the two sites of stimulation was directly measured by a thread from the exposed nerve. Maximum motor nerve conduction velocity was calculated by dividing the nerve distance by the difference in the two latency times.

Guinea pigs were then fixed face upward and succinylcholine chloride (0.5 ml/kg; 40 mg in 2 ml) was injected into the leg muscle. Measurement of Cochlear Microphonics (CM) followed the method of vestibulo-tympanal differential recording (Tasaki et al. 1952). Silver wires (30 μm in diameter) were introduced into the small holes (30-50 μm in diameter) using a micromanipulator. The CM response from a pair of these electrodes was introduced into a high-impedance amplifier and a synchroscope. An audiometer was used as the apparatus of sound stimulus to the guinea pigs. To prevent electrical induction, an iron cover was placed over the receiver of this audiometer and a vinyl tube was inserted into a small hole in the iron cover. Finally, the tip of the vinyl tube was introduced into the external acoustic meatus. The stimulus sound level (dB) was observed from the audiometer, and the intensity function of the CM was measured (test frequency: 4 kHz). An intensity of 0 dB (visual detection level: VDL) was used since this was the stimulus intensity level of 4 kHz which was obtained for CM potentials below 10-20 μV in the control guinea pigs on the CR-oscillator. This level was about 30 dB on the audiometer (pseudo-threshold).

The measurement of Action Potential (AP) was performed by the method of Tasaki et al. (1952) and Teas (1962). AP was induced from the electrode inserted into the small hole of the scala vestibuli, and an indifferent electrode made of silver wire was placed in the neck muscle of the guinea pig. Samples of blood were obtained from the animals after examination.

Analysis were performed by using a Varian AA 175 type flameless atomizer equipped with a D2 lamp background collector. A mixture of nitric acid and perchloric acid was used for the wet digestion of samples. The standard addition method was applied for the determination to eliminate matrix interferences.

RESULTS and DISCUSSION

In each experiment, no animals developed paralysis of

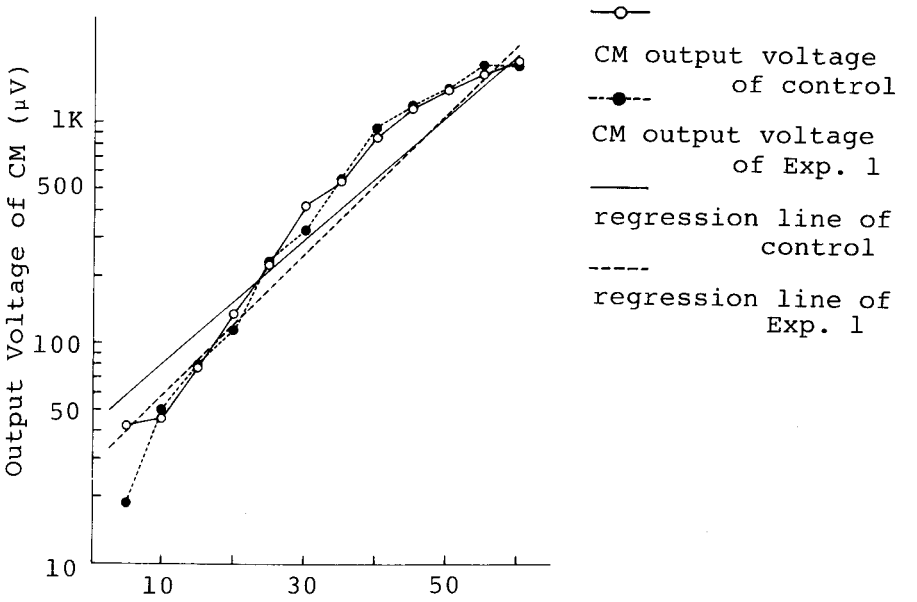

Fig. 1 Intensity Function of CM

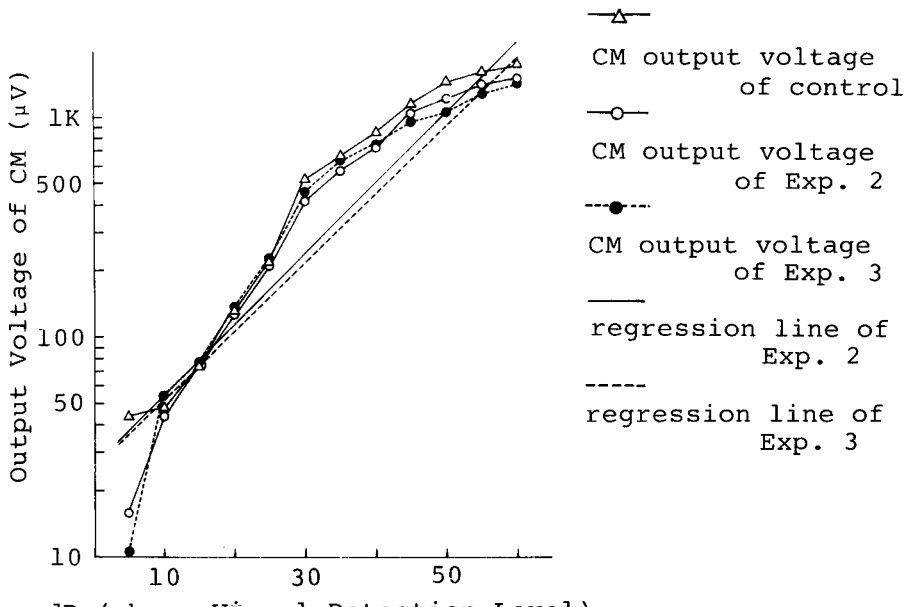

Fig. 2 Intensity Function of CM

the hind legs. The body weight of the guinea pigs in Exp. 1, 50 mg lead acetate (10 mg × 5), changed from 318 g to 405.6 g during the 5 weeks of lead injection. One guinea pig out of 10 died during the 5 weeks. The body weight of the guinea pigs of Exp. 2, 75 mg lead acetate (15 mg × 5), changed from 378.5 g to 502.3 g during the 5 weeks of injection. Five guinea pigs out of 20 died during the 5 weeks. The body weight of the guinea pigs of Exp. 3, 100 mg lead acetate (20 mg × 5), changed from 328.8 g to 425.4 g and 8 guinea pigs out of 20 died during the above injection period.

The results of MCV measurement were as follows:

Control: 39.8 ± 7.5 m/s (M ± SD, n=11)
Exp. 1 : 37.7 ± 9.9 m/s (M ± SD, n=9)
Exp. 2 : 37.4 ± 6.1 m/s (M ± SD, n=15)
Exp. 3 : 36.3 ± 6.6 m/s (M ± SD, n=11)

In the Pb exposed groups, each mean MCV tended to be smaller than that in the control and to decrease with increasing of Pb dose level. However, there was no statistically significant difference among the 4 groups ($F=0.433 < F(0.05)=2.812$). In all experiments (control and Exp. 1-3), regressions between the intensity of sound stimulus and the logarithm of CM output voltage were significantly different. Analysis of variance of regression coefficients were as follows.

Control: CM; $F=389.57 >> F(0.01)=6.89$
Exp. 1 : CM; $F=552.04 >> F(0.01)=7.11$
Exp. 2 : CM; $F=578.74 >> F(0.01)=6.97$
Exp. 3 : CM; $F=258.76 >> F(0.01)=6.93$

Intensity function was obtained in all experiment. However, no significant differences in CM output voltages by analysis of variance were observed (Fig. 1 and Fig. 2).

Examples of maximum AP wave form in Exp. 2 and Exp. 3 are shown in Fig. 3 (A and B), respectively. These photographs of AP were obtained at a sound stimulus of 60 dB above VDL. It was observed that the voltage of peak N_1 and N_2 in Exp. 2 were greater than those in Exp. 3. The mean N_1 values of AP to sound stimulus and regression in each experiment, respectively, are shown in Fig. 4 and Fig. 5. The mean N_1 of maximum output voltage of AP in Exp. 3 was almost the same as those obtained below 10 or 15 dB in controls, and Exp. 1,2. The photographs of AP were obtained at a sound stimulus of 60 dB above VDL. It was observed that the voltage of peak N_1 and N_2 in Exp. 2 were greater than those in Exp. 3. The results of the latency of N_1 were as follows. In control (n=9), it was 3.02 ± 0.067 ms. In Exp. 3 (n=7), that was 3.37 ± 0.07 ms. There were significant differences between the latency of N_1 in control and that of Exp. 3 ($P < 0.05$). However, no significant differences were obtained among the latency

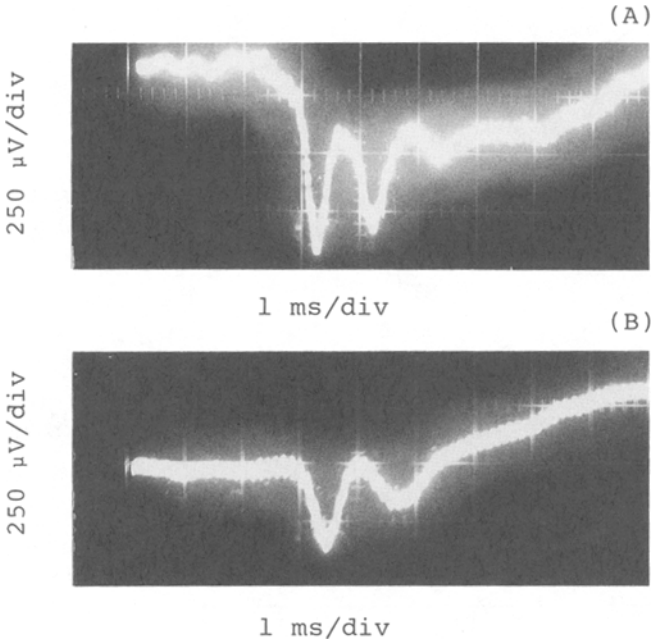

Fig. 3 (A) AP wave form 15 mg/wk, 5 wks to Exp. 2
(B) AP wave form 20 mg/wk, 5 wks to Exp. 3

of N_1 in Exp. 1-3.

The concentrations of blood lead were 4.9 ± 2.9 μg/100 ml, in controls (n=8), 123 ± 39 μg/100 ml, in Exp. 1 (n=8), 102 ± 30 μg/100 ml, in Exp. 2 (n=13) and 134 ± 52 μg/100 ml, in Exp. 3. Significant differences were observed among blood lead concentrations of control and those of Exp. 1,2 and 3 ($P < 0.001$). But, there were no significant differences among blood lead concentrations in Exp. 1,2 and 3. Goździk-Żołnierkiewicz (1969) injected a 1 % water solution of lead acetate into the abdominal cavity of guinea pigs and examined the inner ear with the VIII nerve histologicaly. Although the sensory cells of the inner ear appeared to be normal, the VIII nerve showed axonal degeneration and mild segmental demyelination. The preclinical changes such as vertigo and auditory defects in the central nervous system were also found by lead industrial workers (Ciurls and Ottoboni 1956).

It is reported that the sensory cells of the inner ear have a rather strong resistance to lead exposure (Goździk-Żołnierkiewicz and Moszyński 1969). In our experiment, lead acetate exposure did not induce changes in CM, in either pseudothreshold or maximum output voltages. Davis (1953) reported that the changes in CM (detection level and maximum output

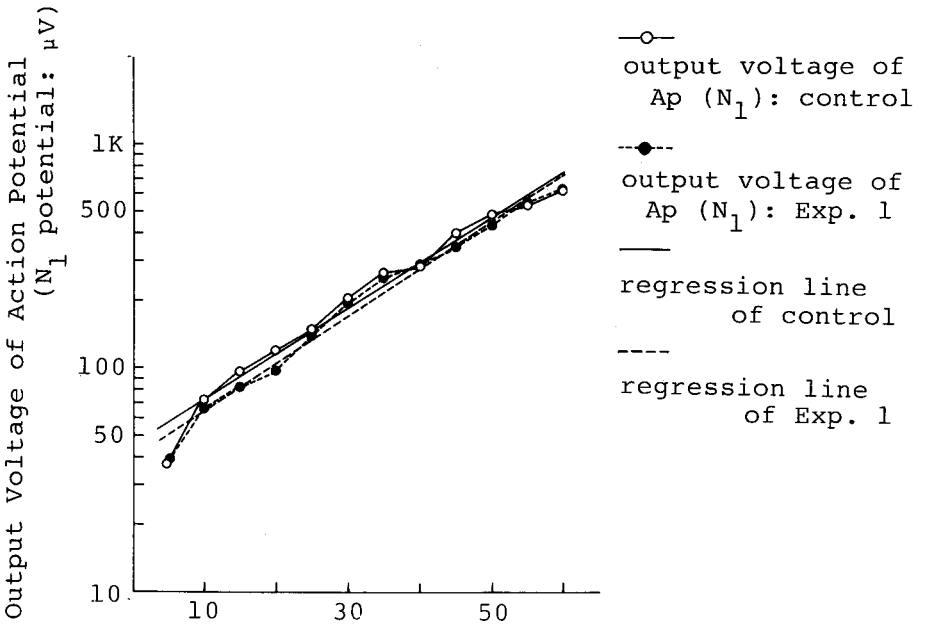

Fig. 4 Output Voltage of Ap (N_1 potential)

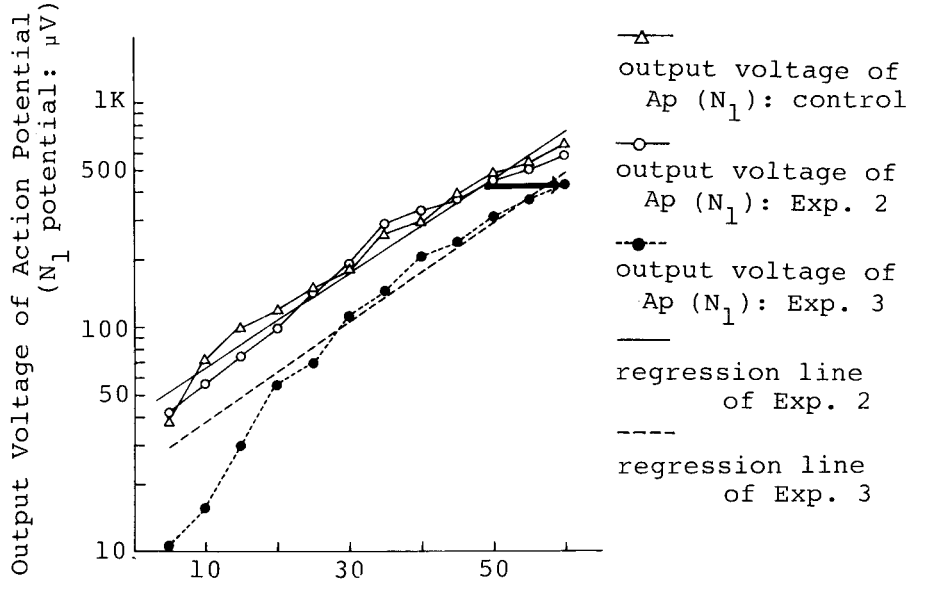

Fig. 5 Output Voltage of Ap (N_1 potential)

voltages) with a pathological change in the sensory cells was proportionate to CM of the normal inner ear obtained below 15-20 dB sound stimulus.

However, it was found that the input-output function of AP was changed as a result of the activity of the cranial VIII nerve. Any event which reduce either the number of fibers responding to the stimulus or the synchrony with which the nerve responds will decrease the amplitude of the action potential (Schmiedt 1984). In our experiments, the maximum output voltage of AP (N_1) in Exp. 3 was smaller than that under other experimental conditions. Above results may be considered to be caused by axonal impairment of the VIII nerve, since our experimental dosage of lead acetate (250 mg/kg) is similar to that reported by Goździk-Żołnierkiewicz.

The pathological findings of lead acetate exposure to guinea pigs studied by Goździk-Żołnierkiewicz were mainly axonal degeneration of the VIII nerve. A decrease in the maximum output voltage N_1 potential was also observed in our previous experiment (Yamamura et al. 1984).

Fullerton (1965) observed correlation between the extent of histological change and decrease of the conduction velocity of the sciatic nerve in chronic lead exposure by stomack tube. Daily exposure, from two days to two years, induced paralysis of the hind limbs and demyelination and axonal degeneration of the sciatic nerve. Segmental demyelination and axonal degeneration occurred in 60 % of the animals examined, segmental demyelination occurred only in 26 % and axonal degeneration in 14 %. Conduction velocity was markedly reduced only in those animals with segmental demyelination.

According to Windbank et al. (1980), 15 % of the established demyelination was observed in the peroneal nerve of the rats fed basic lead carbonate after 50 days or more days. In some species, the predominant change appears to in the central nervous system. However, scanty changes have been described in peripheral nerves (Ferraro and Hernandez 1932).

The reason why no significant difference of MCV was observed in our experiment, might be explained by the following results that segmental demyelination without axonal degeneration was more commonly seen in animals that had received lead acetate over a long period than those in animals treated with acute exposure of lead acetate. Fullerton (1965) observed that a dose of 1.0-0.5 g/kg lead acetate (50 % solution) administered 5

time a week by stomach tube during a few days to 2 years, induced markedly reduced MCV with the segmental demyelination. Otherwise, Goździk-Żołnierkiewicz (1969) reported that the total dose of 300 mg/kg of lead acetate administered one time in week during 7 weeks by peritoneal injection, induced pathological change of the VIII nerve. But, the guinea pigs of this experiment were observed to have mild paralysis of hind limbs.

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REFERENCES

Catton ME, Harrison MTG, Fullerton PM, Kazantzis G (1970) Subclinical neuropathy in lead workers. Br Med J 11: 80-82

Ciurol E, Ottoboni A (1956) Variations of the internal ear in chronic lead poisoning. Exc Med 9: 60

Davis H (1953) Acoustic trauma in the guinea pig. J Acoust Soc Am 59: 1180-1189

Ferraro A, Hernanadez R (1932) A histophathological study of the nervous system of cats and monkeys in the acute and subacute stages. Psychiat Quart 6: 319-350

Fullerton PM (1965) Chronic peripheral neuropathy produced by lead poisoning in guinea-pigs. J Neuropathol Exp Neurol 25: 214-236

Goździk-Żołnierkiewicz T, Moszyński B (1969) VIII nerve in experimental lead poisoning. Acta Otolaryng 68: 85-89

Knecht CD, Crabtree J, Katherman A (1979) Clinical, clinicopathologic and electroencephalographic features of lead poisoning in dogs. J Am Vet Med Assoc 175: 196-201

Mitchell C (1976) Susceptibility to auditory fatigue: comparison on changes in cochlear nerve potential in the guinea pig and chinchilla. J Acoust Soc Am 60: 418-422

Ohnishi A, Dyck PJ (1956) Retardation of Schwann cell division and axonal regrowth following nerve crush in experimental lead neuropathy. Ann Neurol 10: 469-477

Schmiedt RA (1984) Acoustic injury and the physiology of hearing. J Acoust Soc Am 76: 1293-1317

Tasaki I, Davis H, Legouix JP (1952) The space-time pattern of the cochlear microphonics (guinea pig) as recorded by differential electrodes. J Acoust Soc Am 24: 502-519

Teas DC, Eldredge DH, Davis H (1962) Cochlear responces to acoustic transients: an interpretation of whole nerve action potentials. J Acoust Soc Am 59: 143-147

Ursan U, Suciu I (1965) Notes on modern aspects of neurotoxicosis chronic inductrial lead poisoning. J

Hyg Epidemiol Microbiol Immunol IX: 409-420
Windebank AJ, McCall JT, Hunder HG, Dyck PJ (1980) The endoneuronal content of lead related to the onset and severity of segmental demyelination. J Neuropathol Exp Nerolo 39: 692-699
Yamamura K, Kishi R, Maehara N, Sadamoto T, Uchino E (1984) An experimental study of the effects of lead acetate on hearing. Toxcol Lett 21: 41-47

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