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A NEW METHOD OF ASSAY FOR HEAVY METAL DETOXICANTS USING DENTIN OF THE RABBIT INCISOR (I)

BY

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ABSTRACT

Investigation has been made to develop a new method of assay for heavy metal detoxicants by utilizing the deposition pattern of heavy metal salts in the incisal dentin of rabbits. The results obtained are as follows.

1) Under a subcutaneous injection of 500 mg/kg of the lead (II) salt of Amberlite IRC-50 (Pb-A) suspended in 5% gum arabic, measurements were carried out of lead concentration in the blood in all of and its content in the urine in some of animals by the dithizone method. Single injection of this suspension brought about a continuous release of lead ions into the blood for a long period of time. The duration and amount of the release of the ions differed according to the sort of resin in the formation of the lead compound.

2) Lead ions released from Pb-A into the blood were deposited as lead salt in the incisal dentin which showed a continuous appositional growth.

3) The pattern of the deposition of lead salt in the dentin depended upon the time course of lead ion concentration in the blood.

4) The subcutaneous injection of Pb-A had almost no effect on the body weight.

5) From the foregoing results the possibilities were concluded that the assay of heavy metal detoxicants are performed without inducing the death of animals, and that, several detoxicants are assayed simultaneously with the use of one and the same animal, and moreover that, by administrating lead acetate at a certain interval, there are determined, as to the action of detoxicants, the potency, the duration and the time required for appearance.

INTRODUCTION

Heavy metal detoxicants are commonly assayed by their antidotic action against the lethal effect of heavy metals. However, this method is not only low in sensitivity but also poor in getting stable results. It is, therefore, difficult to obtain detailed information on the property of

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detoxicants. This report will be concerned with a new method which permits the assay of heavy metal detoxicants without inducing the death of animals.

1) New Method of Assay

The continuously growing incisor of rodents is widely accepted as one of the most valuable biologic indicators to ascertain the condition of growth, metabolism and so forth[1].

Heavy metals have a strong affinity for bone and teeth, where a large amount of calcium deposition is seen. Heavy metals are deposited in these areas in proportion to the concentration of heavy metal ion in the blood. Okada and Mimura[2] thus, devised an excellent method of time marking in teeth and bone by using intravenous injection of a small dose of lead acetate.

If there is obtained a substance which, when injected, can release heavy metal ion in the blood continuously, it is deposited in teeth and bone as a salt to form the wide deposit layer in the dentin. Heavy metal detoxicans that decrease the amount of heavy metal ion in the blood by forming complexes with it, prevent the depositions of heavy metal salts in the dentin. From the pattern of heavy metal deposition in the dentin, the pharmacological action of the detoxicants may be explored. Such a result could be obtained when lead (II) salt of Amberlite IRC-50 (i.e., Pb-A) was injected.

Materials and Methods

Twelve male rabbits weighing about 2.5 kg were used. Pb-A was crushed to pass through 400 mesh or more. This was suspended in 5% gum arabic and was injected subcutaneously with an 18 gage needle. Animals were divided into 4 groups including the control group (3 animals in each). All animals had an intravenous injection of lead acetate in a dose of 1 mg/kg to leave a time mark in the dentin. As soon as possible after this administration, each of 3 groups was given a subcutaneous injection of 100, 300 and 500 mg/kg of Pb-A respectively, the control being treated by 5% gum arabic alone. The absolute lead content of Pb-A was 256 mg/g. The injections of lead acetate were made at 5 days interval, the body weight being measured each time.

The animals were killed one month after the Pb-A injection. The lower jaws were fixed in 10% neutral formalin and then decalcified in 0.2 N HCl saturated with H_2S. The decalcified jaws were washed and embedded in 15% and then in 30% gelatin. Frozen sections were made at 10–15 μ.

Transverse sections of the incisor were stained with 0.1% $AuCl_3$ to develop the sharp lines and diffuse layer of deposited lead salt, reduced in 5% $NaHSO_3$, and mounted in glycerin jelly. The lead salt in the dentin assumed black or brown color under a microscope.

Results

The subcutaneous injection of Pb-A and the intravenous injections of lead acetate at 5 days interval had almost no effect on the body weight, as shown in Fig. 1, no other significant symptoms being observed.

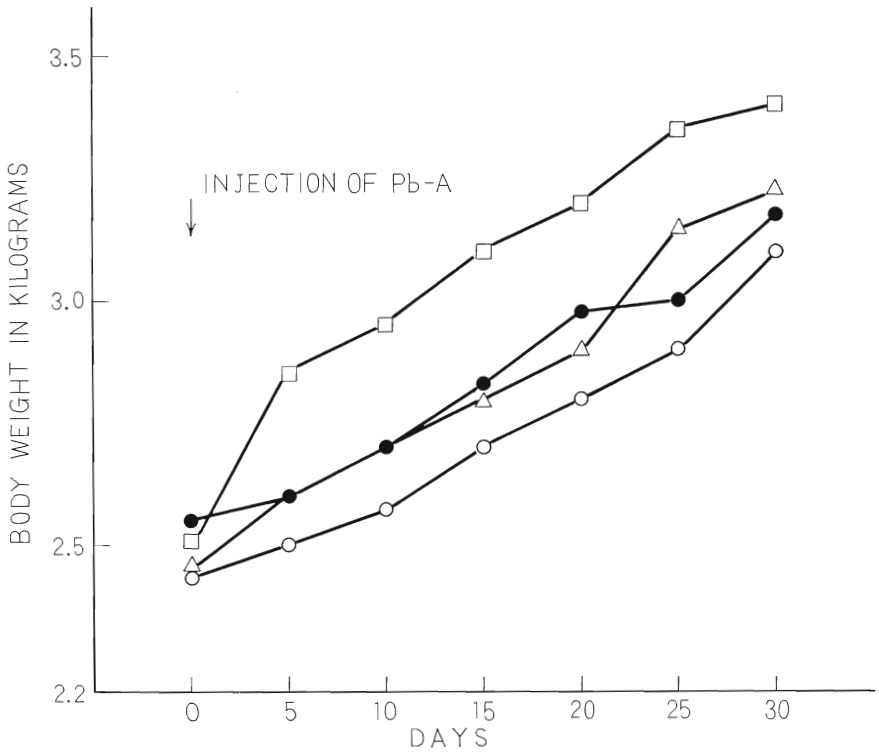

Fig. 1. Graph showing average body weight (kilograms) of 3 male rabbits following subcutaneous injection of Pb-A or 5% gum arabic.
○—○: 500 mg/kg. □—□: 300 mg/kg. △—△: 100 mg/kg. ●—●: Control.

Fig. 2 shows the transverse section of the incisal dentin obtained in the above experiment. Dark black lines appeared by the intravenous injections of lead acetate. The diffuse layer of black or brown color which was formed by the lead salt deposited was obtained by the subcutaneous injec-

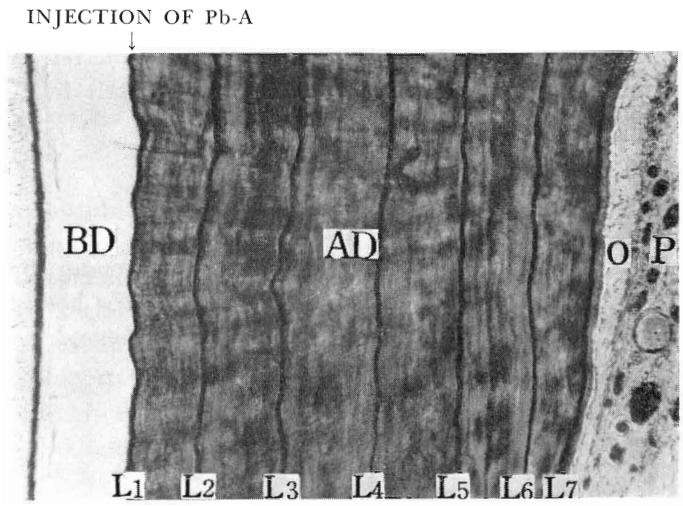

Fig. 2. Photomicrograph of mesial portion in a transverse section of dentin of lower incisor in rabbit. L_1—L_7 are lead lines appeared by intravenous injections of lead acetate at 5 days interval. Appositional growth of dentin is toward right side of photograph. AD: Dentin formed after subcutaneous injection of 500 mg/kg of Pb-A, diffuse deposit layer of lead salt being observed for a month in a similar uniform density. BD: Dentin formed before injection of Pb-A. P: Pulp. O: Odontoblast layer.

tion of Pb-A with a lead content of 500 mg/kg. The diffuse deposit layer appeared immediately after injection. This deposition of lead salt was observed for about a month in a similar uniform density.

II) Relationship between Deposit Pattern of Lead Salt in Dentin and Lead Concentration in Blood

The deposit layer of lead salt in the dentin, as seen in Fig. 2, is considered to be caused by lead ions released gradually from Pb-A into the blood. The following experiment was done to prove this hypothesis and to compare the difference in the release, if any, between lead carbonate and lad (II) salt of Dowex-50 W–X8 (i.e., Pb-D).

Materials and Methods

Nine male rabbits weighing about 2.5 kg were divided into 3 groups (3 animals in each). Each group was given a single subcutaneous injection of suspended Pb-A, lead carbonate and Pb-D with a lead content of 500

mg/kg in 5% gum arabic respectively. All animals had subsequent intravenous injections of lead acetate in a dose of 1 mg/kg at 5 days interval, 7 cc of blood were drawn from the ear vein of each animal 3 hours before injection of lead acetate and at intervals of 3, 6, 12, 24, 48 hours etc. after that during a period of one week. Urine was obtained from some of the animals before injection and every 24 hours after that during one week. Lead concentration in the blood and its content in the urine were measured by the dithizone method[3].

Animals were killed one month after injection of the three sorts of lead salts, and the histological procedures were the same as those described in the section I.

Results

Lead concentration in the blood is shown in Fig. 3. Three hours after the Pb-A injection lead could be detected in the blood. Maximum concentration of lead was seen in the blood 6 hours after injection. After 6 hours the concentration decreased gradually, lead being detected up to 7th day at least. Lead concentration in the blood was less in the Pb-D group than in the Pb-A group. In the lead carbonate group, it was difficult to detect

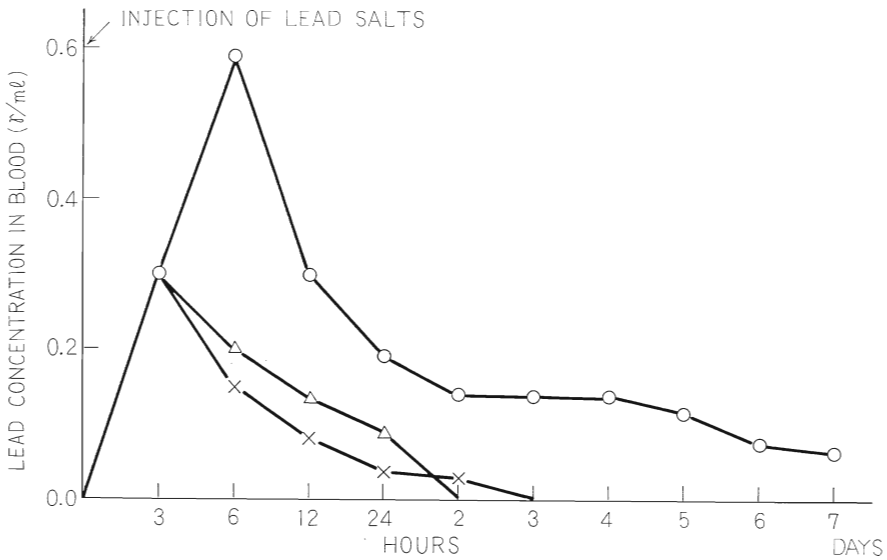

Fig. 3. Graph showing average blood concentration of 3 male rabbits following subcutaneous injection of Pb-A, Pb-D or lead carbonate. ○—○: Injection of 500 mg/kg of Pb-A. △—△: Injection of 500 mg/kg of lead carbonate. ×—×: Injection of 500 mg/kg of Pb-D.

lead in the blood about 4 days after injection. The time course of lead concentration in the blood almost corresponded with the pattern of lead

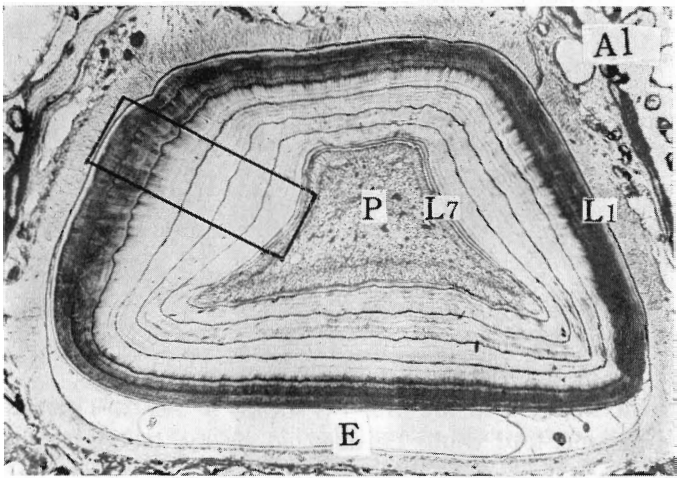

Fig. 4. Photomicrograph of a transverse section of dentin of lower incisor in rabbit. L_1—L_7 are lead lines appeared by intravenous injections of lead acetate at 5 days interval. P: Pulp. E: Enamel. Alv: Alveolar bone.

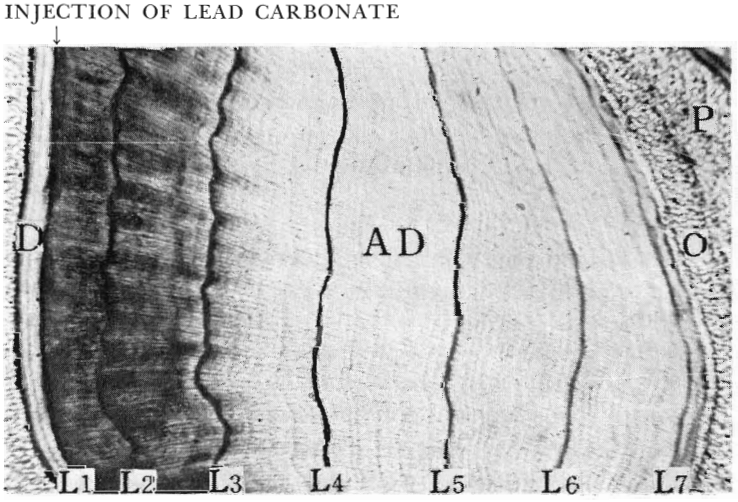

Fig. 5. Enlarged photomicrograph of Fig. 4 for distal portion. L_1—L_7 are lead lines appeared by intravenous injections of lead acetate at 5 days interval. Appositional growth of dentin is toward right side of photograph. AD: Dentin formed after subcutaneous injection of 500 mg/kg of lead carbonate, no deposition of lead salt being observed. D: Dentin farmed before injection of lead carbonate. P: Pulp. O: Odontoblast layer.

salt deposition in the dentin. Lead was also seen in the urine. Its content in the urine showed parallel relation to the concentration in the blood.

Figs. 4 and 5 show the transverse section of the dentin obtained after the single injection of 500 mg/kg of lead carbonate. It should be noted that, approximately 14 days after injection, the deposition of lead salt in the dentin stops and does not occur any longer. The widest deposit layer of lead salt in the dentin (Fig. 2) was obtained from lead ions which were gradually released from Pb-A. In this case the deposit occurred with the appositional growth of dentin.

Discussion

The fact that subcutaneous injection of lead salt produces a wide deposit layer of it in the incisal dentin was already known since the investigation of Okada and Mimura[4], who used lead carbonate. However, there has been no previous report of experiment using lead resinate. As described in the section of results, when lead resinates especially Pb-A were used, the release of lead ions in the blood was much more durable than in the case of lead carbonate. It was also found that the pattern of release differed according to the sort of resin. These results may be utilized to maintain a drug concentration in the blood in experiment as well as in clinic, the latter case being by the oral administration alone.

By giving a single subcutaneous injection of Pb-A, it was possible to observe the continuous release of lead ions during a long period of time. Resulting from continuous appositional growth[5], lead ions in the blood were deposited in the incisal dentin of rodents to provide a built-in smoked drum paper, as seen in Fig. 2. The density of the lead salt deposited in the dentin depends upon the ion concentration in the blood. Thus it is expected that the detoxicants[6] which act to increase the excretion of heavy metal ions induce the decrease of lead salt deposition in the dentin, and that a part of the dentin where the deposition is suppressed by the detoxicants is observed as a white zone. In contrast to this, it is also expected that the detoxicants which induce the increased ion deposition in tissues increase the ion deposition in the dentin, where a distinct black layer is observed. From such deposition pattern of lead salt, the mechanism of action of detoxicants may be revealed, and by using lead acetate injections as a time marker, it is possible not only to assay detoxicants quantitatively but also to determine the time for appearance of action and the duration of its maintenance. The experiments for these possibilities along with the details of method will be described in the second report.

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