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Tohoku Journal of Agricultural Research
Vol. 31, No. 1, August 1980
Printed in Japan

Distribution of Lead and Endotoxin-Detoxicating Activity of Tissue Extracts in Lead-treated Mice

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(Received, April 16, 1980)

Summary

The detoxication rate of endotoxin by extracts from the liver, spleen and lung of normal mice was over 70% and that of the kidney and serum was 51% and 23%, respectively. The detoxicating activity of liver and spleen extracts reduced to two-thirds at 6 hours after a single intravenous injection of lead acetate at the dose of 0.5 mg per 10 grams of body weight. The dose had been known as that which enhanced the susceptibility for an endotoxin. There was no reduction in the detoxicating activity of kidney and lung extracts. The reduction of the activity in liver and spleen playing a main role in the detoxication of endotoxins seems to be one of the causes of the enhanced susceptibility in the lead-treated animals.

The lead concentrations in the liver and spleen extracts were 67 and 29 μg/ml respectively. These values were twice to 8 times those in the kidney and lung extracts and serum. These facts show that the reduction of detoxicating activity is caused by the accumulation of lead.

Gel-filtration of the liver extract of normal mice by Sephadex G-200 gave 4 fractions of protein, 3 of which had the endotoxin-detoxicating activity. These 3 fractions from the lead-treated mice contained lead and had less activity than those from normal mice. Therefore, the lead may act directly on the endotoxin-detoxicating agents to inhibit the activity.

Selye *et al.* (1) researched the pharmacological action of several heavy metals and reported that lead acetate remarkably enhanced the susceptibility of rats to a bacterial endotoxin. This same effect of lead acetate was also confirmed in chickens (2), baboons (3) and mice (4). Also, in our previous paper (5), adequate conditions to produce the enhanced susceptibility to an endotoxin derived from *E. coli* were determined in mice, and an available method was stated for the quantitative assay for small amounts of endotoxin using lead-treated mice. However, the mechanisms of the sensitization by lead acetate are not clear.

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On the other hand, it has been well known that tissue homogenates, serum and plasma in various kinds of animals alter the activities of bacterial endotoxins to reduce their toxicity (6) (7). Therefore, the enhanced susceptibility to endotoxin caused by lead acetate may be due to the inhibition of the endotoxin-detoxicating activity by lead acetate. To prove this assumption, the relation between the distribution of lead injected into mice and the endotoxin-detoxicating activity of tissue extracts was demonstrated in this paper.

Materials and Methods

Endotoxin and Lead Acetate: Lipopolysaccharide from *E. coli* (029: B6) and lead acetate were supplied and prepared in the same manner described in our previous paper (5).

Mice: SPF mice (ddY strain) as previously described (5) were used.

Tissue Extracts: Six hours after an injection of lead with a dose of 0.5 mg per 10 grams of body weight, the mice were sacrificed via decapitation and their blood, livers, spleens, kidneys and lungs were collected. The organs from 3 mice were pooled, weighed and homogenized with 3 volumes of cold Tris buffer solution (pH 7.0 at 37°C) containing 0.25 M sucrose in the Polytron homogenizer under ice-water cooling, and then centrifuged at 10,000 r.p.m. for 30 minutes at 4°C. The supernatants, 25% (w/v) extracts, were diluted with the buffer to make 2% (w/v) extracts. Serum separated at 4°C from pooled clotted blood of 3 mice was diluted with 4 volumes of the buffer. These diluted extracts and serum were used for determination of endotoxin-detoxicating activity, and lead and protein concentrations. Similar materials prepared from normal mice were also employed as the control.

Other extracts (33% w/v) from the liver of the lead-treated and normal mice were prepared by homogenization with 2 volumes of Tris buffer (pH 7.0) containing 10^{-5} M $MgCl_2$ and 0.25M sucrose and centrifuged at 15,000 r.p.m. for 45 minutes. The extracts were used for gel filtration.

Gel Filtration: Two ml of the 33% liver extract was filtered through a column (2.7×60 cm) of Sephadex G–200 with the buffer containing $MgCl_2$ and sucrose as described above. The optical density, at 280 nm, of the filtrate was continuously measured by UV Monitor (Isuco, UA–5) to detect protein elution. The filtrate was divided seriously into 5 ml portions, and each of the portions was used for detection of lead. The portions giving relatively high O.D. value at 280 nm were put together in 25 ml to detect the endotoxin-detoxicating activity. Also, a part of the liver extract used for gel filtration was diluted 25 times with Tris buffer, and the endotoxin-detoxicating activity was detected.

Determination of Lead and Protein: Lead concentration was determined by flameless atomic absorption spectrophotometer (Shimazu, Type–640). Protein concentration was colorimetrically determined with Cu-Folin reagent.

Endotoxin-Detoxicating Reaction: Reaction mixture containing 2.5 ml of diluted tissue extracts or serum, 0.5 ml of endotoxin solution (0.5 mg/ml) and 2.0 ml of phosphate buffer (pH 7.0) was incubated at 37°C for 1 hour and then heated at 100°C for 5 minutes to stop the reaction. Another mixture consisting of 4.5 ml of gel filtrate and 0.5 ml of endotoxin solution (0.5 mg/ml) was also incubated and heated as stated above.

As the control mixture, the buffer solution used in the preparation of the extracts or filtrates was employed in place of them. After the reaction finished, the toxicity of endotoxin in these mixtures was assayed.

Assay of Toxicity of Endotoxin: The toxicity of endotoxin was assayed by a method reported in our previous paper (5). That is, 0.5 mg of lead acetate and 0.1 ml of the reaction mixtures, per 10 grams of body weight, were simultaneously injected into the caudal vein of 8 to 10 ddY male mice, and the titre of residual endotoxin were calculated from mortality within 72 hours according to the formula (5).

Endotoxin-Detoxicating Activity: Endotoxin-detoxicating activity of tissue extracts, serum and gel filtrate was expressed as the detoxication rate of endotoxin, calculated with the following equation.

$$\text{Detoxication rate } (\%) = \left(1 - \frac{\text{residual endotoxin in tested mixture}}{\text{residual endotoxin in control mixture}}\right) \times 100$$

Results

Endotoxin-Detoxicating Activity of Tissue Extracts and Serum: As shown in Table 1, the detoxication rates of endotoxin by liver, spleen and lung extracts from normal mcie were 70 to 80%, and that by kidney extract was about 50%. In lead-treated mice, the detoxication rates by liver and spleen extracts diminished to 2/3 of those in the normal mcie. However, such effect was not observed on other tissue extracts. These facts clearly show that the endotoxin detoxicating activity of liver and spleen was depressed by the lead-treatment. The activity of serum was completely lost in the lead-treated mice. However, this fact does not mean that the activity of serum is easily depressed by lead in comparison with other tissues, because the activity of normal serum was very low in itself.

Lead Concentration of Tissue Extracts and Serum: Concentrations of lead in tissue extracts and serum prepared from the lead-treated mice are shown in Table 2. The concentrations in liver and spleen extracts were 67.1 and 28.9 μg/ml respectively, and the values were 2 to 8 times greater than those in kidney and lung extracts and serum. The lowest concentration was obtained in lung extract. The lead content per unit weight of protein in these materials followed a similar pattern as above, except for a relatively high value in serum.

TABLE 1. *Endotoxin-Detoxicating Activity of Tissue Extracts and Serum from Normal and Lead-Treated Mice*

Materials	Detoxication rate (%)		Depression of endotoxin-detoxicating activity by lead (%)[1)]
	Normal[a)]	Lead-treated[b)]	
Liver	79.5±1.6[2)]	55.5±13.0*	30.2±13.4
Spleen	80.4±5.1	54.8± 7.4**	32.6± 5.5
Kidney	51.4±8.3	61.0±12.9	−9.1± 7.1
Lung	71.5±6.6	75.9±13.9	−7.7±18.4
Serum	23.0	0.0	100.0

1) (a–b)/a×100, 2) Mean±SD, (n=3)
*P <0.1, **:P<0.05 compared to the normal.

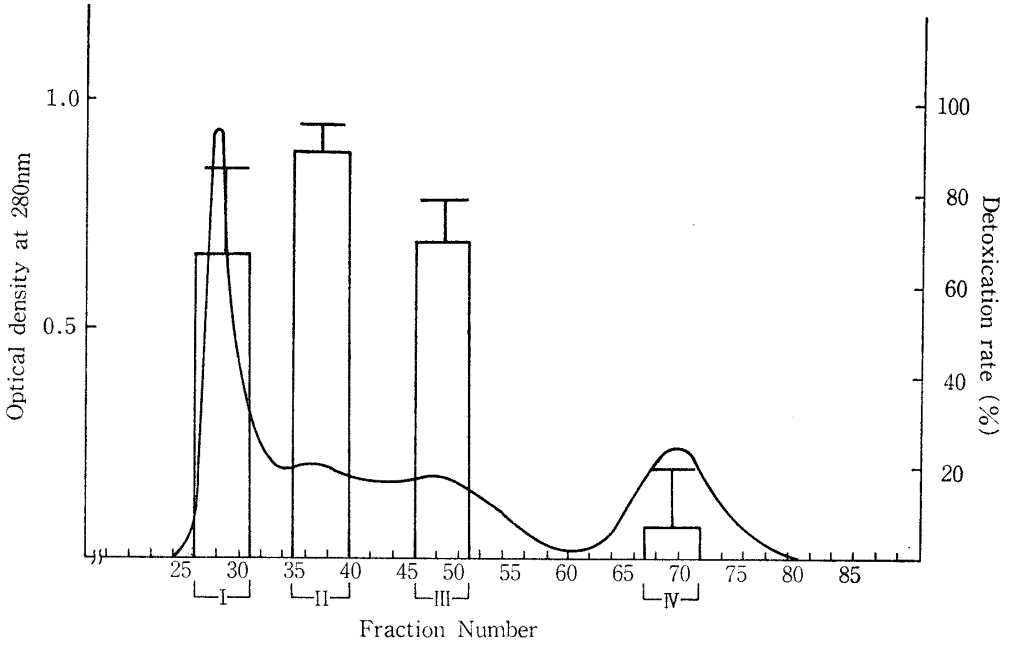
TABLE 2. *Lead Concentration in Tissue Extracts and Serum at 6 Hours after Intravenous Injection of Lead Acetate*

Materials	Lead concentration (μg)	
	Per ml of extract[1)]	Per mg of protein
Liver	67.1±8.2[2)]	0.91±0.14
Spleen	28.9±6.3	0.85±0.15
Kidney	14.8±2.8	0.55±0.15
Lung	7.9±0.7	0.23±0.04
Serum	14.7±2.6	0.65±0.11

1) Tissue extract: 25% (w/v), serum: 20% (v/v)
2) Mean±SD, (n=3)

Fractions Having Endotoxin-Detoxicating Activity in Normal Liver Extract: A liver extract prepared from normal mice produced 4 peaks of protein indicated by O.D. value at 280 nm through gel-filtration with Sephadex G–200, as shown in Fig. 1. The 4 fractions corresponding with these peaks were assayed for their endotoxin-detoxicating activity. The results are also shown in Fig. 1. The highest detoxication rate (90%) of endotoxin was found in the 2nd fraction. The detoxication rate of the 1st and 3rd fractions was 3/4 of the above. The 4th fraction showed no detoxication. Thus, it may be stated that the endotoxin-detoxicating activity of normal liver extract depends on the first 3 protein fractions.

Endotoxin-Detoxicating Activity and Lead Concentration of Gel-Filtrated Protein Fractions from Lead-Treated Mice: Fig. 2 depicts the results of gel-filtration of the liver extract prepared from the lead-treated mice. The filtrate produced 4 peaks of protein identical to those of normal mice, though lead was contained only in the first 3 peaks. These peaks were active fractions on the detoxication in the normal mcie. Total lead content in these fractions was estimated at 98.5 μg, and the recovery of lead was regarded as 100%. The endotoxin-detoxicating activity of the second fraction (Fraction II) was significantly depressed by lead ($P<0.02$) as shown in Table 3. The detoxication rates of endotoxin by the other two fractions containing lead were diminished to 2/3 of the normal mcie. The lead content



Curve shows optical density at 280nm and column shows detoxication rate of endotoxin (m±SD, n=3).

FIG. 1. Endotoxin-detoxicating activity of fractions of normal mouse liver extract divided by gel-filtration with Sephadex G–200.

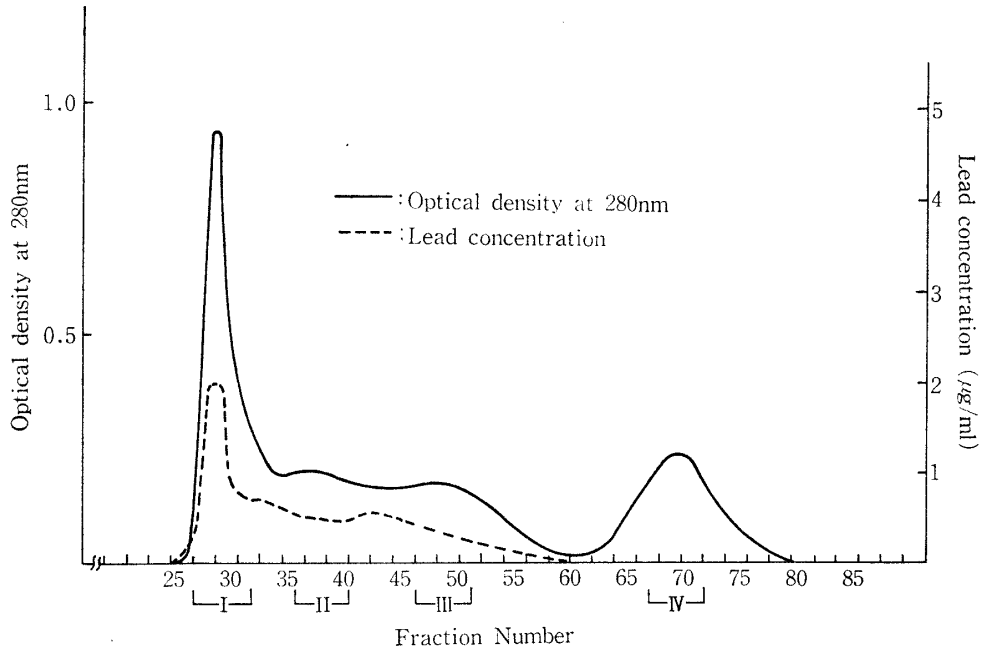


FIG. 2. Protein and lead concentration of the filtrate of lead-treated mouse liver extract through Sephadex G–200 column.

per protein in the first fraction was 1.53 μg/ml, of which value was twice that in the other two fractions.

These facts seem to suggest that lead was bound with endotoxin-detoxicating agents, thought to be proteins, and reduced the detoxicating activity as a result. However, the rate of the impairment was not proportional to the concentration of lead.

TABLE 3. *Endotoxin-Detoxicating Activity of Fractions of Normal and Lead-Treated Mouse Liver Extract Divided by Gelfiltration with Sephadex G-200*

Fractions[1]	Mice	Lead contents μg/mg protein	Detoxication rate (%)	Depression of endotoxin-detoxicating activity by lead (%)[2]
Intact extract[3]	Normal Lead-treated	— 0.94±0.09[4]	91.3± 4.6[4)a)] 67.4± 9.4[b)]*	25.9±10.9[4]
Fraction I	Normal Lead-treated	— 1.53±0.18	68.9±17.1 40.5±28.0	32.6±48.9
Fraction II	Normal Lead-treated	— 0.87±0.15	91.4± 3.3 64.8± 3.7**	29.1± 3.9
Fraction III	Normal Lead-treated	— 0.85±0.13	70.7± 9.0 49.5±20.0	26.9±29.3
Fraction IV	Normal Lead-treated	— 0.00±0.00	7.7±13.3 3.3± 5.7	19.0±26.9

1) Fractions I-IV are shown in Fig. 1 and 2. 2) (a–b)/a×100
3) 1.32% (w/v) liver extract. 4) Mean±SD (n=3)
*: $P<0.1$, **$P<0.02$ compared to the normal.

Discussion

Several mechanisms of enhancement of susceptibility to endotoxins in lead-treated animals has been reported. These include the impairment of reticuloendotherial system (8), disorder of adrenal (1) or liver (9) functions and inactivation of SH compounds (10) by lead. Trejo and DiLuzio (11) compared endotoxin-detoxicating activities of several tissue homogenates from lead-treated rats with those from normal ones, and they showed that lead depressed an ability to detoxify endotoxin in liver and spleen. As the result, they thought that the lead-induced hypersensitivity to endotoxin was due to an impairment of detoxicating activities in these tissues. In contrast, Filkins and Buchanan (12) observed no alteration in hepatic detoxication in lead-treated rats. In our present study, the liver and spleen extracts, which had a relatively high activity of endotoxin-detoxication in normal mice, were depressed in their detoxicating activity by the lead-treatment. However, the other tissue extracts were not affected. Considering that most of the endotoxin injected was accumulated into RES of spleen and liver from circulating fluid (13), the depressed activity of endotoxin-detoxication in

these tissues is thought to be one of the causes of the enhancement of susceptibility to endotoxin induced by lead acetate.

Hoffman et al. (8) showed ultramicroscopically that lead injected intravenously into rats was taken up by RES in liver and spleen. Our result also showed that lead was detected at higher concentration in the liver and spleen extracts than in other extracts of lead-treated mice. Also, the endotoxin-detoxicating activity of these two extracts was significantly reduced in the lead-treated mcie. These facts show that the depression of detoxicating activity was cuased by the accumulation of lead.

On Sephadex G–200 column, elution of lead was accompanied with three protein fractions that corresponded with those which had the endotoxin-detoxicating activity in normal mice. The lead containing fractions had a lower activity of detoxication than normal ones. These facts suggest that the lead combined with the endotoxin-detoxicating agents, which are thought to be proteins, and reduced their activity.

However, in these fractions, no correlation was observed between the lead content in protein and the depression of the endotoxin-detoxicating activity. This suggests that the lead may have also combined with proteins other than the endotoxin-detoxicating agents.

The endotoxin-detoxicating activity of normal liver extract depends mainly on the activity of Fraction II, and the activity of the fraction was significantly depressed in lead-treated mice. Therefore, the results suggest that depression of detoxicating activity of lead-treated mouse liver extract was caused by the reduction of the activity of this fraction.

Acknowledgements

We would like to thank Dr. K. Kubota and Dr. H. Takahashi for their interest and encouragement, and Dr. K.T. Suzuki for his helpful discussions. We would also like to thank Mr. K. Matsushige for his useful advice in the determination of lead content.

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