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Induced Accumulation of Citrate in Therapy of Experimental Lead Poisoning.* (22468)

JOAN F. FRIED, MARCIA WHITE ROSENTHAL, AND JACK SCHUBERT.
(Introduced by A. M. Brues.)
Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill.

Chelating or complexing agents are widely used to counteract the toxicity of metal ions and to accelerate the elimination of radioelements from the body. Since the distribution of administered water-soluble chelating agents is primarily extracellular it is unlikely that these chelating agents are in sustained contact with cellular components. For this reason we have explored the possibility of interfering with metabolic processes *in vivo* so that certain compounds which are naturally present in the body and which possess chelating or complexing properties might be maintained in abnormally high concentrations within the cells and thus be utilized to modify metal toxicity. Citric acid is a compound which is intimately involved in the Krebs cycle and which possesses complexing properties. Citrate ion, Cit^{3-}, forms a stable complex with Pb^{++} ($PbCit^{-}$) under physiological conditions; the logarithm of the formation constant for this reaction is 5.74(1). For these reasons, lead was chosen as the toxic agent and citrate as the metabolic chelating agent. Sodium citrate in clinical use has resulted in symptomatic relief in some cases of lead poisoning(2-4). However, it was necessary to administer large doses at frequent intervals over fairly long periods of time, because citrate ion, administered either orally or parenterally, disappears rapidly by excretory and metabolic pathways(5). These facts suggested that an induced maintenance of high citrate levels *in vivo* might be of greater therapeutic value than the repeated administration of exogenous citrate(6). Administration of sodium fluoroacetate (FA) in lethal doses has been shown by Peters *et al.*(7) to produce synthesis of fluorocitric acid which acts as a competitive inhibitor in the enzymatic destruction of citric acid in the tissues(8-10). It has been shown(6) that a single injection into rats of a small, non-lethal dose of FA causes a pronounced accumulation of citric acid in many tissues though not in blood. While the control levels of citrate in the kidney and spleen were roughly 20 μg and 70 μg per gram fresh tissue respectively, maximum levels of more than 10 times normal were reached in the kidney, spleen, and heart within 4 to 6 hours and high levels were maintained for several more hours.

In the present experiment the survival of animals given toxic doses of Pb^{++} ion and treated with small, sublethal amounts of fluoroacetate has been compared with that of citrate-treated and untreated animals.

Materials and methods. The animals used were 78- to 181-day-old Sprague-Dawley female rats weighing between 225 and 270 g. In each series of experiments animals were

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TABLE I. Effect of Citrate on Survival of Pb^{++} Poisoned Animals, 4 Groups.

Treatment post Pb^{++}			Survival	Time to 50% death (days)
1 min.	3 hr	6 hr		
C*	0	0	0/10	.63
C	C*	0	0/10	.67
C	C	C*	1/10	.67
S*	S*	S*	2/10	.79

* C = Citrate at 250 mg/kg; S = Saline.

evenly selected from 2 age groups; differences between these 2 groups were not statistically significant in terms of the experimental results. The animals were maintained on a diet of Purina chow and water *ad libitum* throughout the experiment. They were grouped together in such a manner as to minimize "cage effects." Lead was administered to all animals in a single injection into the tail vein as a 6.4% solution of lead nitrate ($Pb(NO_3)_2$), in doses ranging from 50-70 mg/kg as Pb. The FA-treated animals received intraperitoneal injections of a 0.005% solution of sodium monofluoroacetate (0.30 mg/kg per injection) at 22 and 5 hr prior to and at 8, 19, and 25 hr after the lead injection (a total of 1.5 mg/kg as FA over a 3-day period). Preliminary experiments indicated that this dosage schedule provided the maximum degree of protection with the minimum of toxic effects. The citrate-treated animals received intraperitoneal injections of 3% sodium citrate solution (250 mg/kg) after the lead injection at the time intervals listed in Table I. Control animals received saline intraperitoneally in equivalent volumes at corresponding time intervals.

Results. Three series of experiments were performed. In the first, 30-day survival was compared in FA-treated and untreated animals following an approximate LD_{90} dose of lead nitrate (70 mg/kg as Pb). The 2 groups (40 treated animals, 70 untreated) each contained an equal number of animals aged 114 and 160 days. The survival in the FA-treated group was 53%, while that in the untreated (saline control) group was 10%. The difference in survival data for the 2 groups was statistically significant: $P << 0.01$. There was no significant difference in time to 50% death, which was 1½ days.

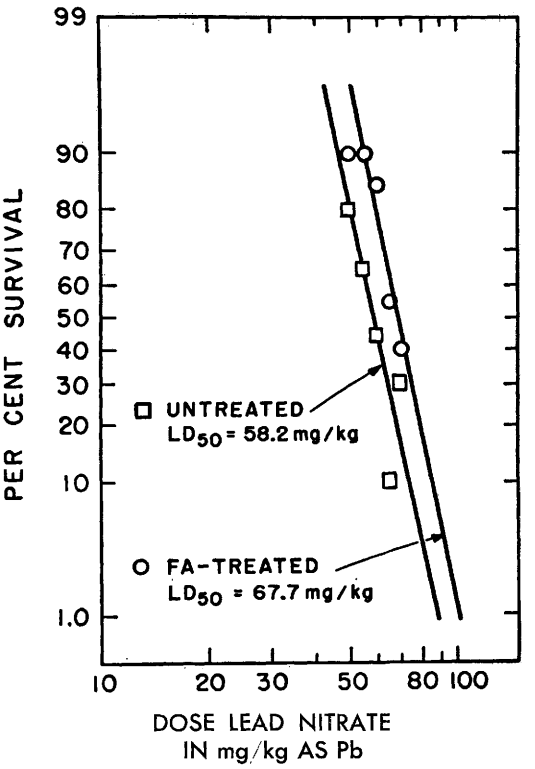

FIG. 1. Survival curves of fluoroacetate-treated and untreated (saline control) groups. Each point represents data from 20 animals except at the lowest dose for the control group and the 2 lowest for the FA-treated, where 10 animals were used.

The second series of experiments was designed to evaluate the shift in the LD_{50} value of Pb for the FA-treated as compared with the untreated. One hundred and seventy 78- and 136-day-old rats were used. Probit curves of the survival data and the LD_{50} values for the FA-treated and untreated groups (Fig. 1) reveal an increase in the LD_{50} of lead nitrate from 58.2 mg/kg (± 4.3%) for the untreated (saline controls) to 67.7 mg/kg (± 3.1%) as Pb for the FA-treated. This difference is significant at the 5% level ($.05 > P > .02$). The curves show no significant difference in slope ($t_b = 0.32$).

A third experiment was performed to determine the effect of repeated intraperitoneal administration of citrate. Animals (181-day-old) were all given lead nitrate (60 mg/kg as Pb) followed at various time intervals by citrate. The results (Table I) reveal no sig-

nificant differences in survival among these groups, and for this reason no further survival studies were undertaken with administered citrate.

Discussion. The similarity in slope of survival curves obtained from the FA-treated and untreated animals supports the explanation that the mechanism of protection is a simple one in which the concentration of toxic lead ions is lowered by the *in vivo* formation of the complex $PbCit^-$. Even though only partial protection was obtained by treatment with FA, the experimental results demonstrate the feasibility of utilizing an induced *in vivo* stimulation of a naturally occurring chelating or complexing agent for the treatment of metal poisoning.

It is conceivable that other metabolic inhibitors may be even more effective in poisoning by lead and other metal ions, since they may induce the accumulation of other naturally occurring chelating agents with more favorable or more prolonged tissue distributions relative to that of the metal. In the present experiment, gross observations at autopsy and hematuria indicated that kidney damage contributed to the death of the lead-poisoned animals. Thus it is possible that the major protective effect of FA resulted from the elevation of intracellular citric acid levels in the kidney. It can be estimated from earlier data (6) that the citrate concentrations in the FA-treated animals were maintained at about 0.5 mg/g of spleen and about 0.3 mg/g of kidney through the course of treatment, but remained near normal levels (~0.03 mg/g) in the blood. Thirty minutes after the injection of 250 mg/kg of sodium citrate the blood citrate level was about 0.6 mg/g whole blood but returned to the normal level within 1½ hours; all other tissue levels remained essentially unchanged. Sustained high citrate levels in the blood have been reported following injection of guanidine compounds in rabbits(12), but we were unable to reproduce this result in rats. Metabolic inhibitors which would induce the accumulation of naturally occurring chelating agents with stronger formation constants for the toxic metal under physiological conditions(11) would also be more effective.

Summary. The concept of interference in a metabolic cycle as a means of modifying metal toxicity has been tested. The accumulation of citric acid in certain soft tissues of the rat has been induced by administration of small, non-lethal doses of sodium fluoroacetate. This has been found to give partial protection to rats acutely poisoned with lead nitrate. Of rats given the LD_{90} of lead nitrate, 53% survived when treated with sodium fluoroacetate. The LD_{50} of lead nitrate was increased from 58.2 mg/kg (as Pb) in saline controls to 67.7 mg/kg in fluoroacetate-treated rats.

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