THERAPY AND PREVENTION OF LEAD POISON- ING WITH CALCIUM VERSENATE*

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A number of reports⁽¹⁻⁶⁾ indicate that administration of an organic chelating agent, calcium disodium ethylenediamine tetracetate (Ca EDTA) markedly stimulates elimination of lead from the bodies of patients suffering from lead poisoning. This is accompanied by significant clinical improvement of the patients. The majority of these clinical reports concern the use of parenteral (intravenous) administration of Calcium Versenate in acute lead poisoning in children and adults. There are a few published reports on the oral administration of the drug^(7,8); these indicate that Ca EDTA is effective orally, but that oral therapy does not eliminate as much lead as can be accomplished by parenteral therapy.

In Japan, potential, chronic lead poisoning with symptoms including coproporphyrinuria, abdominal pain, and anemia, has frequently been reported among lead workers, notably in storage battery and lead refining plants. The liver concentrate extracted from cattle liver according to a special procedure⁽⁹⁾ devised by one of us (KT) was applied to those workers, as one of the preventive therapeutic measures for the chronic lead poisoning. The administration of the liver concentrate improved the symptoms moderately, especially the anemia associated with this poisoning, but the amount of lead in the urine was not decreased.

We therefore became very much interested in trying Ca EDTA in these cases. A supply of parenteral and oral Ca EDTA was obtained and used on a number of hospitalized patients with acute lead poisoning (parenteral therapy), as well as in patients with subacute or chronic lead poisoning, who were still actively employed (oral therapy).

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In our animal experimental work, which was done at the same time as the clinical tests, we paid particular attention to the liver functions, particularly the albumin and globulin ratio, and the total protein in the serum. In addition, the distribution of lead in the blood of dogs with artificial lead poisoning, as well as other symptoms of lead poisoning were observed closely. To our knowledge, such studies have not been done previously. In the lead workers who received oral therapy, a close check was kept on their symptomatology; in addition blood and urinary lead determinations were done once monthly for 10 months.

METHODS AND MATERIALS

The present study is divided into three parts:

1) Investigations of the effect of intravenous injection of Ca EDTA upon liver function, particularly on A/G ratio in the serum of lead poisoned dogs; on the change of the pattern of lead distribution in the blood after intravenous administration of the drug, and on the difference in lead content in the organs between the Ca EDTA administered dog and the control.

2) Observation of the change in the amount of lead and its distribution, in the blood and in the urine of the lead workers, with chronic potential lead poisoning, after intravenous administration of Ca EDTA.

3) Studies on the lead eliminated from the urine after oral administration, as compared with that after the intravenous administration; on the monthly changes of the amount of lead in the blood and urine of the lead workers in a small lead refining plant, over a long period of oral administration; and on the changes of symptoms including anemia due to the lead absorption by these workers.

In the first experiment, 3 mg of lead per kilogram of body weight (in the form of lead acetate) was injected subcutaneously three times weekly for a total of ten times in about three weeks. Two and half ml of 20% Ca EDTA, diluted to 300 ml in 5% glucose solution, was given three times weekly, i.e. 0.5 gram to each, given by intravenous drip over a period of one hour on the days when lead was not administered. The amount of 0.5 g of Ca EDTA is calculated to be in excess of the equivalent of 3 mg of lead to be chelated. The U.S.P.H.S. standard method was applied to analysis of lead. The serum total protein was estimated by the Kjeldahl method, and protein fractions in the serum were determined by means of the sodium sulfite precipitation method.

In the second clinical experiment, a few lead workers suffering from chronic lead poisoning, with a slight subjective complaint of abdominal pain, and moderate

anemia, but not disabled for work, were admitted to the university hospital. These were treated with Ca EDTA, and some of the results will be reported here. The method of therapy in these patient is indicated in Fig. 6.

In the third clinical experiment, the effect of oral Ca EDTA on the lead refining workers, with chronic lead poisoning, was observed before the drug was given. At the end of May 1954, the amount of lead in the blood and urine were estimated, and the clinical pictures such as calcium level, specific gravity of whole blood, hemoglobin value, red cell count, hematocrit, coproporphyrinuria, lead line of the gum, and also other subjective symptoms were evaluated. All this was done once monthly during therapy with the exception of December to February; during this period the therapy was discontinued as a control measure, and it was resumed at the end of January. It was not possible to check throughout the whole period as we had anticipated. The daily dose of the drug given to the workers was one gram in June, July and August, two grams in September and October, and four grams in February. The total doses administered to each person, from June to October, and February are shown in Fig. 12. During the therapy, the workers were kept occupied as usual, and the rates of monthly working hours are indicated in Fig. 12, in comparison to the figure for May as 100%.

RESULTS

1) *First experiment*

The data on hemoglobin, red cell count, basophilic stippled cell count, reticulocyte count, white cell count, and the body weight of the Ca EDTA injected dog as well as the control dog are shown in Fig. 1 and Fig. 2. From these figures, it is apparent that Ca EDTA is very useful in preventing the onset of poisoning, as well as in recovery from the poisoning symptoms. Although the total protein in the serum of the control dog was decreased and A/G ratio of serum was also diminished to 0.5 on the 42nd experiment day (Fig. 3), the Ca EDTA injected dog recovered the pre-experimental level in total protein, and the A/G ratio reached nearly the same level on the same day. A significant difference of protein fractions between two dogs was not apparent, although γ -globulin of both dogs was increased gradually.

Changes of distribution of lead in the blood are shown in Fig. 4 which indicates that the amount of lead in the plasma was only about 25 γ /dl even when that of the red cells reached 409 γ /dl in the control dog on the thirty-sixth day of the experiment. In the Ca EDTA administered dog, however, the lead distribution evidently differed from that of the control; namely, on the same

Fig. 1

Fig. 2

○—○: CaEDTA (↑ each 0.5 g in 5% glucose)
△—△: Control (5% glucose only)

Fig. 1 & 2 Effect of CaEDTA injection on symptoms of lead poisoning. (dogs)

Fig. 3 Effect of CaEDTA injection on plasma protein in lead poisoning. (dogs)

Fig. 4 Changes of distribution of lead in blood one hour after intravenous injection. (dogs)

Fig. 5 Lead amount in urine and feces. (dogs) (Determined before every inj. of Ca EDTA).

experiment day, the lead content of the plasma increased up to 60 γ /dl, although that of the red cells was estimated only as 100 γ /dl. (Fig. 4) Lead content in the urine and feces, collected shortly before each Ca EDTA injection, was analysed. The results showed that the urinary lead excretion in the treated dog was between 0.01 mg and 0.48 mg throughout the whole experiments, while that of the control dog had already reached 2.0 mg on the twenty-second experimental day. There would appear to be hardly any difference between the two dogs in the trend of lead excretion from the feces. (Fig. 5) It is our opinion, however, that the essential nature must be quite different, as pointed out in the "discussion". On the 45th experiment day the dogs were killed under

Table 1
Lead amount in organs (Dogs)

	Liver	Kidney
Ca EDTA Administered Dog	1.21 mg %	0.77 mg %
Control	2.31	2.52

the anesthesia by ether and the quantity of lead in the kidneys and livers were estimated. The quantities in both organs of the treated dog were approximately one half of the control in the liver, and one third in the kidney. (Table 1)

2) *Secend experiment* (Clinical study, intravenous injections)

There is good evidence that intravenous administration of Ca EDTA is effective in acute lead poisoning. It seems, therefore, unnecessary to report here all results obtained by the authors. On the other hand, some results concerning distribution of the lead in the blood, and the trend of lead elimination from the urine in a case of chronic, potential lead poisoning should be reported. Our method of treatment by parenteral Ca EDTA administration is shown in Fig. 6. The maximum excretion appeared in the urine within three to six

Fig. 6 Changes of lead content in urine after intravenous administration of CaEDTA.
(Lead-worker. 2 weeks after isolation from lead exposure)

hours after every intravenous drip injection. But the maximum lead amount was decreased gradually with time. After interrupting the injections, from the twenty-first to the thirty-first day of admission, the maximum amount of lead after the first injection after resumption of treatment remained nearly the same as the preceeding amount. This phenomenon seems to indicate that lead from the hard tissue penetrated into the blood stream during eleven days of interruption of the treatment. A virtually similar trend was observed in all the other treated cases.

The amount of lead in the whole blood usually decreased one hour after injection, although a faint rise was seen on about the twentieth day. With continued treatment, the lead in the blood decreased gradually. The lead in the plasma did not rise as high as that seen in the experimental dogs, although

Fig. 7 Changes of lead level and its distribution in vein-blood after intravenous administration of CaEDTA.
(Lead worker. 2 weeks after isolation from lead exposure)

some slight increases were apparent immediately after injections. (A discussion of this contradiction will follow later.) (Fig. 7)

Fig. 8 Lead content in urine after oral administration of CaEDTA.

Fig. 9 Comparison of maximum urinary lead contents between intravenous and oral administration of CaEDTA.

3) *Third experiment* (Prophylactic oral, therapeutic administration to lead workers)

The maximum lead excretion was seen in the urine, three to six hours after a single oral administration, (Fig. 8) The rise of the maximum urinary lead concentration is on the order of one tenth to one twentieth, compared with that seen with parenteral administration. (Fig. 9) Fig. 10 and 11 show the trends of the lead levels, in both the urine and blood, from May, when the drug had not been administered, to March of the next year. During this period oral therapy was given as shown. Samples for analysis were collected after a two day interruption of therapy to avoid the concurrent effect of the drug on the increased level of lead in the urine or blood. In July, about one and a half months after beginning of treatment, the lead levels in both the urine and blood remained the same as the pre-experimental level. In August, September and October, they decreased gradually in general, although some exceptions were recognized. This may be attributed to the variable range of lead levels in both the urine and blood. A slight rise was seen in the urine, but not in the blood again in November, about twenty days after cessation of the therapy. The decrease of the urine lead content, from May to October was not statistically significant, whereas the decrease of the blood lead level, from May to November, was significant ($P < 0.05$). In January, twelve weeks after cessation, they increased again to a level almost equal to the preexperimental level. The levels in March, only one month after resumption of treatment, remained nearly the same as in January, just as was seen in May and July, although the dose of the drug was

Fig. 10 Monthly change of lead content in urine.

Fig. 11 Monthly change of lead content in blood.

increased to four grams a day during this period.

Among the many features of the estimated clinical pictures, the data on the whole blood gravity are presented here, as a representative feature of chronic, potential lead poisoning.

Fig. 12

The increase or decrease of the blood gravity was almost symmetrically parallel with the lead level in the urine or blood. The increase, from May to

November, was statistically significant ($P < 0.05$). These changes could be reasonably correlated with the view of the interrelation between the lead content in the body, and the grade of anemia due to the poisoning.

DISCUSSION

It is well established that Ca EDTA is highly effective in mobilizing, *in vivo*, and in causing rapid excretion of the lead in the urine, particularly after parenteral administration.

P. E. Karpinski, et al.⁽¹⁾, noted that administration of Ca EDTA was followed by prompt rise in the level of lead in the blood, indicating mobilization of lead from extravascular tissues. In our clinical experiments, however, this was seen rarely, although a slight rise occurred in a few patients. This difference in results might be attributed to the fact that Karpinski's cases were acute, and ours were chronic, and in chronic cases the soft tissue presumably contained significantly less lead than is seen in acute cases.

At the end of the first course of treatment (in clinical experiment) the lead levels in the blood and urine were obviously decreased. From the standpoint of the trend of the treatment courses, the lead levels on the first day of the second treatment course should be lower than the last day of the first treatment course. Actually, however, the lead levels on the first day of the second treatment course showed nearly same values as were seen on the last day of the first treatment course. This is thought to be due to diffusion of lead (perhaps chiefly from the bone) into the blood stream during the period of interruption of therapy. According to our types of experiments, the results lead us to agree with Karpinski's corollary that the storage places for lead in the body are not reached readily by Ca EDTA.

The lead distribution in arterial blood, as shown by animal experiment, was changed one hour after EDTA injection, although it was apparently not seen in the cases of chronic lead poisoning. This is probably due to the fact that the lead released from erythrocytes does not remain in the blood stream, but is lost rapidly, as H. W. Ryder⁽¹⁰⁾ has reported. We utilized different sites for taking the sample blood; in the dogs, it was taken from the artery, where the Pb EDTA was formed and remained as it was, before reaching the kidneys for filtration to the urine. In the lead workers it was taken from the vein and most of the Pb EDTA has already been excreted in the urine from the kidneys before the sample was taken. There is another problem concerning the change of the distribution and the means of increase of the lead in the serum. We believe it reasonable to assume that the lead should have been liberated from

the extravascular tissues, and also from the red corpuscles in which most of the lead was found, as has been shown by many investigators.

The decrease of the A/G ratio was not prevented fully, even in the Ca EDTA treated dog, despite the fact that we were able to keep other findings, particularly red cell count, and hemoglobin nearly within normal limits. Other investigations have reported that the A/G ratio is decreased very early in chronic lead poisoning^(11,12). In order to ascertain to what extent this change of the A/G ratio is due to the impairment of the liver by lead, further study is necessary. It was noted, however, that the decrease was slighter, and recovery was more rapid in the treated dog.

In our other experiments⁽¹³⁾ on dogs and goats poisoned by injections of lead, the lead excretion from the feces was initially extraordinarily high, and it began to decrease as the liver function was impaired. Again, in this experiment the control dog only with lead showed the same trend. The decrease of lead excretion at the terminal stage, in the treated dog, indicates recovery from the lead poisoning, as well as diminution of the amount of lead retained in the body. (Table 1) The decrease in the control dog may, however, indicate the result of excretory disturbance, due to the impairment of liver function.

The maximum increase of the amount of lead in the urine by a single oral dose was only one tenth to twentieth of that seen with an intravenous injection. If the chelating ability of Pb EDTA by intravenous injection is assumed to be one hundred per cent, that by oral administration would range between five to ten per cent. This indicates that the absorption of oral Ca EDTA from the intestinal tract might be five to ten per cent of the total administered dosage, and agrees with the report of Foreman⁽¹⁴⁾ as well as of Sidbury⁽⁷⁾. Foreman reported that the excretion of Ca EDTA (oral administration), from the urine was 4.2~2.0 per cent of the dosage administered. Absorption from the intestine may be very low, due to the decomposition of Ca EDTA, according to our experiment in vitro, made with the artificial stomach juice in which calcium was liberated and precipitation was apparent. Administration of enteric, coated tablets should be tried in the future.

Oral administration of Ca EDTA as prophylactic therapy for lead workers proved successful, and its use for a long period divulged no harmful reactions. The problem of dosage is still unsolved, because increase of the daily dose has not improved the response. As the lead concentration in environmental air varies over a wide range, and the absorption of lead into the body also varies, the dosage should be adjusted to the symptoms, not to the amount of lead of the air. In the present experiment, however, a significant difference in effective-

ness between one gram, and four grams, daily was not apparent. It may be that there is a limit of absorption from the gut, and further study is required to determine the optimal daily dose. At all events, it is important to keep in mind that when Ca EDTA is administered orally, improvement in symptoms, and decrease in urinary lead will not be seen (to a significant degree) for some time, usually about 2 months, and increase of the daily dosage (orally) will not hasten this response. Short term oral therapy has little value prophylactically, unless the workers are removed completely from lead exposure. It is, furthermore, advisable to conduct additional long term studies (for 1 year, or more) to determine whether deleterious reactions may be seen from long term administration.

SUMMARY

Experiments on the effectiveness of Ca EDTA upon lead poisoning were undertaken, on dogs and lead workers.

1. In the animal experiments the following conclusion were reached.
 - a. The parenteral administration of Ca EDTA changed the distribution of lead in arterial blood, and plasma lead was increased. This is thought to come from the red corpuscles in which the greatest portion of lead in the blood was found when lead absorption occurs, and also from the extravascular tissues.
 - b. Decrease of A/G ratio was not prevented by the administration of the drug, although the symptoms, including anemia, were kept normal. This indicates that the decrease of A/G ratio appears in the earlier stage of lead poisoning, with the exception of coproporphyrinuria, as has been stated previously.
2. Clinical and prophylactic use of the drug yielded the following results.
 - a. Intravenous therapy did not increase the lead concentration in the blood but the urinary lead showed its highest level in the urine after three hours administration.
 - b. Higher urinary lead excretion by oral administration was observed in only a few patients in contrast with the results seen by intravenous injection. This indicates that the absorption from the intestine is very low, following the oral administration.
 - c. Oral administration (which was extended over a period of more than six months) for the purpose of preventing chronic, potential lead poisoning, was successful in decreasing both the blood lead and urine lead quantity, in preventing appearance of the symptoms, and in improving anemia.
 - d. Further study is necessary to determine a proper, daily, oral dosage.

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