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ALTERATION OF SODIUM AND POTASSIUM MOBILIZATION AND OF ADRENAL FUNCTION BY LONG-TERM INGESTION OF LEAD

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Abstract—Serum sodium concentration was markedly decreased by long-term (12 weeks) ingestion of lead above 5 mg $Pb \cdot kg^{-1} \cdot day^{-1}$, whereas serum potassium concentration was notably decreased by the long-term (12 weeks) ingestion of lead above 2 mg $Pb \cdot kg^{-1} \cdot day^{-1}$. Urinary sodium and potassium in fasted rats were increased markedly 24 hr after a single lead dose (200 mg Pb/kg, o.p.) [Y. Suketa, S. Hasegawa and T. Yamamoto, *Toxic. appl. Pharmac.* **47**, 203 (1979)]. In contrast, urinary excretion of sodium or potassium in non-fasted rats was not changed significantly by 2 weeks of lead ingestion at 200 mg $Pb \cdot kg^{-1} \cdot day^{-1}$. Renal activities of Na^+,K^+-ATPase and K^+-dependent phosphatase were decreased to 50–70% of control values by long-term (12 weeks) ingestion of lead (above 5 mg $Pb \cdot kg^{-1} \cdot day^{-1}$).

Sandstead *et al.* [1] reported that humans who exhibited signs of lead intoxication after long-term ingestion of moonshine whiskey usually showed decreased renin activity, aldosterone secretion, and plasma sodium concentration. However, such changes could not be attributed solely to the toxic effect of lead or ethanol.

To understand the mechanism of the disorder of sodium balance produced by long-term ingestion of moonshine whiskey, we studied the alteration of sodium mobilization during long-term (12 weeks) ingestion of lead using rats as experimental animals. On the one hand, Na^+,K^+-ATPase has been recognized as the sodium pump [2]. On the other hand, the K^+-sensitive site of Na^+,K^+-ATPase has been shown to be the same as the K^+-sensitive site of K^+-dependent phosphatase [3]. Thus, renal K^+-dependent phosphatase activity was examined in association with changes in sodium and potassium mobilization in rats caused by long-term (12 weeks) ingestion of lead.

METHODS

Treatment. Male Wistar albino rats weighing 95–105 g were used in this study. The rats were fed basal diet MF (Oriental Yeast Co. Ltd., Tokyo, Japan) and were housed in an air-conditioned room at 22°. After being kept on the same basal diet and water *ad lib.* for 5 days, rats received lead acetate (0, 2, 5, 20, 50 or 200 mg $Pb \cdot kg^{-1} \cdot day^{-1}$) for 12 weeks, consecutively. The animals were housed two to a cage, and the urine was collected at 0, 2, 4, 6, 8, 10 or 12 weeks after the start of each lead administration. Water and food were provided *ad lib.* except during the times the urine was collected after lead administration was started. An effect of lead administration on body weight and on intake of water and food was not observed ($P > 0.05$).

At least eight animals were used for each group. Serum and tissues from animals were pooled individually and the assays for each variable were performed for each animal.

Preparation of microsomes. At the time of sacrifice, the animals were anesthetized with ethyl ether and killed by cardiac puncture. The kidneys were immediately placed in ice-cold 0.25 M sucrose. The renal microsomes were prepared in ice-cold 0.25 M sucrose according to the method of Jørgensen [4]. The heavy microsomal fraction was used in this experiment.

Enzyme assay. Renal microsomal K^+-dependent phosphatase activity was assayed according to the method of Forte *et al.* [5] applied by Suketa *et al.* [6]. The enzyme activity was measured at 37° in a final volume of 2 ml containing 30 mM Tris–HCl (pH 8.0), 3.7 mM $MgCl_2$, 25 mM KCl, and 3.7 mM *p*-nitrophenylphosphate. The reaction was terminated with 2 ml of 0.1 N NaOH, and the amount of *p*-nitrophenol liberated was measured by the increase in absorbance at 410 nm using a Beckman spectrophotometer. Renal microsomal Na^+,K^+-ATPase activity was assayed according to the method of Jørgensen [4].

Determination of metals. The concentrations of Na^+ and K^+ were determined in uring and serum by the method of Willis [7] using a Hitachi model 518 digital atomic absorption spectrophotometer. The concentrations of lead in microsomes and adrenal glands were estimated by the method of Gross and Parkinson [8] using a Perkin–Elmer model 403 atomic absorption spectrophotometer with flameless equipment.

Determination of aldosterone. Aldosterone concentration was measured using an Aldosterone

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Radioimmunoassay Kit of the CIS-Sorrin Ltd. Co. (Paris, France).

Materials. Aldosterone-5′-triphosphate (ATP), disodium salt, was obtained from the Teijin Ltd. Co. (Tokyo, Japan). *p*-Nitrophenylphosphate, disodium salt, and glycol ethylenediaminetetraacetic acid (EGTA) were from the Wako Pure Chemical Inc. Ltd. Co. (Osaka, Japan). Disodium ATP and disodium *p*-nitrophenylphosphate were converted to the Tris salts by passage through a chilled column of Dowex 50W-X8 cation-exchange resin in the Tris form.

Statistical analysis. Differences between control and experimental groups of animals were analyzed by Student's *t*-test.

RESULTS

Alterations in serum sodium and potassium following long-term ingestion of lead. The responses of serum sodium and potassium concentrations to long-term ingestion of lead were examined to determine the progression to chronic renal symptoms of lead poisoning. The serum sodium concentration was decreased significantly after ingestion of lead for 12 weeks at doses of, or above, 5 mg $Pb \cdot kg^{-1} \cdot day^{-1}$ (Fig. 1A). This lead dose (5 mg $Pb \cdot kg^{-1} \cdot day^{-1}$), however, caused only a slight increase (of 15%) in urinary sodium excretion, and the increase was not significantly different from the control ($P > 0.05$).

On the other hand, the serum potassium concentration was decreased markedly after ingestion of lead for 12 weeks at a dose at, or above, 2 mg $Pb \cdot kg^{-1} \cdot day^{-1}$ (Fig. 1B). The decreases of serum sodium and potassium concentrations after long-term ingestion of lead reached plateaus with lead doses above, or at, 20 mg $Pb \cdot kg^{-1} \cdot day^{-1}$ (Fig. 1A and B).

Elevation of urinary excretion of sodium and potassium after long-term ingestion of lead. It has been suggested that a decrease in serum sodium concentration after long-term ingestion of lead is due to an elevation of urinary sodium excretion [9].

Changes in urinary sodium excretion following long-term ingestion of various doses of lead (0, 2, 5, 20, 50 or 200 mg $Pb \cdot kg^{-1} \cdot day^{-1}$) are presented in Table 1. Urinary sodium excretion was elevated significantly after more than 4 weeks of lead ingestion at a dose of 200 mg $Pb \cdot kg^{-1} \cdot day^{-1}$ and by more than 6 weeks of lead ingestion at 50 mg $Pb \cdot kg^{-1} \cdot day^{-1}$ as shown in Table 1.

The decrease in serum potassium concentration after long-term ingestion of lead suggests the possibility of an association with an elevation in urinary potassium excretion, similar to the urinary sodium excretion shown in Table 1. The patterns of urinary potassium excretion following long-term ingestion of various doses of lead (0, 2, 5, 20, 50 or 200 mg $Pb \cdot kg^{-1} \cdot day^{-1}$) are shown in Table 2 to have been similar to the patterns of urinary sodium excretion.

These observations demonstrate that the decreases of serum sodium and potassium concentrations were associated with elevations of the urinary excretion of sodium and potassium after consecutive long-term ingestion of lead (shown in Fig. 1 and Tables 1 and 2).

Changes in renal K^+-dependent phosphatase and Na^+,K^+-ATPase activities after long-term ingestion of lead. The active transport of sodium and potassium ions in animals systems is known to be affected by a membrane-bound enzyme, the sodium- and potassium-activated adenosine triphosphatase (Na^+,K^+-ATPase). On the other hand, Robinson [10, 11] distinguishes between β-sites for K^+, at which K^+ activates the Na^+,K^+-ATPase and α-sites for K^+, at which K^+ activates the K^+-dependent phosphatase. The differences and similarities between the change in renal K^+-dependent phosphatase activity and that in Na^+,K^+-ATPase activity after long-term ingestion of lead were, therefore, of interest.

Renal activities of K^+-dependent phosphatase and of Na^+,K^+-ATPase decreased to 50–70% of control values after long-term (12 weeks) ingestion of lead at, or above, 5 mg $Pb \cdot kg^{-1} \cdot day^{-1}$, as shown in Fig. 2. The response, at each lead dose, of renal K^+-

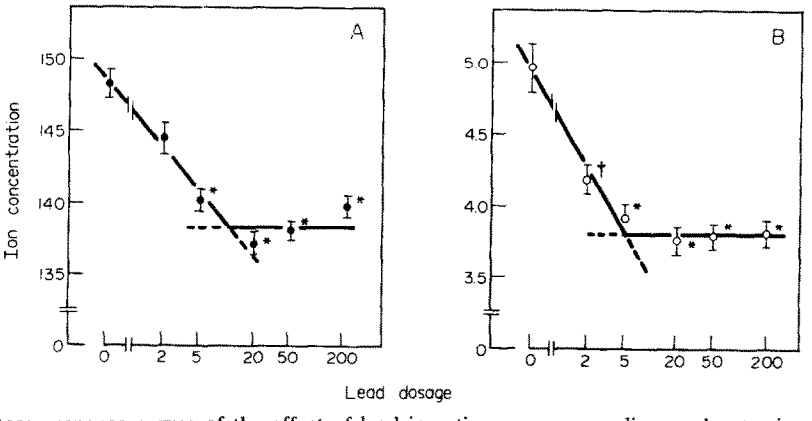

Fig. 1. Dose–response curves of the effect of lead ingestion on serum sodium and potassium. Each day for 12 weeks lead acetate (0–200 mg $Pb \cdot kg^{-1} \cdot day^{-1}$) was adminstered by stomach tube as an aqueous solution. The rats were killed 12 weeks after the last dose. Ion concentration is expressed as μequiv./ml. The values are average data from six rats; standard errors are indicated by bars. Panel A: serum sodium (●). Panel B: serum potassium (○). Significant difference from the respective control: (*) $P < 0.02$, and (†) $P < 0.05$.

Table 1. Effects of long-term ingestion of lead on urinary sodium excretion*

Lead dose ($mg \cdot kg^{-1} \cdot day^{-1}$)	Urinary sodium excretion [$\mu moles \cdot (100\,g\ rat)^{-1} \cdot day^{-1}$]						
	Duration of lead ingestion (weeks)						
	0	2	4	6	8	10	12
Control (none)	120.7 ± 6.2	112.1 ± 10.6	115.9 ± 20.2	122.4 ± 5.3	119.5 ± 5.0	118.1 ± 4.1	114.9 ± 8.6
2		112.1 ± 6.0	113.5 ± 12.3	109.0 ± 6.0	111.4 ± 5.0	105.8 ± 3.1	126.7 ± 3.8
5		117.8 ± 7.4	112.1 ± 22.0	115.2 ± 5.0	112.3 ± 8.6	102.5 ± 8.4	132.7 ± 3.1
20		114.7 ± 12.0	104.9 ± 13.9	148.6 ± 8.9	125.5 ± 2.4	117.6 ± 3.4	150.9 ± 6.5†
50		102.2 ± 3.4	110.0 ± 3.8	154.8 ± 3.6‡	133.9 ± 4.3	140.6 ± 6.0†	185.3 ± 6.2‡
200		132.0 ± 5.8	264.2 ± 8.2‡	304.6 ± 20.1‡	339.1 ± 10.1‡	355.1 ± 9.6‡	331.1 ± 16.4‡

* Values are averages obtained from three or four pairs of rats ± S.E.
† Significantly different from control, $P < 0.05$.
‡ Significantly different from control, $P < 0.02$.

Table 2. Effects of long-term ingestion of lead on urinary potassium excretion*

Lead dose ($mg \cdot kg^{-1} \cdot day^{-1}$)	Urinary potassium excretion [$\mu moles \cdot (100\,g\ rat)^{-1} \cdot day^{-1}$]						
	Duration of lead ingestion (weeks)						
	0	2	4	6	8	10	12
Control (none)	556.9 ± 31.2	525.6 ± 62.4	516.0 ± 59.5	556.8 ± 31.2	547.2 ± 16.8	554.4 ± 12.7	540.2 ± 14.4
2		590.4 ± 21.6	456.9 ± 40.8	549.6 ± 28.8	573.6 ± 36.0	532.8 ± 12.0	541.0 ± 2.5
5		630.0 ± 21.7	492.0 ± 38.4	554.4 ± 29.8	592.8 ± 33.6	573.6 ± 24.4	559.4 ± 18.2
20		630.0 ± 24.0	516.5 ± 57.6	592.8 ± 14.4	660.9 ± 14.4†	607.2 ± 19.2‡	612.7 ± 9.7†
50		580.8 ± 9.6	528.1 ± 55.2	712.8 ± 21.6†	662.4 ± 19.2†	612.0 ± 7.2†	612.7 ± 16.8†
200		664.8 ± 43.2	708.0 ± 56.6‡	722.4 ± 52.8†	787.2 ± 34.8†	720.0 ± 33.5†	754.3 ± 30.8†

* Values are averages obtained from three or four pairs of rats ± S.E.
† Significantly different from control, $P < 0.02$.
‡ Significantly different from control, $P < 0.05$.

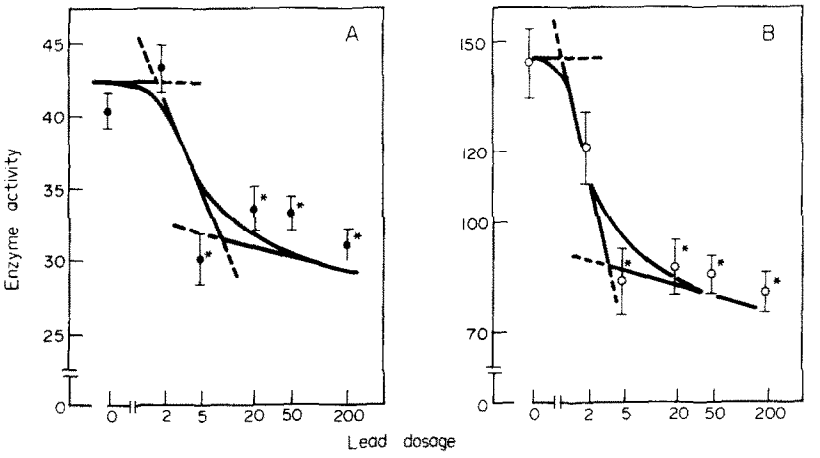

Fig. 2. Responses of renal K^+-dependent phosphatase and Na^+,K^+-ATPase activities to various doses of lead. Rats were killed after long-term (12 weeks) ingestion of lead acetate (0–200 mg $Pb \cdot kg^{-1} \cdot day^{-1}$). Enzyme activities are expressed as nmoles product $\cdot$ (mg protein)$^{-1} \cdot min^{-1}$. Lead dose: mg $Pb \cdot kg^{-1} \cdot day^{-1}$. The values are average data from six rats; standard errors are indicated by bars. Panel A: renal K^+-dependent phosphatase activity (●). Panel B: renal Na^+,K^+-ATPase activity (○). Significant difference from the respective control: (*) $P < 0.02$.

dependent phosphatase activity was very similar to that of the corresponding renal Na^+,K^+-ATPase activity, as shown in Fig. 2. Thus, *in vitro* effects of lead on renal K^+-dependent phosphatase and Na^+,K^+-ATPase activities were examined, using various metals as potent inhibitors [12, 13] of Na^+,K^+-ATPase activity, as shown in Fig. 3. The inhibitory effects of the various metal ions on renal K^+-dependent phosphatase activity were approximately the same as the effects on the corresponding renal Na^+,K^+-ATPase activity (shown in Fig. 3A and B). Moreover, the curves for Pb^{2+}, VO_3^- and Hg^{2+} were roughly similar for both enzyme activities (Fig. 3A and B).

The diminution of these enzyme activities after long-term ingestion of lead was thought to be due to lead accumulation in renal microsomes. Lead accumulation in renal microsomes was markedly augmented after 12 weeks of lead ingestion at doses above 5 mg $Pb \cdot kg^{-1} \cdot day^{-1}$, as shown in Fig. 4.

Renal Na^+,K^+-ATPase and K^+-dependent phosphatase activities after long-term ingestion of lead were examined after addition of EDTA or EGTA to the incubation medium in order to determine whether the suppression by lead was due to a primary or a secondary effect of lead. The decrease in renal K^+-dependent phosphatase activity, produced by various doses of lead, was partially reversed by the addition of EDTA or EGTA (Table 3). The data (in Table 3) indicate that the enzyme activity in the control preparation (no lead treatment) was enhanced by EDTA/EGTA. If this enhancement was due to an effect on metals that were contaminants of the reaction mixture, i.e. present in the $MgCl_2$, KCl or NaCl, they would have competed with lead.

The effect of Mg^{2+} concentration on inhibition of renal K^+-dependent phosphatase activity by lead ions was examined in the presence of various concentrations of magnesium ions at a fixed concentra-

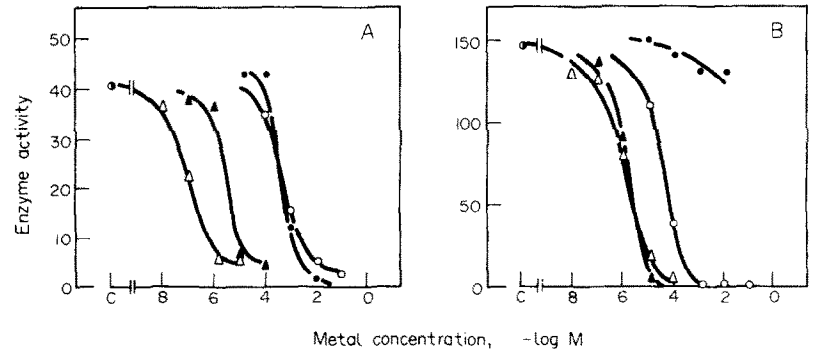

Fig. 3. Inhibitory effects of metals on renal K^+-dependent phosphatase and Na^+,K^+-ATPase activities. Enzyme was incubated in the standard incubation medium with, in addition, the concentrations of Pb^{2+} (○), AsO_4^{3-} (●), VO_3^- (▲) and Hg^{2+} (△) indicated, as described in Methods. Enzyme activity is expressed as nmoles *p*-nitrophenol, or P_i, liberated per mg protein per min. Panel A: renal K^+-dependent phosphatase activity. Panel B: renal Na^+,K^+-ATPase activity.

Table 3. *In vitro* effects of EDTA and EGTA on changes in renal K^+-dependent phosphatase activity after long-term ingestion of lead*

Lead dose ($mg \cdot kg \cdot day^{-1}$)	Enzyme activity [nmoles *p*-nitrophenol · (mg protein)$^{-1}$ · min^{-1}] None	T/C	With EDTA	T/C	With EGTA	T/C
Control (none)	40.3 ± 0.6	100	56.7 ± 3.0	141†	66.8 ± 0.8	166†
2	43.4 ± 2.7	108 (100)	49.5 ± 1.4	123 (114)‡	55.2 ± 2.1	137 (127)‡
5	30.0 ± 1.6	74 (100)	37.2 ± 0.3	92 (124)‡	45.9 ± 3.4	114 (153)†
20	33.4 ± 1.9	83 (100)	40.1 ± 4.0	100 (120)‡	49.1 ± 5.4	122 (147)†
50	33.2 ± 1.2	82 (100)	40.6 ± 1.3	101 (122)‡	46.6 ± 0.1	116 (140)†
200	30.9 ± 1.2	77 (100)	38.2 ± 0.4	95 (124)‡	47.1 ± 2.6	117 (152)†

* Rats were killed 12 weeks after long-term ingestion of lead acetate (0, 2, 5, 20, 50 or 200 mg $Pb \cdot kg^{-1} \cdot day^{-1}$). The enzyme was preincubated with buffer, cations and EDTA (0.5 mM) or EGTA (0.5 mM), for 10 min at 37° before adding *p*-nitrophenylphosphate to measure the enzyme activity. Values are expressed as average data of six rats ± S.E. T/C: Ratio of treated to control values. Numbers in parentheses are the percentages of control values (without EDTA or EGTA).

† $P < 0.02$.

‡ $P < 0.05$.

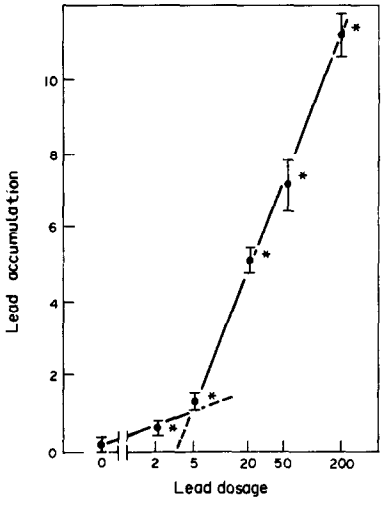

Fig. 4. Relationship between long-term ingestion of lead and its accumulation in renal microsomes. Rats were killed after long-term (12 weeks) ingestion of lead acetate (0–200 mg $Pb \cdot kg^{-1} \cdot day^{-1}$). Lead content: nmoles/mg protein. The values are average data from six rats; standard errors are indicated by bars. Significant difference from the control: (*) $P < 0.02$.

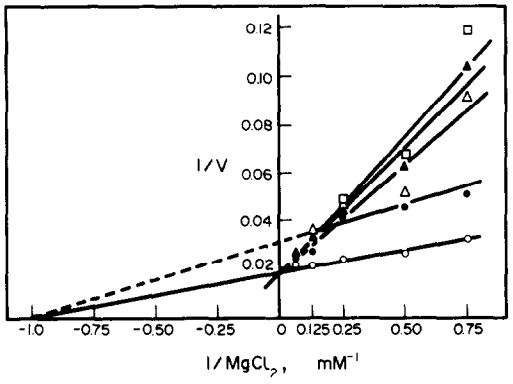

Fig. 5. Lineweaver–Burk plots of renal K^+-dependent phosphatase activities with $MgCl_2$ in the presence and absence of metals as inhibitors. The enzyme preparation was incubated in the standard medium but with the concentrations of $MgCl_2$ indicated, in the absence of metals as inhibitors (○) and in the presence of 0.25 mM Pb^{2+} (●), 2.5 μM Hg^{2+} (△), 0.1 μM VO_3^- (▲) or 0.5 mM AsO_4^{3-} (□). 1/*V*: [nmoles]$^{-1}$ · [mg protein · min].

Table 4. Changes in urinary aldosterone excretion during long-term ingestion of lead*

Lead dose ($mg \cdot kg^{-1} \cdot day^{-1}$)	Urinary aldosterone excretion [$pg \cdot (100\ g\ rat)^{-1} \cdot day^{-1}$] Duration of lead ingestion (weeks) 0	4	8	12
Control (none)				
20	14.5 (100)	6.9 (47.6)	<2 (<13.8)	<2 (<13.8)
200		<2 (<13.8)	<2 (<13.8)	<2 (<13.8)

* Values are averages of duplicate examinations from collected urine samples of four rats. Numbers in parentheses show relative values.

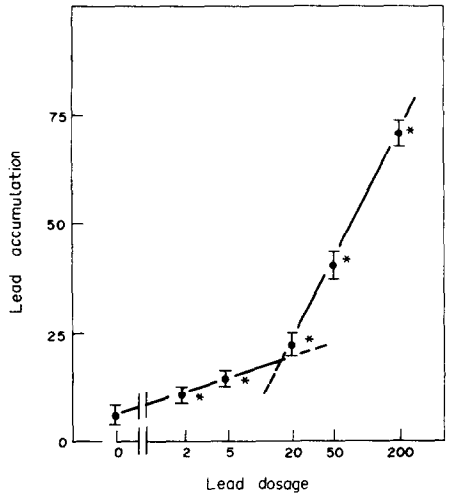

Fig. 6. Relationship between long-term ingestion of lead and its accumulation in adrenal glands. Rats were killed after long-term (12 weeks) ingestion of lead acetate (0–200 mg $Pb \cdot kg^{-1} \cdot day^{-1}$). Lead content: nmoles/g fresh tissue. The values are average data from six rats; standard errors are indicated by bars. Significant difference from the control: (*) $P < 0.02$.

tion of lead (0.25 mM) as compared with vanadate (0.1 μM), mercury (2.5 μM) and arsenate (0.5 mM) ions. As shown in Fig. 5, the apparent K_m values of the enzyme, in the presence of lead ions, derived from the biphasic $1/V$ vs $1/MgCl_2$ plot, were about 1.0 and 4.3 mM. Lead for the K^+-dependent phosphatase activity was found to produce competitive inhibition in the presence of high Mg^{2+} but to produce non-competitive inhibition in the presence of low Mg^{2+}. On the other hand, the plots of $1/V$ vs $1/MgCl_2$ for vanadate, mercury and arsenate ions of the K^+-dependent phosphatase activity are straight lines with intercept of $1/V$ where $V_{max} = 55$ nmoles·(mg protein)$^{-1}$·min^{-1}. Inhibition by vanadate, mercury or arsenate of the K^+-dependent phosphatase activity was found to be competitive with Mg^{2+}.

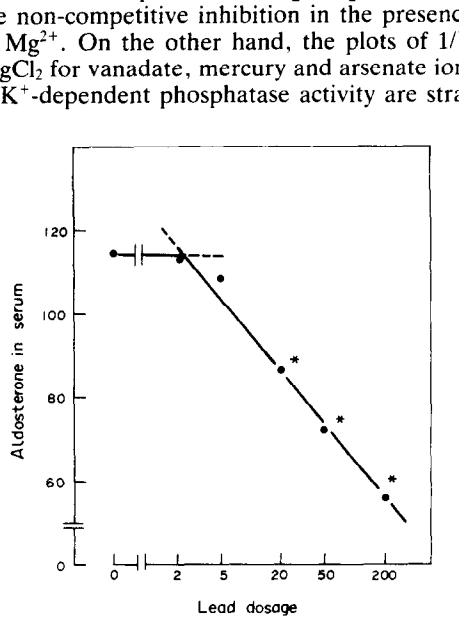

Fig. 7. Response of aldosterone in serum to lead dose. Rats were killed after long-term (12 weeks) ingestion of lead acetate (0–200 mg $Pb \cdot kg^{-1} \cdot day^{-1}$). Aldosterone in serum: pg/ml. The values are average data of three samples [each sample consisted of combined serum (equi-volume) from two rats]. Significant difference from the control: (*) $P < 0.02$.

Alteration of adrenal function by long-term ingestion of lead. The reduction of renal K^+-dependent phosphatase and Na^+,K^+-ATPase activities by lead doses was not reversed completely by addition of EDTA or EGTA as shown in Table 3.

Thus, the reduction of these enzyme activities was postulated to be due to suppression of enzyme protein synthesis as a secondary effect of the lead dose, in addition to direct inhibition of these enzyme activities as a primary action of the lead. Lead accumulation in adrenal gland was determined in order to understand the effect of lead on adrenal gland. The lead content of adrenal gland was elevated significantly after 12 weeks of lead ingestion at doses above 2 mg $Pb \cdot kg^{-1} \cdot day^{-1}$, as shown in Fig. 6.

Aldosterone in urine and serum was measured to determine the changes in adrenal function in rats after long-term ingestion of lead. Serum aldosterone contents linearly decreased, with logarithmically increasing doses of lead (2–200 mg $Pb \cdot kg^{-1} \cdot day^{-1}$) for 12 weeks consecutively, as shown in Fig. 7. Urinary excretion of aldosterone was decreased to 13.8% of control level after 8 weeks of lead ingestion (20 mg $Pb \cdot kg^{-1} \cdot day^{-1}$), respectively, as shown in Table 4. Urinary excretion of sodium was demonstrated to be elevated in association with attenuation by lead of serum aldosterone content.

DISCUSSION

In these experiments, serum sodium and potassium concentrations were demonstrated to be decreased significantly by long-term (12 weeks) ingestion of lead acetate of only 2 or 5 mg $Pb \cdot kg^{-1} \cdot day^{-1}$. The decrease by lead of serum sodium and potassium was found to be associated with elevation of urinary sodium and potassium excretion in rats after long-term ingestion of small lead doses (2 or 5 mg $Pb \cdot kg^{-1} \cdot day^{-1}$), similar to that in rats after single oral doses of lead (200 mg Pb/kg) [9, 14]. A sub-lethal dose of lead acetate has been reported to be 6000 mg Pb/kg, o.p., in rats [15].

Renal K^+-dependent phosphatase activities were found to be markedly suppressed in association with changes in mobilization of sodium and potassium ions after long-term ingestion of small doses of lead. Renal K^+-dependent phosphatase activity was decreased, similar to the decrease of renal Na^+,K^+-ATPase activities, after long-term ingestion of lead.

Renal Na^+,K^+-ATPase activity is known to be stimulated by aldosterone [4]. Recently, Suketa *et al.* [16] reported that reduction of renal K^+-dependent phosphatase and Na^+,K^+-ATPase activities after a single oral dose of lead acetate (200 mg Pb/kg) was reversed significantly by aldosterone.

In these experiments, we demonstrated that the enzyme activities and aldosterone excretion were reduced, and that the enzyme inhibition was only partly reversed by exposure to chelators.

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