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LEAD TRANSFER IN MATERNAL MILK, AND THE ABSORPTION, RETENTION, DISTRIBUTION AND EXCRETION OF LEAD IN SUCKLING MICE

by

Charles Arthur Keller

MASTER

Submitted in Partial Fulfillment

of the

Requirements for the Degree

DOCTOR OF PHILOSOPHY

Supervised by Richard A. Doherty, M.D.

Toxicology Training Program
Department of Radiation Biology and Biophysics
School of Medicine and Dentistry

University of Rochester

Rochester, New York

1980

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Vita

Charles Arthur Keller was born on [redacted]. He graduated from our Lady of Lourdes High School (Poughkeepsie, New York) in 1972. He received the B.S. degree cum laude in Biology from Union College in 1976. He began studies in the Toxicology Training Program at the University of Rochester School of Medicine and Dentistry in September, 1976. During this period he was supported by the Pharmacological Sciences Training Grant from the Public Health Service. Dr. Richard A. Doherty was his research advisor.

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Abstract

Young children and young experimental animals are known to be more sensitive to the toxic effects of lead exposure. Studies were conducted to define differences between suckling and adult mice in absorption, retention, distribution and excretion of lead which might account for the age differences in lead toxicity. In addition, a lactating mouse model was developed to study the quantitative aspects of lead transfer from lead-exposed mothers to suckled offspring.

Suckling mice were found to absorb and retain a greater fraction of an oral lead dose than did adult mice. It was proposed that increased intestinal pinocytotic activity in suckling mice could account for the increased absorption relative to that observed in adults. The validity of the hypothesis was supported by the results of numerous experiments which examined lead uptake from the intestine of suckling animals. Pinocytotic activity in the small intestine was quantitated by the measurement of mucosal uptake of polyvinyl-pyrolidone (^{131}I-PVP), a biologically inert macromolecule. Pinocytotic activity and lead uptake (in vivo) were found to be greatest in the distal small intestinal tissue. Uptake of PVP and lead were very much less in adult small intestinal tissue. Cortisone pretreatment results in precocious cessation of pinocytotic activity in the intestine of suckling mice. Cortisone pretreatment of 6 day old mice produced decreases in carcass lead retention and pinocytotic activity when lead was orally administered at 12 days of age.

Pretreatment with cortisone was also found to reduce lead absorption following intraluminal lead injection (in situ) into the ileum of suckling mice. However, cortisone pretreatment did not affect in situ lead absorption from the jejunum of suckling mice. Cortisone pretreatment of adult mice had no effect on whole body lead retention or intestinal tissue content of lead or PVP following an oral dose. The data indicate that the distal small intestine is the site of active pinocytosis of lead, and that pinocytosis is the major mechanism involved in lead absorption in suckling mice.

Single doses of lead were administered orally to suckling and adult mice. Developmental differences were observed in the percentage of lead retained in the whole body. Both groups exhibited dose-independent lead retention when doses of 4 to 445 mg/kg were administered, indicating a first-order absorption process for each age group. However, a much greater proportion of the administered lead dose was retained when carrier-free ^{203}Pb was administered. The results were consistent with mechanisms of gastrointestinal lead absorption comprising two or more processes.

Lead distribution and elimination from organs differed between suckling and adult mice. Developmental differences were observed in organ lead concentration for kidneys and brain following oral doses. Relative distribution of lead to the brains of suckling mice were greater than to adult brains. Lead uptakes into brain and bone were linearly related to lead dose for both suckling and adult age groups. The relationships between lead concentrations of blood and organs

were shown to be non-linear relative to lead dose. Whole body and bone lead elimination rates were reduced in suckling compared to adult mice. Brain lead elimination rates did not differ in suckling and adult mice.

A lactating mouse model was developed to study lead transfer to suckling offspring. Lead was mobilized from the bone of lactating mice and was transferred in milk to suckling offspring from mothers which had previously ingested lead in the drinking water. The net transfer of lead was greatly increased when the lactating mother ingested lead in drinking water continuously during lactation. Relative lead transfer to suckled offspring during lactation greatly exceeded transfer to fetuses during gestation. Lactation resulted in an increased rate of maternal lead elimination following single lead doses as well as after cessation of chronic lead exposure. Lead concentration in milk exceeded plasma concentration by a factor of approximately 25, indicating that there is a physiological process(es) which establishes a large milk to plasma concentration ratio. A similar calcium concentration ratio was also observed. The mouse model which was developed could provide useful information on estimating potential hazard associated with lead exposure of women of child-bearing age.

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PUBLICATIONS

Work presented in this thesis has been published and presented as follows:

1. "Effect of Age and Dose on Lead Retention." Abstract, Toxicol. Appl. Pharmacol. 48:A70, 1979; presented at 1979 Society of Toxicology Meeting.
2. "Role of Pinocytosis in Lead Absorption." Abstract, Physiologist 22:67, 1979; presented at 1979 Fall American Physiological Society Meeting.
3. "Possible Relationship Between Lead and Calcium Transport into Milk of the Lactating Mouse." Abstract accepted for presentation, 1980 Society of Toxicology Meeting.
4. "Lead and Calcium Distributions in Blood, Plasma, and Milk of the Lactating Mouse." J. of Lab. Clin. Med. 95:81, 1980.
5. "Effect of Dose on Lead Retention and Distribution in Suckling and Adult Female Mice." Toxicol. Appl. Pharmacol. in press, 1980.
6. "Distribution and Excretion of Lead in Young and Adult Female Mice." Environ. Research in press, 1980.
7. "Correlations Between Lead Retention and Intestinal Pinocytosis in the Suckling Mouse." Accepted for publication, Am. J. Physiol.
8. "Bone Lead Mobilization in Lactating Mice and Lead Transfer to Suckling Offspring." (Submitted for publication.)

List of Terms Used

The following terms have been used throughout this thesis and are defined as follows:

a) Fraction of Initial Dose (FID)

FID = (ng of Pb in sample, time t) ÷ (ng Pb in whole body, time 0)

FID is used to relate the total lead content of the whole body, carcass, or organs to the initial dose of lead administered. (FID x 100 = % of Initial Dose)

b) Body Concentration Ratio (BCR)

BCR = (ng of Pb/g organ, time t) ÷ (ng of Pb/g whole body, time t)

BCR is a measurement which enables the comparison of lead concentration in an organ relative to the "average" whole body lead concentration at the time of necropsy. It is especially useful in comparing the distribution of lead to organs when differing initial lead doses are administered.

c) Multiple of Initial Body Concentration (MIBC)

MIBC = (ng of pb/ml fluid, time t) ÷ (ng of pb/g whole body, time 0)

MIBC is a data transformation which normalizes fluid lead concentration for variance in the initial lead dose. It is essentially equivalent to measurement of concentration (ng/ml) except that there is correction for initial dose.

Each of these measure (FID, BCR, and MIBC) are used to quantitate lead contents of the whole body, carcass, blood or organs of individual mice, since the initial dose (ng of Pb whole body, time 0), the initial concentration (ng of Pb/g whole body, time 0) or the body concentration (ng of Pb/g whole boyd, time t) can be measured in each mouse. This enables a higher degree of precision than if group "average" initial doses, initial concentrations or body concentrations were used as denominators in the above equations.

I. INTRODUCTION

The history of human lead exposure begins with the mining of the metal during Roman times. Grandjean (1978) calculated that current lead intake is approximately two orders of magnitude greater than intake in prehistoric man. Ericson et al. (1979) has determined that skeletal lead levels are three orders of magnitude greater in present-day skeletons than in ancient Peruvian skeletons. As is the case with many toxic chemicals, the hazards associated with occupational exposure to large amounts of lead became apparent before the age of scientific investigation. With the realization of these spectacular toxic effects (encephalopathy, serious anemia) exposure levels have been reduced with a consequent shift in the observed toxic effects. Current lead toxicologic research is aimed at estimating safe exposure limits relative to subtle neurological and behavioral effects.

Lead hazard-benefit analysis is unusual in that current permissible occupational exposure levels of some workers may result in observable biological effects. Generally an attempt is made to limit human exposures to toxic substances to levels at which no biological or toxic effects are observed. A "safety factor" of one or more orders of magnitude is often applied when determining permissible levels of human exposure from the results of animal studies. The use of "safety factors" are common when animal toxicity data are extrapolated to human exposure limits so as to account for species differences in toxicity, metabolism or pharmacokinetics. In the

case of lead exposures, either occupational or environmental, it is critical that the biological and toxicological effects of lead be distinguished. Controversy often arises in discussion of whether an observed biological effect constitutes a toxic effect. If the biological effect does not result in an immediate or future decrement in biological function or developmental potential, and does not produce an irreversible biological change then the effect cannot be considered a manifestation of toxicity. At 40 mg/100 ml blood lead concentration hematopoietic effects are evident, but due to the reserve adaptive capacity of hematopoiesis the biological effect cannot be considered a toxic effect. However, this biological effect provides a useful clinical index for monitoring an individual's exposure to lead. The large capital costs involved in decreasing occupational lead exposure makes it uneconomical to reduce lead levels to the lowest technicologically feasible level in all work environments. Becasue of this, regulatory agencies in the United States, using available scientific data, have targeted the permissible occupational exposure (50 mg/m^3 air concentration, 8 hr. average) to result in blood lead concentrations below 40 mg/100 ml in 70% of those exposed (Bingham, 1978). At this blood lead concentration there is concern about potential toxic effects of lead on the peripheral nervous system. Lead induced neurological and behavioral effects in adults have been reviewed by Repko and Corum (1979).

While non-occupational cases of lead toxicity are rarely seen in adults, a large number of children probably are being adversely

affected by lead. The removal of lead pigments in paint from the child's environment and the dramatic reduction in the use of leaded gasoline are expected to reduce childhood lead exposure. The extent of lead exposure which consitutes a hazardous level for children is controversial. Similarly, the proper biological indicator(s) for measurement of the toxic effects of lead exposure is currently being debated. The choice of a biological measure of potential toxicity is made based upon the correlation observed between the biological manifestation of lead exposure and a presumed toxic effect. Biological manifestations in current use include blood lead concentration, urinary lead excretion, delta-aminolevulinic acid (S-ALA), aminolevulinic acid dehydratase (ALAD) in erythrocytes, zinc protoporphyrin (ZPP; also known as free erythrocyte porphyrin in older literature) and bone or dentine lead concentrations (reviewed by Posner, 1977).

The debate over childhood lead exposure has been stimulated by the recent publication of Needleman et al. (1979). Their work indicates that there is an excellent correlation between dentine lead concentration and behavioral deficits in children. While their behavioral performance tests do not include new or more sensitive indicators of toxicity, the use of dentine lead concentrations represents an important application of pharmacokinetic parameters to clinical studies. By the time most children are tested for behavioral deficits (in the primary grades) current lead exposure may be well below that which has occurred previously. Even if lead exposure has been relatively constant, blood lead concentrations will reach an

equilibrium plateau (Benson et al., 1976) within a period of months. Blood lead would not indicate cumulative lead exposure. However, due to the long biological half-life of bone lead (Rabinowitz, 1976; Batschelet et al., 1979; Sugita, 1979) it would be expected to be a better indicator of past history of lead exposure. Needleman et al. (1979) found a significant correlation between increasing dentine lead and decreasing classroom performance. Experimental design provided controls for most, but not all, complicating variables.

These findings represent a direct application of animal research studies to the difficult problem in cross-sectional studies of identifying individuals with excessive exposure to lead. Additionally, most studies which attempt to identify children exposed to excessive amounts of lead are retrospective, since screening programs usually begin in the primary grades after the age when lead ingestion is thought to be greatest. Longitudinal studies, conducted over a period of years, would provide both valuable research data and the identification of those exposed to excessive amounts of lead. However, the costs and complications of conducting longitudinal studies necessitates the development and verification of cross-sectional screening protocols.

Given the large variations in individual performance and childhood development which are observed, it is critical that a toxicologically valid indicator of lead exposure be employed in order to find strong positive correlations between exposure and effect. It is likely that improved measures of behavior and

performance will further increase the sensitivity of dentine lead screening. Furthermore, it is probable that the estimated no-effect level of lead exposure will be lowered as more sensitive and reliable behavioral tests are developed.

The cause of pediatric lead intoxication has been the subject of extensive speculation. It has been hypothesized that young children are at greater risk of intoxication because of increased exposure as a result of hand to mouth activity and ingestion of non-food materials (PICA), increased gastrointestinal absorption of a given dose, increased organ retention of lead, differential distribution to sensitive organs (principally the brain) and/or increased sensitivity of the developing brain to the toxic effects of lead.

This thesis is limited to a consideration of developmental differences between young and adult animals in lead absorption from gastrointestinal tract, in organ distribution of lead, and in lead excretion. The findings have direct relevance in delineating possible approaches to assessing the impact of human lead exposure and to defining a susceptible subpopulation of individuals. It is apparent that in the case of human lead exposure the permissible lead concentrations in air, water or diet must be a function of the exposed individual's age.

The remainder of the thesis will report the development of an animal model for assessing the absorption, distribution and excretion of lead in young animals, and for estimating the transfer of lead from exposed lactating mothers to suckled offspring.

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concentration is the most widely employed index medium for the evaluation of current lead exposure. Thus, there is no available comparison between milk lead concentrations and blood or plasma lead concentrations. If such data existed then correlation between maternal exposure and milk concentration could be evaluated. Additionally, the reported human studies do not provide information relating to possible differences in the recent exposure history of the mother, which as will be shown with mouse data may be an important parameter determining milk lead levels. Human data from a cross-sectional population study do not provide a basis for determining the effect of past lead exposures on milk lead concentration. The relationship between human milk lead concentrations and blood or plasma lead concentrations must be determined in order to assess the applicability of animal studies to the potential hazard of exposure of lactating mothers to lead.

The transfer of lead to neonates suckled by lead exposed mothers has been studied experimentally. Pentschew and Garro (1966) found that lactating rats fed a 3% lead diet produced milk containing a lead concentration of 45.9 µg/ml. This was the first experiment which demonstrated neonatal toxicity in suckled animals in the absence of maternal toxicity. Unfortunately their report did not provide information on the maternal body burdens or maternal blood levels of lead. Michaelson and Sauerhoff (1974) measured milk lead levels of 25 µg/ml in lactating rats which had been fed a 2.7% lead diet for 16 days. No blood or body burden data were provided.

Kostial and Momcilovic (1974) studied lead and calcium retention in rat pups of 4 and 15 days of age suckled by mothers given a single intravenous injection of radiolabeled lead and calcium. The authors performed a single whole body radioactivity determination of the entire litter 2 days following injection. Four day-old pups retained 10% and 15 day-old pups, 14% of the initial maternal lead dose. Calcium retentions by the litters at these two ages were 37.9% and 51.9% of the initial maternal dose. The authors hypothesized (but provided no supporting data) that mammary transport of lead might be related to active calcium transport. Whole body retentions in lactating rats were reduced compared to control females. The data presented indicated that milk is an important route of lead elimination in the rat and that lead secretion in milk may contribute significantly to increased elimination of lead in the lactating compared to the nonlactating rat.

Bornschein and co-workers (1977) have studied lead retention in 12 day-old rat pups suckled by mothers exposed to 1.1 mg/ml of lead in the drinking water. Milk lead concentrations of 2.5 and 1.5 µg/ml were found in milk from two mothers. Lead body burdens in pups cross-fostered to these mothers averaged 26.3 and 15.0 µg per pup after 24 hours.

Lorenzo and associates (1977) have studied the kinetics of lead distribution in lactating rabbits after an intravenous dose of either 0.4 or 2.0 mg/kg of lead acetate. While plasma and blood levels declined from maximum values rapidly following injection,

milk levels did not peak until 8 days after lead administration. Milk lead concentrations on day 8 were 8-fold greater than blood lead concentrations. However, this experiment is flawed by a number of inconsistencies. The terminal phase plasma lead levels are approximately 50% of the whole blood lead concentrations. If an hematocrit of 50% is assumed, the distribution of lead in blood was 33% in plasma and 67% in the erythrocytes. This value does not agree with published data for lead distribution in blood of humans or rats. The authors fail to explain the apparent discrepancy. The observation that milk lead concentrations reach a maximum 8 days after administration is in disagreement with data from lactating mice to be presented. The lag period is difficult to explain considering the kinetics of lead in the blood. It could be the result of an additional compartment interposed between plasma and milk, a delayed excretion of newly formed milk (however a delay of 8 days is unlikely) or to lead interactions with mammary tissue. The authors did not discuss possible mechanism responsible for the lag period between peak concentrations in blood and milk.

The mechanism of lead transport between plasma and milk has not been elucidated. Any hypothesis used to explain milk uptake of lead must be consistent with the observation that milk:plasma ratios are always greater than 1:1. It is suggested that a milk:plasma ratio greater than one could be sustained by one or both of two mechanisms: 1) active transport of lead from plasma into the mammary cells or organelles and /or 2) different chemical forms of lead in

plasma and milk which prevents free diffusion of lead and a consequent concentration equilibrium.

It has been shown that calcium concentrations in milk of dairy animals exceed plasma concentrations by 10 to 14 fold (Smith, 1959). However, large species differences are known to exist in milk calcium concentrations (O'Connor and Fox, 1977). Calcium ion concentrations in cow's milk in the range of 0.08 to 0.136 mg/ml and plasma concentrations of 0.036 to 0.076 mg/ml have been reported (Twardock and Comar, 1961). The data indicate an ionic milk:plasma calcium ratio of between 1 and 4 to one. Studies using the lactating mouse model were conducted to obtain basic information on the transfer of lead in milk to suckling offspring. In view of human exposure to lead, experimental work was conducted in order to establish an appropriate index medium for the estimation of lead transfer in milk. The index medium should be easily sampled from the exposed individual, should provide a direct correlation with milk lead concentration, and should allow for lead analysis using established analytical techniques. Plasma lead concentration is shown to be a more appropriate index medium than whole blood, but in most circumstances blood lead concentration is an adequate indicator of milk lead concentration.

In addition, basic information was obtained concerning the physiological processes involved in the transport of lead into milk. A milk:plasma lead concentration ratio of 23:1 was measured, and this was determined to be similar to the milk:plasma calcium

concentration ratio. Further experimentation yielded quantitative data concerning the mobilization of lead from bone during lactation and the transfer of lead from chronically exposed mothers to suckled pups. The extrapolation of data from the mouse model to human exposures is discussed in reference to the potential toxicological hazard associated with occupational exposure of women to lead.

2. Gastrointestinal Absorption of Lead

2.1 Developmental Differences in Absorption

Increased lead absorption in children in comparison to adults has been established by metabolic balance studies. Alexander et al. (1973) reported absorption of 53% and retention of 18% of ingested lead in healthy children 3 months to 8 years of age. Average lead ingestion during this 3-day balance study was 10.6 µg/kg daily. In a more complete study, Ziegler et al. (1978) demonstrated absorption of 41.5% and retention of 31.7% of ingested dietary lead. Multiple 3-day balance studies were performed on infants 14 days to 2 years of age, with carefully controlled intake during the 3 days prior to the start of the balance period. Ingested lead was controlled within the range of 30 to 130 µg/day. The authors also claimed a significant inverse relationship between calcium intake and retention. In both studies absorption was defined as lead intake minus fecal lead excretion, and retention, as absorption minus urinary excretion. These definitions fail to account for lead which is absorbed from the gastrointestinal tract and then excreted back into the feces, e.g. in bile. Thus the values given for lead

absorption must be underestimates of what was actually absorbed from the gastrointestinal tract into blood. Data of Quarterman et al. (1977) and results to be presented in the experimental results section indicate that biliary excretion of lead is an important route of elimination in rodents.

The fraction of an oral dose of lead absorbed from the gastrointestinal tract of adults is undoubtably less than absorption in young humans. Hursh and Suomela (1968) have shown that adults absorb 1.3 to 16% of an oral lead dose. This value was estimated by extrapolation from the amount of lead excreted in the urine during a 24 hour period to an approximate value for absorption. The extrapolation was based on observed lead clearance into urine after an intravenous injection of lead. The required assumption is that an oral and an intravenous dose are excreted similarly.

Lead absorption from the gastrointestinal tract of experimental animals has shown excellent correlation with the human lead balance studies. Kostial et al. (1971) administered carrier-free ^{203}Pb in supplemented cow's milk to 5 to 7 day old rat pups. The fraction of initial dose retained in the carcass (whole body minus G.I. tract) 40 hours after administration was 0.42, and at 80 hours, 0.53. Four-month-old females retained only 0.01 of the oral dose. A more extensive study of the ontogeny of lead absorption was conducted by Forbes and Reina (1972) with rat pups 16 to 89 days of age. Carrier-free ^{212}Pb was administered *per os* to groups of six rats and the animals were sacrificed 8 hours later. The G.I. tract

was flushed at autopsy. Retention of lead in the 16 to 22 day old pups ranged between 74% and 90% of the administered dose. A sharp transition at 24 days of age was noted with only 42% retained in the carcass. Rats 32 days old showed the same retention as did rats of 89+ days of age (approximately 16% retention). Forbes and Reina hypothesized that the sharp transition in lead absorption is related to the cessation of pinocytosis (gut closure) in the weanling rat.

Increased lead retention in mouse pups of various ages (compared to adults) will be reported in the EXPERIMENTAL RESULTS section. The observations of the above authors have been confirmed and extended to doses which are more realistic in terms of actual lead exposures than are the previously reported studies using carrier-free doses.

2.2 Mechanism of Gastrointestinal Absorption of Lead

The mechanisms responsible for lead absorption in both young animals and adults have not yet been elucidated. While large differences between suckling and adult animals in the fraction of an oral lead dose absorbed have been observed, no mechanism(s) has been demonstrated to account for this difference. The factors affecting lead absorption in adults have been extensively examined, but the experimental difficulties associated with neonatal models have limited investigation of lead absorption in young animals.

The interaction of dietary components with lead absorption in adults has been extensively studied. However, specific mechanism(s) by which dietary calcium, iron, phosphorus, milk,

protein, and fat act to modify lead absorption have not been resolved. Investigations to date have identified those age and dietary parameters which correlate with increases or decreases in lead retention but have not progressed beyond speculation concerning possible biochemical or physiological mechanisms. While it is important to identify those factors which might place an individual at increased risk to intoxication, research priority must be focused on the development of a theoretical basis for the observed effects and resultant application of this knowledge to the prediction of hazard associated with varying exposures or dietary parameters. It is doubtful that a single absorption mechanism can account for the variety of observed dietary effects on lead absorption.

2.2.1 Effect of a milk diet on lead absorption. A milk diet fed to adult rats has been shown to increase lead absorption from the gut. Kello and Kostial (1973) fed six-week-old rats diets of rat chow, cow's milk, powdered milk, and a combination diet of cow's milk and chow for a period of one week prior to, and one week after per os or intraperitoneal dosing with 0.2 μg of lead. The investigators determined that calcium, phosphorus, and caloric intakes were approximately equal for all dietary groups. Rats receiving the milk diets had higher intakes of fats and proteins but lower intakes of carbohydrates, Vitamin D and iron. After oral administration of lead, retention was increased in animals fed the milk diets. However, retention of an intraperitoneal dose was also increased significantly in animals fed milk diets. With powdered

milk and cow's milk diets the absolute increases in retention between the milk and the chow diets were nearly equivalent for either route of administration. The authors suggested that the experimental vs. control diet retention ratios provided supportive evidence for the hypothesis that milk increases lead absorption from the G.I. tract. However, they failed to explain the significantly increased retentions in the i.p. dose groups which paralleled the increase in the p.o. groups in terms of percentage of dose retained. The differences in retention could, on this basis, be explained not by increased lead absorption but by a decrease in lead excretion. This could occur if enterohepatic recirculation of biliary excreted lead is increased in animals fed milk diets. No kinetics or excretion data were presented which exclude this possibility. Though it is possible that the milk diets increase absorption of the orally administered lead dose in adults a more complex interaction cannot be ruled out.

Furthermore, other investigators have failed to confirm the results of Kello and Kostial. Meredith et al. (1977) found that administration of a ^{203}Pb dose in either whole milk or skim milk did not increase lead retention in adult rats relative to controls. Likewise, Garber and Wei (1974) found no difference in lead retention in adult mice following oral lead administration in milk or water.

Furthermore, the effect of a milk diet in neonates on lead absorption cannot be controlled properly. Due to the nearly continuous feeding of pups by the lactating mother it may not be

possible to duplicate the feeding schedule with artificial diets of varying composition without changing other parameters. It is possible that increased lead absorption in young animals is the result of a diet consisting solely of milk. The sharp decrease in lead absorption between 18 and 23 days of age in rodents corresponds with the period of weaning. In children increased lead absorption is evident even when milk is only a minor part of the total diet.

2.2.2 Effect of dietary calcium on lead absorption. The calcium content of the diet has been shown to affect the retention of lead in adults. Six and Goyer (1970) demonstrated increased kidney, femur and blood lead levels when rats were fed a low level (0.1%) calcium diet rather than a normal (0.7%) calcium diet and were provided drinking water containing 200 mg/ml lead. In a further experiment this group demonstrated dose related lead concentrations in femur and kidney when the drinking water contained 3 to 200 mg/ml lead (Mahaffey et al., 1973). Femur and renal lead contents were greatly increased by feeding a low calcium diet compared to a normal calcium diet. These experiments show an inverse relationship between dietary calcium intake and lead retention.

Quarterman and Morrison (1975) have demonstrated that a low calcium diet (0.2%) increased carcass retention of lead after 8 weeks as compared with a normal calcium diet (0.65%) when animals were fed 200 mg/g of lead in solid food. The low calcium diet did not affect retention of parenterally administered lead (100 mg per day for 15 days) which was administered during the closing weeks of a 7 week diet experiment.

Barton et al. (1978) found that pre-feeding of adult rats with diets containing 2.3 to 13.8 g/kg of calcium had no effect on the absorption of a 5 mg/kg intraluminal lead dose from the small intestine. However, when lead was administered in combination with $CaCl_2$ increasing calcium concentration correllated with decreased lead absorption. Furthermore, they interpreted their data to indicate that lead has affinity for two calcium binding proteins (CaBP) in the intestinal mucosa, and that calcium acts as a competitive inhibitor of lead binding to these proteins. Lead exhibited greater affinity for non-vitamin D inducible CaBP than for vitamin D sensitive CaBP. Their data provide strong evidence for the involvement of the calcium transport system in lead absorption in adult rats.

Though available data document strong correlations between absorption of lead and calcium in adults, there have been no published reports concerning the possible relationship between lead and calcium absorption in young animals. Examination of the possible relationship between lead and calcium absorption in young animals is hampered by the inability to perform dietary studies using controlled variation of diets in preweanling animals. Suckling in rodents is a nearly continuous process and it is unlikely that suckling rodents could be manually fed at periodic intervals without introducing additional complicating variables and affecting normal weight gain. Furthermore, the constancy of milk composition from lactating mothers precludes significant dietary modification by maternal treatment. Currently available information indicates that increased lead

absorption in young animals does not involve an absorptive pathway shared with calcium. Bruns et al. (1977) presented data which indicated that vitamin-D inducible CaBP in the duodenum is constant between 4 and 9 weeks of age, but thereafter declines to a level which was 30% of the initial value by 30 weeks of age. Armbrecht et al. (1979) have demonstrated that active calcium transport by Wilson-Wiseman everted gut sacs and CaBP content of the duodenum decreased between 3 weeks and 6 months of age in the rat. Ueng et al. (1979) did not detect CaBP in the intestinal mucosa and could not induce CaBP with $1,25(OH)_2\text{-}D_3$ treatment until 5 days of age in the rat. These observations are in direct conflict with the hypothesis of developmental changes in lead absorption in the rat. Forbes and Reina (1972) demonstrated that the fraction of an oral dose absorbed before 5 days of age is as great as that absorbed in animals 5 to 21 days of age. Furthermore, lead absorption was shown to decrease sharply to adult levels before 32 days of age. Thus there is a poor correlation between the developmental timing of lead absorption and active calcium transport. The hypothetical involvement of intestinal pinocytosis in the absorption of calcium, as well as lead, in suckling mice will be briefly discussed in the GENERAL DISCUSSION AND SUMMARY.

2.2.3 Other dietary factors. A large number of dietary factors other than milk and calcium have been implicated in lead absorption in adult rodents. Various factors have been shown to increase or to decrease lead absorption (or lead retention). Barltrop and Khoo (1975) demonstrated increased lead retention when

diets were deficient in either calcium or phosphorus. Conversely, lead retention was decreased by phosphorus or calcium dietary levels 4-fold greater than in control diets. Levels of phosphorus or calcium 2-fold greater than in control diets did not reduce lead retention. Quarterman and Morrison (1975) also showed that decreased dietary levels of calcium or phosphorus resulted in increased lead retention following chronic lead feeding. Quarterman et al. (1978) confirmed the earlier observation that lead retention is reduced by high dietary intake of calcium or phosphorus.

Quarterman et al. (1977) presented data indicating that the addition of phospholipids or bile salts to adult rat diets resulted in a 2 to 3 fold increase in retention of a carrier-free dose of ^{203}Pb.

El-Gazzar et al. (1978) found that lead concentrations in erythrocytes and bone following chronic feeding with 100 µg/ml lead in water were decreased by concurrent feeding of 50 µg/ml zinc in the water. Fine et al. (1976) reported that lead absorption in adult dogs was increased by a magnesium-free diet as compared to a diet containing 10 meq/kg of magnesium.

The effect of iron deficiency on lead absorption and retention in adult animals has been extensively studied. Mahaffey-Six and Goyer (1972) demonstrated increased lead retention in bone, liver and kidney when adult rats were fed an iron deficient diet (5 µg/g) compared to an adequate iron diet (25 µg/g). Bone lead content was increased 3-fold and soft tissues by somewhat smaller amounts

when rats were fed an iron deficient diet. Ragan (1977) reported that lead body burdens following a single dose of ^{210}Pb (0.3 mg) were increased 6-fold in 68-day-old rats fed an iron deficient diet since weaning. Barton et al. (1978) found that lead absorption was decreased by oral administration of ^{203}Pb in a 1mM $FeCl_2$ vehicle. In addition they present evidence for the cross-affinity of a mucosal binding protein for both iron and lead. They estimated a molecular weight of 370,000 for this protein and determined that it has a much higher affinity for iron than for lead. In contrast, Robertson and Worwood (1978) were unable to demonstrate a direct relationship between iron and lead absorption in adult rats. Although absorption of ^{59}Fe tracer was increased by prior feeding of an iron deficient diet, ^{203}Pb absorption was not affected. In addition, iron deprivation had no effect on subcellular distribution of ^{203}Pb in the intestinal mucosa, while ^{59}Fe distribution was markedly affected. ^{59}Fe was bound to ferritin or mucosal transferrin, and very little could be removed by exhaustive dialysis. In contrast, ^{203}Pb was bound to a protein intermediate to these in molecular weight, which was largely dialysable.

2.2.4 Application of adult dietary studies to assessment of the hazard of lead absorption in the young. Considerable caution must be exercised in using data relating dietary effects on lead retention obtained in adults to predict effects in young animals or children. Authors of these studies have discussed the possibility that poor diet may be a contributory factor in increasing childhood

lead absorption. It is hypothesised that dietary insufficiency (protein, iron, and calcium) may be most severe in those children with the greatest potential exposure to lead (urban children living in deteriorating housing). This extrapolation could only account for a small portion of the large differences observed in lead absorption between children and adults when they are fed adequate diets.

Even when adult animals are fed grossly inadequate diets the magnitutde of lead absorption (or retention) does not approach that observed in young of the same species. Furthermore, it can be assumed that the milk diet provided by the lactating mother must be nearly optimal in the evolutionary sense in terms of providing for normal growth and maturation in the offspring. While dietary deficiencies may contribute to increased lead absorption or retention in adult humans and post-suckling children, dietary factors cannot fully account for lead absorption differences in rodents. If the physiological or biochemical processes involved in lead absorption in young animals are distinctly different from those in adults, then the validity of studies utilizing adult animals for the assessment of potential hazard in young animals is questionable.

2.2.5 Role of pinocytosis in lead absorption.

Pinocytosis of intestinal contents is the principle mechanism by which macromolecules are transported intact into the intestinal mucosa in young animals. The mechanism provides the biological benefit that immunoglobulins in the mother's milk (colostrum) are absorbed without degradation into the neonatal circulatory system.

Pinocytotic uptake of immunoglobulins clearly occurs in pigs and rodents, but its involvement in human infants has not been established (see Section II-3, DISCUSSION). It is proposed that orally administered lead may bind to pinocytosed macromolecules or be in the form of inorganic lead colloidal precipitates which are transported into the intestinal mucosa. The metabolism of macromolecules within the pinocytotic vesicle is poorly understood. However, lead absorption could occur regardless of whether proteins are metabolized (by lysosomal action) or are absorbed intact. The increased absorption of lead in neonates could be the result of an increase in available absorptive surface area due to formation of pinocytotic vesicles.

The chronology of ontogenic changes in lead absorption are closely correlated with the developmental timing of gut "closure", which results in a sharp decline in pinocytotic activity in the gastrointestinal tract at the time of weaning. The sharp decrease in lead absorption noted by Forbes and Reina (1972) in rats at 21 days of age has also been observed in mice. Retention of a per os dose of lead in 22-day-old mice was markedly reduced as compared to younger pups and resembled the much lower retention which was found in adults (Section II-3, DISCUSSION). It has been hypothesized that increased intestinal pinocytotic activity in the young animals results in the observed increases in the retention of lead and other metals in neonates. However, no experimental evidence to document that a connection exists between macromolecule and lead absorption has been published. Furthermore, experimental proof is lacking to

support the claim that increased pinocytotic activity is quantitatively sufficient to account for the ontogenic differences.

Pinocytotic activity in the rat neonate has been shown to decline abruptly between 16 and 22 days of age (Clarke and Hardy, 1971). Uptake of a 0.1 to 1.0 mg dose of polyvinyl pyrrolidone (PVP) was found to be linear for rats of ages 16 days or less, with uptake into the intestine of 60% or more of initial dose. In 21-day-old rats uptake of only 5% of the dose of PVP occurs.

Daniels and co-workers (1973) have shown that treatment with large doses of cortisone acetate resulted in premature gut closure (decrease in pinocytotic uptake of PVP to nearly zero) in 5 and 12-day-old rats 4 and 6 days (respectively) after administration. Induced gut closure was histologically similar to natural closure. The authors also showed that plasma levels of corticosterone increase dramatically with age, and that these changes in steroid levels correlate chronologically with natural closure. It is theorized that closure is due to the migration of pinocytotic incapable (mature) mucosal epithelium ascending the villi from the crypts of Lieberkuhn. The time required for the mature cells to replace the pinocytotic-capable cells could explain the observed delay in induced closure. The time course of transition in pinocytotic activity corresponds to epithelial cell turnover time.

Studies were conducted utilizing suckling and adult mice to ascertain the relationship between intestinal pinocytotic activity and lead absorption. The results of these studies are

presented in Section II-3. Strong correlations were found between pinocytosis and lead absorption in 12-day-old mice following *per os* lead administration, as well as following *in situ* lead instillation into the intestine. Cortisone pretreatment produced a dose-related decrease in lead retention following oral lead dosing.

II-1: Distribution and Excretion of Lead in Young and Adult Female Mice

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Abstract

The kinetics of whole body lead elimination and organ distribution were studied in 10 day old and adult female mice following a single dose of lead. Necropsies were performed periodically during the 50 day experiment to assess organ lead distributions and lead elimination. Between days 15 and 50 excretion of lead was found to occur nearly equally through urinary and fecal routes. Whole body lead retention during the terminal elimination phase was observed to have a half-time similar to that of lead retained in femur. Rates of lead elimination from femur and from whole body of young mice were apparently less rapid than comparable elimination rates in adult mice. Lead fluxes from the brains of young and adult mice were closely approximated by single component exponential equations. The results suggest that a three-compartment model does not adequately account for the kinetics of lead distribution and retention in mouse brain after a single dose.

Introduction

The pharmacokinetics of lead have been investigated in a number of species, including man. Rabinowitz et al. have has shown that lead distribution and clearance in adult humans can be described by a three compartment kinetic model (Rabinowitz et al., 1975, 1976). He suggested that these compartments may represent bone, soft tissues, and blood. Castellino and Aloj (1964) have reported that during the two weeks following lead administration, lead pharmacokinetics in adult rats are consistent with a three compartment model for elimination of lead from the blood. Momcilovic and Kostial (1974) have observed differences in uptake and elimination of lead in organs of adult and suckling rats following intraperitoneal lead administration. The short duration of their experiment (8 days) limited their observations to uptake and initial elimination of lead from organs. Quarterman and Morrison (1978) have studied whole body lead retention in rats of 0 to 9 months of age following administration of a single intraperitoneal dose. Age-related differences were found in the percentage of lead retained in the whole body, with increasing age associated with decreasing lead retention eight days after lead administration. Interpretation of the results of these experiments is complicated by the possibility that absorption of lead from the peritoneal cavity may be a relatively slow process and consequently may affect the observed results.

Since the brain is an important target organ of lead toxicity during chronic exposures (Task Group on Metal Accumulation, 1973;

World Health Organization, 1977), it is important that the distribution and elimination of lead in the brain be defined in greater detail. Blood is currently considered an important indicator for estimation of risk of toxicity in individuals exposed to lead. However, details of the relationship between blood and brain lead concentrations have not yet been accurately delineated. It has been suggested (Grandjean, 1978; Barry and Mossman, 1970) that the half-time of lead in adult human brain is somewhat longer than in other soft tissues. Barry and Mossman (1970) reported that elevated lead concentrations were found in the brain and bone of a retired lead worker at the time of autopsy. Lead concentrations in other soft tissues were not greater than values found in individuals with no known occupational exposure to lead. This observation suggests that elimination of lead from the brain is slower than lead elimination from other soft tissues. Thus it has not yet been established that blood lead concentration is an accurate and reliable indicator of brain lead concentrations after chronic lead exposure. It has also not been possible to resolve whether the brain constitutes a separate physiological compartment for lead, or whether the behavior of lead in the brain is similar to lead uptake and elimination in other soft tissues. Since only a very small fraction of an administered dose is initially distributed to the brain, analysis of lead concentration in the blood, or of whole body lead retention, is not sufficiently sensitive to allow definition of an additional compartment representing the brain.

We report kinetics of distribution and elimination of lead in the whole body and organs (including brain) of mice following a single dose. In addition, the terminal lead clearance component, representing lead clearance from bone is estimated for young and for adult mice. Our results reveal differences in distribution and elimination of lead in adult and 10 day old mice during a 50 day period following a single intravenous or *per os* dose of lead.

Materials and Methods

Adult (98 to 112 days of age) and suckling (10 days of age) female C129F_1 mice (BALB/c female X 129 male) produced in our Inbred Mouse Unit were maintained in stainless steel metabolism cages and were fed a pelleted diet (Agway RMH 2000) and tap water ad libitum. The diet contains approximately 1 µg of lead/g, 9.3% fat, 18.4% protein, 1.1% calcium, 0.8% phosphorous and 4% fiber. Adults were housed 3 mice per cage. Pups were housed 3 per cage with a lactating mother plus two additional non-treated littermates until 25 days of age, when the mothers and non-treated mice were removed from the cages. Animals were maintained at an ambient temperature of 22°C with a light period of 5:00 a.m. to 7:00 p.m. (EST).

Lead-210 (^{210}Pb) (Amersham Corp.) was mixed with lead acetate (Fisher Scientific Co., ACS) to obtain two solutions with different specific activities to be administered to adults and 10 day old pups. A single dose of 4.45 ± 0.18 (SD) mg/kg (as elemental lead, 4.0 µCi/mg lead) was administered by tail vein injection to adult mice (weighing 24.47 ± 1.48g). A single per os dose of 5.22 (± 0.31) mg/kg (24.0 µCi/mg lead) was administered to 10 day old pups (weighing 7.53 ± 0.52g) by stomach intubation. The dose was administered to both adults and pups in 150 mM sodium acetate adjusted to pH 6.2 (5.0 ml/kg of body weight). The initial doses and daily whole body lead (^{210}Pb) retentions were determined in each mouse in the 70 x 130 mm center well of a 135 mm diameter x 160 mm deep thallium-activated sodium iodide crystal, utilizing the 47 Kev gamma emission of ^{210}Pb.

^{210}Pb content of sealed standards was determined daily to correct for radioactive decay and for changes in instrument efficiency, as well as to correct for differences in efficiency between the two instruments used for ^{210}Pb determination. Feces and urine were separated and collected from cages on a weekly basis and at the time of necropsy.

Groups of 6 adults and 6 pups were necropsied 0.075, 0.25 (or at 0.40 days for 10 day olds), 1, 2, 6, 15 and 50 days following lead administration. Mice were killed by CO_2 asphyxiation and then decapitated to enable blood collection in volumetric capillary tubes and subsequent drainage of blood from the carcasses. At each necropsy kidneys, liver, whole femur (right side only), skull section (containing parts of parietal, interparietal and frontal bones), and brain (cerebrum, cerebellum and brain stem) were collected from each animal. In addition, the gastrointestinal tracts were removed from the younger mice and divided for analysis of tissue and intraluminal lead contents of stomach, small intestine and colon. Organs were weighed immediately following dissection. ^{210}Pb content of each organ was determined with a Beckman 300 gamma spectrometer and total lead retention was calculated from the specific activity of the administered lead.

Whole body lead retention and organ content are expressed as Fraction of Initial Dose (FID) retained at each of the sampling times. Whole body lead retention (FID) was fitted to exponential equations using a computer program which minimizes the sum of the

squared deviations to obtain the "best-fit" (Knott, 1979). Lead elimination from the brain and femur were determined by least squares linear regression of the logarithms of the FID retained for stated time points.

Results

Whole body retention and cumulative fecal and urinary excretion of lead in adult female mice following a single intravenous injection are shown in Figure 1. On day 50 of the experiment the adult mice weighed 26.6 ± 1.0 (SD) grams. In adults there were no significant differences in organ weights (kidney, liver, brain) among the various necropsy groups. The whole body retention curve was fitted mathematically by a three component exponential equation:

$$\text{FID retained} = 0.41\ e^{-0.90t} + 0.12\ e^{-0.21t} + 0.47\ e^{-0.0050t}$$

The estimated half-time ($t_{\frac{1}{2}}$) of the observed terminal component of lead elimination (days 15 through 50) is 138 days. Only 1.03 ± 0.80 (SD)% of the body burden on day 15 was found in the skin and hair indicating that lead excretion via hair has little effect on whole body lead elimination. Lead retention (FID) in tails of the i.v. dosed adults was uniform among the various necropsy groups (0.0497 ± 0.0294,SD). The small amount of lead in the tail was probably sequestered in bone, although some of the retained lead could be the result of a partial subcutaneous initial injection. Total recovery

(whole body retention at necropsy plus cumulated urinary and fecal lead) of the administered lead dose was 101.8 ± 1.6(SD)% in cages housing adult mice. Initial excretion of lead occurs primarily via feces; between days 0 and 7, 74.2% of total excreted lead was in feces. However, between days 15 and 50, only 47.3% (range: 40.4 to 51.2%) of the total excreted lead was found in feces.

Whole body lead retention and cumulative excretion of lead in 10 day old mouse pups following per os administration are plotted in Figure 2. On day 50 of the experiment the 60 day old mice weighed 22.0 ± 1.2 (SD) grams. Lead retention in the suckling pups between days 0 and 2 represents absorbed and non-absorbed lead in the carcass and gastrointestinal tract. Lead retention between days 3 and 50 represents the FID absorbed minus the FID excreted. Only 3.1 ± 0.4% of the body burden on day 15 was found in the skin and hair of young mice. Analysis of lead elimination was limited to estimation of the terminal component of elimination. The best fit for the terminal component in terms of the FIDs retained between days 29 and 50 yielded an estimated $t_{\frac{1}{2}}$ of 182 days in these young mice. Lead retention between days 29 and 50 yielded a better fit to a single exponential equation than alternative possibilities. The rationale for this analysis is discussed in a later section.

Cumulative total excretion, i.e. total administered lead recovered in urine and feces (and the non-treated mother) is also plotted in Figure 2. Total recovery of the administered dose was 101.0 ± 0.5% (SD) in cages housing young mice. Due to the presence

of the lactating mother in the cages during the initial 15 days of the experiment, urinary and fecal excretion cannot be individually quantitated. The mother is thought to consume a significant fraction of neonatal excretion, and thus the distribution of excreted lead in the cage would partially reflect the adult pattern of lead excretion. It would be expected that most of the lead in pup urine ingested by mothers would not be absorbed and would appear in maternal feces deposited in the cage. After removal of the mother from the cage it was determined that between days 22 and 50 in cages housing young mice, 42.1% (range: 35.9 to 47.5%) of total excreted lead was in feces. Very little of the lead administered per os was eliminated until day 3 when there was an abrupt onset of lead excretion. Changes in the total lead (FID) contained in the tissue and luminal contents of segments of the gastrointestinal tract are plotted in Figure 3. It is evident that lead is rapidly cleared from the stomach through the small intestine to the colon. A long residence time in the colon is responsible for the observed pattern of whole body retention on days 1 through 3 (Figure 2).

The retention of lead in organs of adult female mice following i.v. administration of lead is shown in Figure 4. Maximum levels in liver and kidney are rapidly reached (6 hours or less), while maximum concentrations in femur and brain occur between 2 days (brain) and 6 days (femur) following injection. Lead elimination from kidney and liver can be described by a multiple component exponential equation. However, the limited number of data points is insufficient for

accurate curve fitting. Femur and brain exhibit apparent single component exponential elimination rates. Estimated lead elimination (days 6 through 50) from femur is 110 days (95% C.I.:95 to 130 days). The value for the $t_{\frac{1}{2}}$ of lead elimination from the bone is in close agreement with the terminal component of whole body clearance.

Similar patterns of lead elimination from organs are evident in 10 day old mouse pups following per os lead administration (Figure 5). The fraction of administered lead retained in organs is reduced compared to adults because of the limited absorption of lead from the gastrointestinal tract. Only 11.3% (± 0.5) of the initial dose was retained (whole body) 3 days after lead administration. The estimated $t_{\frac{1}{2}}$ (between days 1 and 50) for lead elimination from femur was 208 days (95% C.I.:83 to 413 days) which is similar to the value calculated for the terminal component of whole body lead elimination.

The data presented in Figures 4 and 5 indicate that lead elimination from the brains of young and adult mice can be described by single component exponential equations. In brains of young mice an elimination $t_{\frac{1}{2}}$ of 31 days between days 1 and 50 (95% C.I.:26 to 40 days) was determined compared to an elimination $t_{\frac{1}{2}}$ of 49 days between days 2 and 50 (95% C.I.:39 to 64 days) in adult brains.

Lead concentrations (ng/ml or ng/g) in blood, organs and bone of young and adult mice are presented in Table 1. Lead concentrations relative to dose are lower in young mice because of the difference in route of administration. Lead concentrations in adult

femur and skull are similar throughout the 50 day period despite the difference in type of bone sampled (long vs. flat bone). In young mice increased lead concentrations occur in the skull compared to the femur. This may be the result of rapid ossification of the skull in suckling mice of 10-12 days of age.

Blood lead concentrations (ng/ml) following i.v. administration of lead to adults and p.o. administration to pre-weanling mice are plotted in Figure 6. Rapid clearance of lead from blood occurs in both groups. The more rapid decline in blood lead between days 15 and 50 in the weanling mouse may be the result of growth-related "dilution" of the lead body burden. As the mouse grows in size, the volume of blood and the whole body mas increase, resulting in additional decrease in blood and whole body lead concentrations which are independent of processes lead elimination.

Figure 1: Whole body retention (●) and cumulative excretion of lead in feces (■) and urine (▲) of adult female mice following i.v. lead administration on day 0. (S.D.'s for lead retention are less than vertical dimensions of symbols.) Each excretion value is the arithmetic mean of collections from two cages.

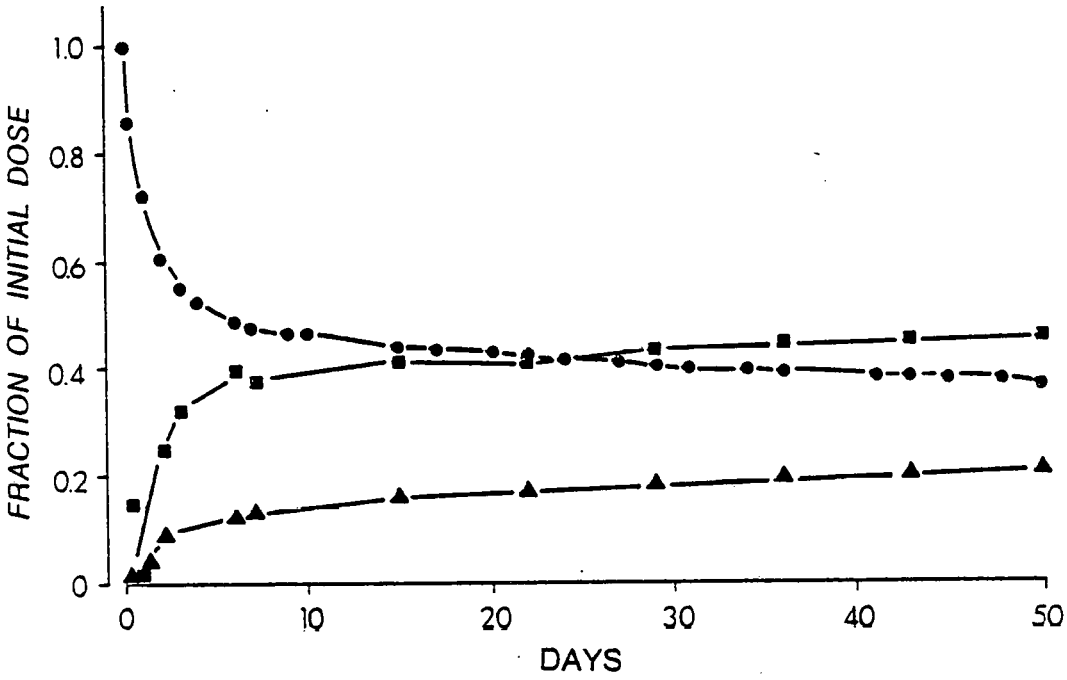
FRACTION OF INITIAL DOSE
1.0
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
DAYS

Figure 2: Whole body retention (◯) and cumulative excretion (▽) of lead in young mice following p.o. lead administration at 10 days of age (day 0). See text for definition of retention. Vertical bars represent ± 1 S.D. Each excretion value is the arithmetic mean of collections from two cages.

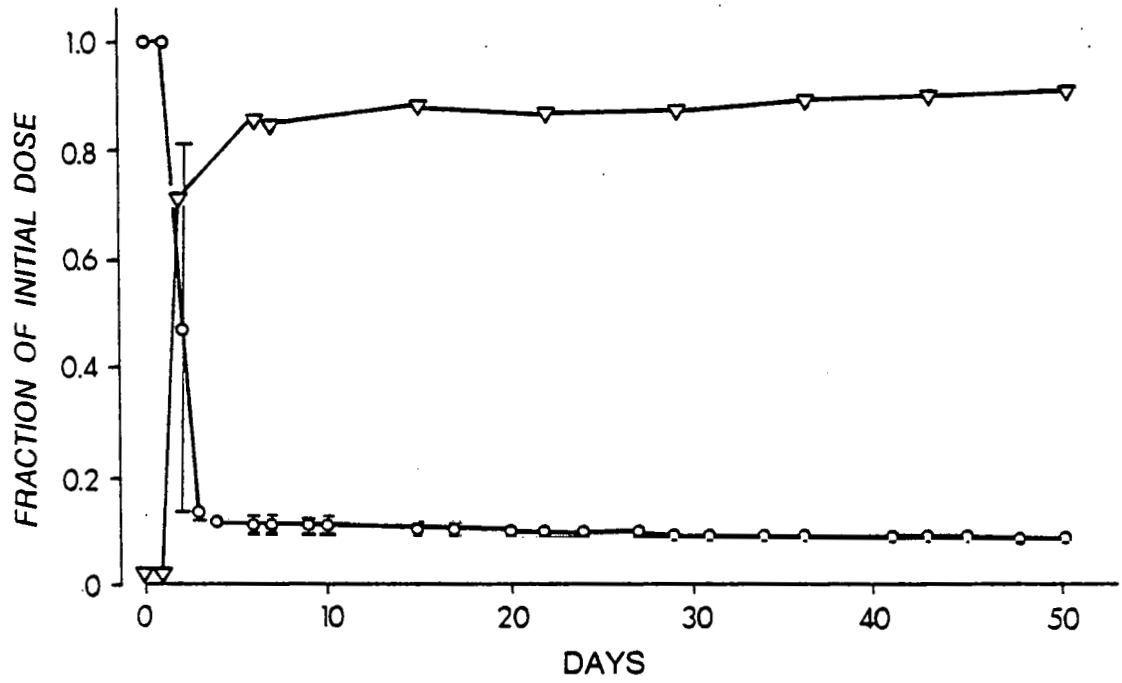
FRACTION OF INITIAL DOSE
1.0
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
DAYS

Figure 3: Gastrointestinal lead content expressed as Fraction of Initial Dose in 10 day old mice (n=6/time point) following p.o. lead administration. Stomach (△), small intestine (□), and colon (○). Vertical bars (± 1 S.D.).

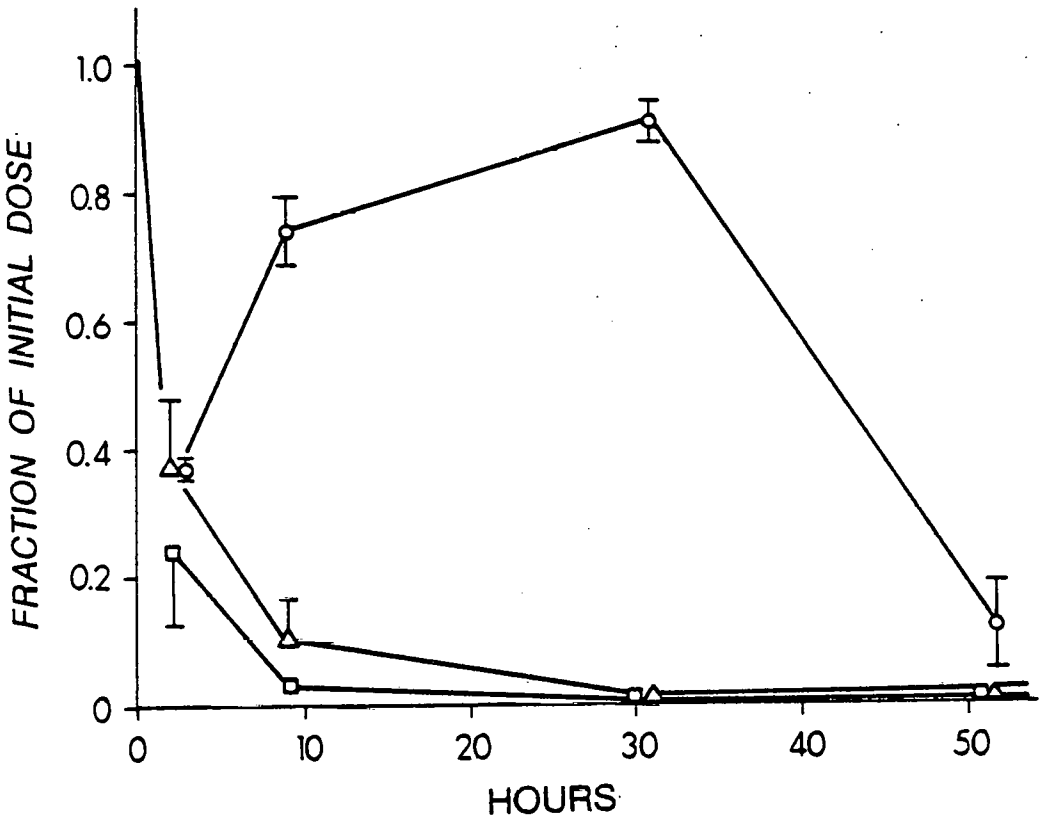
FRACTION OF INITIAL DOSE
1.0
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
HOURS

Figure 4: Lead retention in organs of adult mice (n = 6) following i.v. lead administration (day 0), femur (◆), kidneys (▲), liver (■), brain (●). Vertical bars (± 1 S.D.).

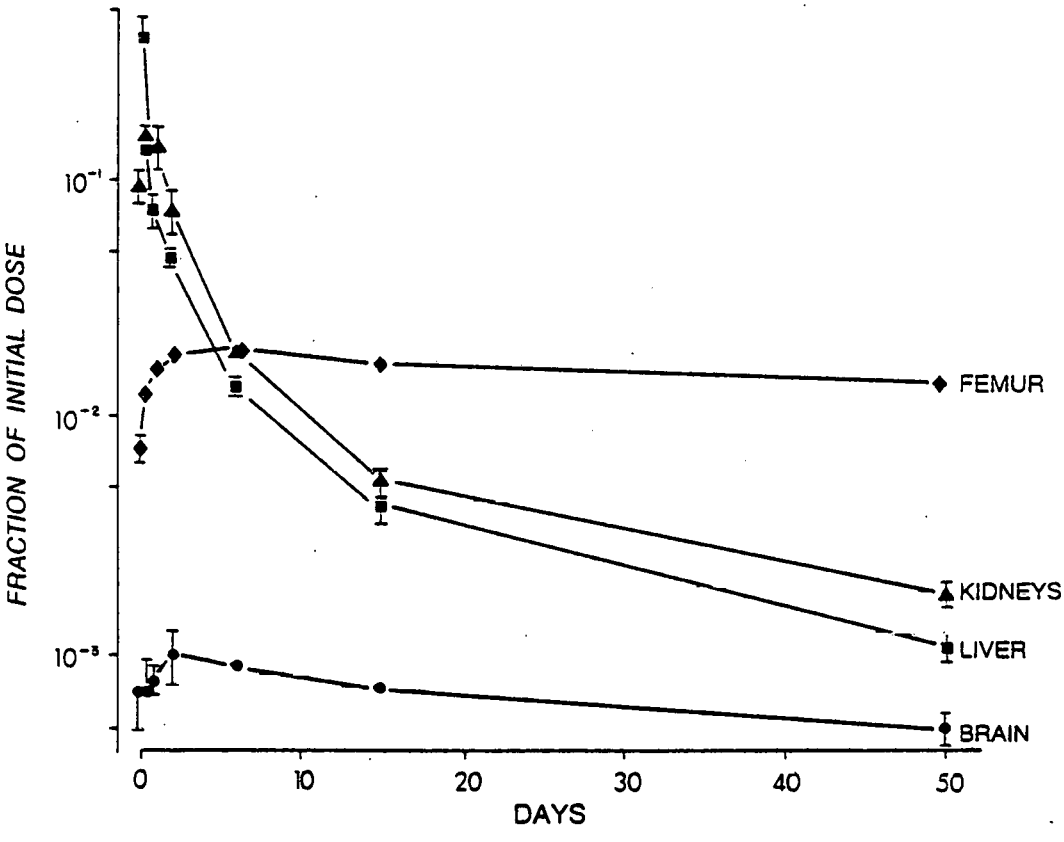
FRACTION OF INITIAL DOSE
10^{-1}
10^{-2}
10^{-3}
FEMUR
KIDNEYS
LIVER
BRAIN
0
10
20
30
40
50
DAYS

Figure 5: Lead retention (expressed as Fraction of Initial Dose) in organs of young mice (n=6/time point) following p.o. administration of lead at 10 days of age (day 0). Femur (◇), kidneys (△), brain (○) and liver (□). Vertical bars (± 1 S.D.).

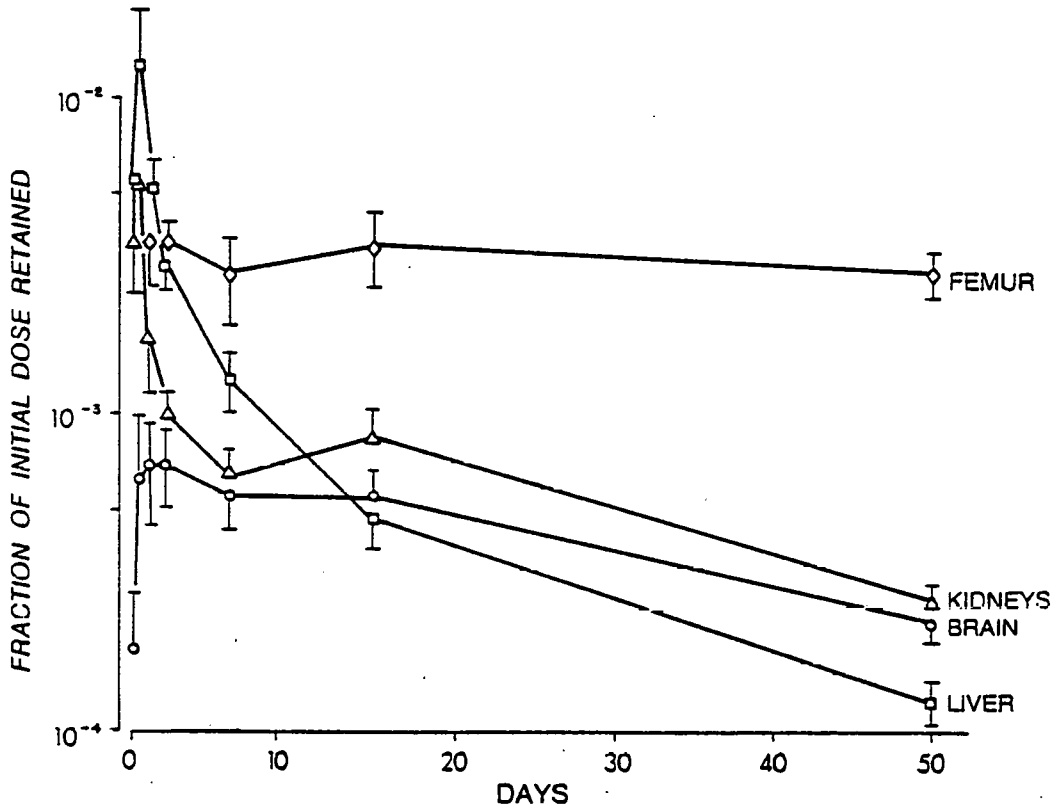
FRACTION OF INITIAL DOSE RETAINED
10^{-2}
10^{-3}
10^{-4}
0
10
20
30
40
50
DAYS
FEMUR
KIDNEYS
BRAIN
LIVER

Figure 6: Whole blood lead clearance in adult mice (●) following i.v. lead administration and in young mice (○) following p.o. administration on day 0. Vertical bars (± 1 S.D.).

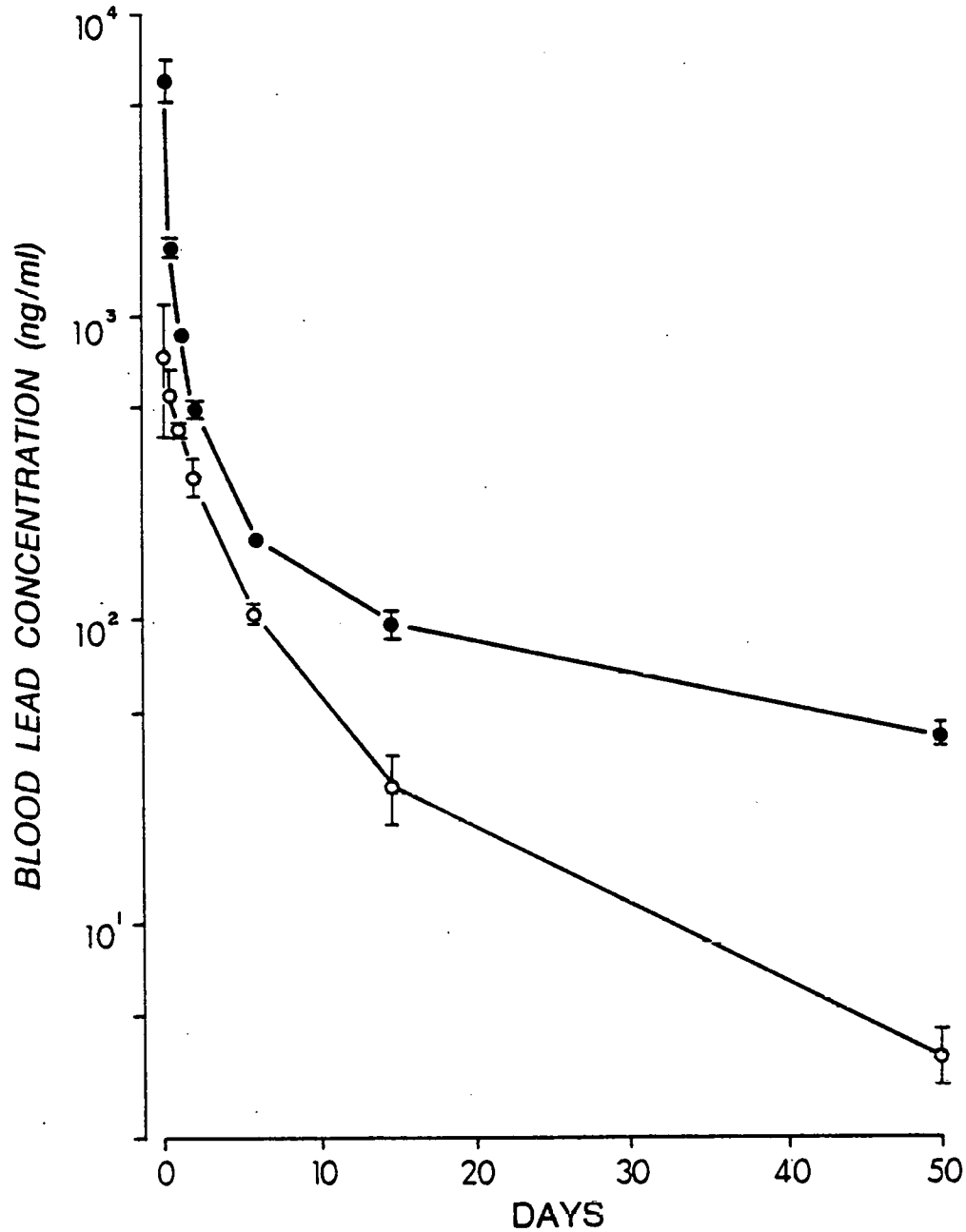
BLOOD LEAD CONCENTRATION (ng/ml)
10⁴
10³
10²
10¹
0
10
20
30
40
50
DAYS

Table 1: Lead concentrations (ng/ml or ng/g, ± SE) in blood, organs and bone of young (p.o.) and adult (i.v.) mice following a single lead df 5 mg/kg.

		Time (days)						
	Tissue	0.08	0.3	1	2	6	15	50
Young mice	Blood	749 ± 174	550 ± 56	437 ± 11	304 ± 18	87 ± 17	29 ± 3	3.7 ± 0.3
	Brain	21 ± 5	68 ± 17	69 ± 10	71 ± 10	51 ± 5	47 ± 5	18 ± 1
	Kidney	1571 ± 230	1960 ± 500	632 ± 76	355 ± 22	165 ± 13	151 ± 15	28 ± 1
	Liver	976 ± 175	1863 ± 360	736 ± 78	404 ± 34	114 ± 10	30 ± 3	4.3 ± 0.3
	Femur	---	---	5310 ± 486	5460 ± 652	3550 ± 216	3250 ± 362	1710 ± 130
	Skull	1770 ± 400	6650 ± 1950	8890 ± 1140	8000 ± 740	6050 ± 620	4400 ± 320	3050 ± 230
Adults	Blood	6010 ± 400	1700 ± 50	873 ± 14	504 ± 19	185 ± 4	97 ± 4	42 ± 2
	Brain	152 ± 21	153 ± 20	181 ± 14	216 ± 26	204 ± 6	159 ± 5	106 ± 11
	Kidney	31600 ± 2540	51600 ± 4050	40900 ± 3600	20600 ± 1960	5840 ± 170	1550 ± 65	483 ± 33
	Liver	45100 ± 3500	14900 ± 700	8050 ± 430	4870 ± 130	1360 ± 120	393 ± 43	99 ± 7
	Femur	10300 ± 700	18400 ± 550	24400 ± 600	25400 ± 700	26000 ± 450	23200 ± 600	18600 ± 400
	Skull	7480 ± 550	17100 ± 700	23700 ± 1000	24700 ± 1200	17700 ± 1200	24900 ± 300	22900 ± 900

Discussion

Lead distribution and elimination in adult and young mice were studied during a 50 day period following a single dose. Direct comparisons between the two age groups are restricted by several factors. Lead was administered *per os* to 10 day old pups since it was not possible to administer compounds by the i.v. route in these small animals. The blood lead data presented in Figure 6 indicate that the oral lead dose administered to 10 day old mice is rapidly absorbed. Therefore, for the purpose of kinetic analysis, oral administration results in rapid delivery of a single dose to the blood.

Analysis in young mice is further complicated by growth and maturation of the animal. Jugo (1977) has reported that renal maturation results in increased lead binding in kidneys. We have confirmed this in mice 7 to 21 days of age. A progressive increase in lead binding is evident at necropsy 6 days following a single dose of lead *per os*. We have also observed decreased retention of lead in brain and liver with increasing age in mice from 7 to 21 days old (unpublished results). Increases in whole body (and organ) weight result in a "dilution" of lead concentration which is independent of organ lead elimination processes. It is likely that the relatively larger decrease in blood in lead concentration in young compared to adult mice between days 15 and 50 was to a considerable extent the result of growth related concentration dilution.

Because of the complexity of changes due to growth and maturation, curve fitting of the whole body retention curve of young mice was

limited to determination of the terminal elimination component. By 39 days of age (experiment day 29), mice are sexually mature and have reached a relatively stable body weight. The terminal elimination components of whole body retention in adult and young mice were significantly different. It was also found that lead elimination from the femur has an estimated terminal elimination component $t_{\frac{1}{2}}$ similar to the observed terminal component of whole body clearance (138 vs. 110 days, and 182 vs. 208 days, for adult and young mice respectively). These results are in close agreement with published values for long term lead elimination in rats following a single dose. Torvik et al. (1974) found a terminal component $t_{\frac{1}{2}}$ of 108 days for femur in a 140 days study. Barton et al. (1978) estimated a terminal component with a $t_{\frac{1}{2}}$ of 160 days for whole body retention. Castellino and Aloj (1964) determined that lead elimination from the femur during a period of 14 days could be described by a single component exponential equation, and that the $t_{\frac{1}{2}}$ of bone lead was approximately 70 days. The similarity of the $t_{\frac{1}{2}}$ of lead in bone and the $t_{\frac{1}{2}}$ in whole body during the terminal elimination phase is consistent with sequestration of nearly all of the long term body burden of lead in bone (Smith and Hursh, 1977). The data presented in this report also show that whole body elimination of lead during the terminal elimination phase is reduced in young compared to adult mice. This finding is consistent with the hypothesis that lead which is sequestered in growing bone is less accessible to elimination processes, and is supported by data showing a longer $t_{\frac{1}{2}}$ in bone from young compared to adult mice.

It should be noted that the results which indicate a $t_{\frac{1}{2}}$ of lead in bone of approximately 100 days may be an underestimate of the actual $t_{\frac{1}{2}}$ of bone lead. This could be the result of extrapolation of relatively long $t_{\frac{1}{2}}$'s from experiments of less than 6 months duration. It is possible that bone lead may be sequestered in two compartments, one of which is relatively mobile and has a $t_{\frac{1}{2}}$ of 100 days or less, and another which has a $t_{\frac{1}{2}}$ of many years. The relatively short life span of rodents does not allow accurate estimation of elimination processes with $t_{\frac{1}{2}}$'s approaching (or greater than) the duration of the animal's lifespan.

Our results (Figure 1) indicate that following an i.v. dose in the adult mouse, fecal excretion of lead was the principle pathway of elimination during the initial period following administration (days 0 to 7). During this period there was also rapid elimination of lead from the liver (Figure 4). Klaasen and Shoeman (1974) reported that feces of adult rats contained 90% of the lead excreted during the 10 day period following a single i.v. dose of 3.0 mg/kg. We have determined that during the terminal elimination phase, lead excretion occurred nearly equally through urinary and fecal routes in both young and adult mice. The similarity of the fraction of total excretion excreted in urine during the terminal elimination phase in young and adult mice indicates that age of the animal at the time of lead administration has little influence on the long term pathway of lead excretion. Pounds et al. (1978) reported a similar pattern of fecal and urinary excretion in young and adult

Rhesus monkeys following an acute oral dose of lead. The mechanism responsible for the difference in initial compared to later distribution of lead excretion between urine and feces has not been defined. Vander et al. (1977) have found that increasing doses of lead result in decreasing renal clearance of lead relative to body burden. They hypothesize that lead tubular reabsorption is decreased with low renal tubule lead concentrations because of the binding of lead to a limited quantity of high affinity chelators. At higher lead concentrations, excess lead would be available for reabsorption due to saturation of these high affinity chelators. This mechanism could account for the differences in lead excretion which occur with time following a single dose.

The data presented in Figures 4 and 5 show that in both adult and pre-weanling mice the maximum observed lead levels in the kidneys and liver were reached by the time of the second necropsy (either 0.25 or 0.40 days). In adult mice the lead contents (FID retained) of the kidneys and liver are similar throughout the 50 days of the experiment. The observation that the elimination rates of lead from the kidneys and liver are nearly equivalent probably indicates that these organs are part of the same pharmacokinetic compartment. In the pre-weanling mice, the pattern of lead elimination from the kidneys and liver is complicated by differential rates of organ growth and maturation. The apparent increase in kidney lead retention observed between days 6 and 15 is consistent with a developmental increase in renal lead binding capacity.

In both adults and pre-weanling mice, lead concentrations in the femur and brain rise more slowly than in liver and kidneys. Maximum observed lead retention in brain and femur occurred between 1 and 6 days following lead administration. The increasing retention of lead in the femur is consistent with sequestration of the lead body burden in the bone. Lead elimination from the brain of young mice is apparently equivalent to or more rapid than lead elimination from adult brain (Figures 4 and 5). It has been observed that following a single dose a larger percentage of the administered or absorbed dose is distributed to the brain of suckling compared to adult mice and rats (Keller et al,1979 ; Momcilovic et al., 1974). Our data shows that 50 days following lead administration the FID retained in brains of young mice is similar to the FID retained in kidneys, and greater than the FID retained in livers. In adult mice the FID retained in brain is much less than the FID retained in either kidneys or liver. Increased lead distribution to the brain may increase the susceptibility of young animals to the neurotoxic effects of lead. Our data indicate that lead elimination from the brain of young mice is equal to or more rapid than elimination from adult brain and therefore probably is not a factor in the special sensitivity to the neurotoxic effects of lead in suckling animals (Pentschew et al., 1966; Michaelson et al., 1974). Compared to the adult, the uptake and elimination of lead in the brain of the young animal appears to differ from uptake and elimination in the other soft tissues studied. The possibility exists that the brain may

represent a pharmacokinetic compartment different from the lead retention compartment which includes the kidneys and liver. However, kinetic analysis of whole body lead retention or of blood lead elimination are not sensitive enough to discern a compartment which contains less than 1% of the body burden of lead.

Because of the known neurotoxicity of lead it is important that lead pharmacokinetics which relate to brain lead concentration be investigated during chronic lead exposures. Currently available data is insufficient for determining whether brain lead levels can be accurately predicted from blood lead concentrations during chronic lead exposures. In view of the widespread use of blood lead concentrations in lead screening programs for determining individuals at risk for lead neurotoxicity, better definition of the accuracy of blood lead concentration as an indicator of brain lead concentration is urgently needed in order to evaluate the reliability of use of blood lead concentration for predicting risk of neurotoxicity.

II-2: Effect of Dose on Lead Retention and Distribution in Suckling and Adult Female Mice

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Abstract

Effect of Dose on Lead Retention and Distribution in Suckling and Adult Female Mice. Single doses of lead (trace to 445 mg/kg) were administered per os to suckling and adult mice. Both groups exhibited dose-independent lead retention when doses of 4 to 445 mg/kg were administered. However, developmental differences in the Fraction of Initial Dose (FID) retained were evident for all doses administered. A much larger FID was retained in both age groups following administration of carrier-free ^{203}Pb. The results are consistent with a mechanism of gastrointestinal lead absorption comprising two or more processes. Developmental differences were also observed in organ lead concentration relative to whole body concentration for kidneys, skull, and brain six days following lead administration. Lead retentions (relative to whole body retention) in brain and in bone were linearly related to dose of lead administered in both suckling and adult age groups. Though uptake of lead into brain and into femur was observed to be directly related to dose over a wide range, relative blood lead concentrations were not linearly correlated with dose administered. The relationships between lead concentrations of blood and organ(s) were also shown to be non-linear relative to dose. However, blood lead concentration was found to be a reliable indicator of kidney and liver lead concentrations following an acute lead exposure.

Introduction

Previous studies have indicated that the Fraction of an Initial Dose (FID) of lead which is absorbed and retained following oral lead administration is not linearly related to the amount of lead administered. Conrad and Barton (1978) reported that the FID's retained in adult rats following intragastric administration of lead (5×10^{-6} mg/kg through 5 mg/kg) were dose dependent. They observed that 4 hours after administration of doses between 5×10^{-6} and 5×10^{-2} mg/kg, 4.2 to 5.5% of the administered doses were retained. However, when a dose of 5 mg/kg was administered only approximately 1% was retained. Garber and Wei (1974) reported that following p.o. doses of 0.2, 2 and 20 mg/kg of lead the percentage of lead absorbed at 4 hours was not affected by the magnitude of the dose. Baltrop and Khoo (1975) studied lead uptake from ligated gut loops in adult rats and reported a maximum absolute rate of lead transfer across the gut wall which was independent of dose for higher lead doses. They calculated a maximal possible daily gut absorption of 580 µg of lead for adult rats and suggested that the mechanism(s) reponsible for lead absorption might be saturable if large single doses were administered *per os*.

Developmental differences in lead absorption have been reported in experimental animals and in man. However, very little information is available concerning absorption and organ distribution over a larger range of doses of lead in neonatal and young animals. We report the absorption and organ distribution of lead in 10 day old and adult mice following single *per os* doses of trace to 445 mg/kg.

Materials and Methods

Suckling (10 days of age) and adult (73 to 84 days of age) female C129F_1 mice (BALB/c female x 129 male) were produced in our Inbred Mouse Unit, housed in stainless steel cages, and fed a pelleted diet (Agway RMH 2000) and tap water ad libitum (less than 5 ng of lead/ml). The diet contained 1.1% calcium, 0.8% phosphorous, 9.3% fat, 18% protein, 4% fiber (manufacturer's analysis), and 0.86 µg of lead/g (determined by flameless atomic absorption spectroscopy). Adults were housed 3 mice per cage; suckling pups, 5 per cage with a lactating mother. Animals were maintained at 22°C with a light period between 5:00 a.m. and 7:00 p.m. (EST). Mice were not fasted prior to lead administration.

Lead-203 (^{203}Pb, carrier-free, as lead acetate, New England Nuclear) was mixed with lead acetate (Fisher Scientific Co.) in 150 mM sodium acetate to obtain five solutions of various lead concentrations each containing an equal concentration of ^{203}Pb. Single per os doses (stomach intubation; 5.0 ml/kg of body weight) were administered (between 10 a.m. and 12 p.m.) to each dose group of 6 adult mice (mean body weight = 21.52 ± 0.39 grams, S.D.) or 5 suckling pups (7.96 ± 0.28 grams). Groups of mice received 4, 19, 104 or 445 mg/kg of lead (as elemental lead). The highest dose (445 mg/kg) was administered only to adult mice. In addition, the carrier-free ^{203}Pb isotope was administered to adult and to suckling mice (approximate dose = 1×10^{-8} mg/kg).

Initial dose and daily whole body ^{203}Pb retention were determined in each mouse in the 70 x 130 mm center well of a 135 mm diameter x 160 mm deep thallium activated sodium iodide crystal utilizing the 279 Kev gamma emission of ^{203}Pb. ^{203}Pb content of sealed standards were determined in each session to correct for radioactive decay and changes in instrument efficiency, as well as to standardize the two instruments used. Cumulative feces and urine were collected on days 1, 2, 4 and 6 of the experiment. Total recovery of administered lead in whole body plus cumulative excretion on day 6 was 99.2 ± 11.3% (SD). Necropsies were performed on all mice 6 days following lead administration. Mice were killed by CO_2 asphyxiation and then decapitated to enable blood collection in volumetric capillary tubes, and to allow subsequent drainage of blood from the carcass. Brain (cerebrum, cerebellum, brainstem), kidneys, liver, stomach, small intestine, colon, whole femur (right side only), and skull section (containing parts of parietal, interparietal, and frontal bones) were collected from each animal and were immediately weighed. Organ lead retention was determined by counting each organ in a Beckman 300 Gamma Spectrometer.

Whole body lead retention is expressed as Fraction of Initial Dose (FID) retained on each of the 6 days following lead administration. Relative lead retention in organs collected on day 6 is expressed as Body Concentration Ratio (BCR).

FID = (ng of Pb whole body, day n) ÷ (ng of Pb in whole body, day 0)

BCR = (ng of Pb/g organ, day 6) ÷ (ng of Pb/g whole body, day 6)

BCR is a transformation which enables comparison of lead concentration in an organ relative to the "average" whole body lead concentration at the time of necropsy. It is a useful measure of relative organ distribution and retention of lead since it normalizes for variations in gastrointestinal absorption of lead following an oral dose. Differences in whole body and organ lead retention among experimental groups were tested for statistical significance using the Duncan multiple range test (Nie et al., 1975). The ratios of organ lead concentration (ng/gm) to blood lead concentration (ng/ml) were converted to $e^{\log[(ng/g) \div (ng/ml)]}$ (personal communication, H.T. Davis). This transformation was used as a check on the probabilistic assumptions required for the statistical analysis, and it produced results nearly identical to those determined when the data was not transformed.

Results

On day 6 of the experiment the adult mice weighed 21.49 ± 1.92 (SD) grams (99.9% of initial weights). There were no significant differences among dose groups in body or organ weights. The results (Figure 7) indicate that in adult female mice within the dose range of 4 to 445 mg/kg, lead retention (FID) on day 6 did not differ significantly ($p<0.01$). Mice given the carrier-free dose had significantly greater retention (FID) (4.3 fold greater) of lead on days 4 through 6 compared to animals given higher doses ($p<0.01$). In the highest dose group (445 mg/kg) an apparent toxic effect (lead-induced constipation) was observed, which was responsible for the increased lead retention in this group between days 0 and 4. This effect has

been reported in humans occupationally exposed to lead (Irwig et al., 1978). The quantity of fecal pellets was markedly reduced in cages housing these animals, especially during the first three days following lead administration. A 7% decrease in mean body weight was also noted in the highest dose group on day 3, but by day 6 weights had returned to normal. One animal in the 445 mg/kg dose group died on day 4.

Suckling pups weighed 9.85 ± 0.66 (SD) grams on day 6. There were no significant differences in body weights among the dose groups. Retention (FID) of lead in suckling pups (Figure 8) did not differ significantly between days 0 and 6 over a dose range of 4 through 104 mg/kg. Lead retention (FID) was significantly increased in the carrier-free dose group compared to the higher dose groups ($p<0.01$). The retention of lead in suckling pups was approximately 9-fold greater than for adults for corresponding doses. Very little lead was excreted by suckling mice during the first 24 hours following lead administration. Lead transit through the stomach and small intestine was rapid, but transit and clearance of lead in the colon occurred more slowly and retarded whole body elimination of lead in the suckling mouse (Keller and Doherty, 1979).

The lead concentration in brain is 8 to 16 times greater in suckling versus adult mice (for doses of 4 to 104 mg/kg) (Table 2). While the BCR in kidneys was shown to be decreased in suckling versus adult mice (Figure 9A), the absolute lead concentrations are equal to or greater than lead concentrations in adult kidneys. The

organ lead concentrations resulting from the carrier-free dose are probably insignificant, since the endogenous lead concentration is much greater. However, similar patterns of organ concentration differences between pups and adults are observed for both carrier-free as well as larger doses.

Increasing lead dose is associated with a decrease in BCR retained in adult kidneys, but not in pup kidneys (Figure 9A). Lead retention in kidneys of adult mice was significantly greater than retention in suckling pups for all doses ($p<0.01$), except for 445 mg/kg ($p<0.05$). The adult carrier-free and 4 mg/kg dose groups had significantly greater renal lead retention ($p<0.01$) relative to dose than did higher dose groups. The adult 19 mg/kg dose group had significantly greater ($p<0.05$) retention of lead in kidneys (BCR) than did the 445 mg/kg dose group.

Relative lead retention in brain (BCR) was 1.5 fold greater in suckling than in adult mice ($p<0.01$) but was dose independent among both suckling and adult animals, i.e. there were no significant differences in the BCR retained among dose groups of adults or suckling pups (Figure 9B). Relative lead concentrations in brain (BCR) were much lower than in other organs which were examined.

Dose dependence of retained lead in liver of both adults and suckling pups is apparent, with increasing dose associated with a decreasing BCR retained (Figure 9C). There were significant differences ($p<0.01$) in liver BCR retained between adults and pups for the three lowest doses administered. In adults there were signifi-

cant differences ($p<0.01$) in BCR retained among all combinations of dose groups except those given 104 and 445 mg/kg. In pups only the carrier-free dose resulted in a significantly greater liver BCR compared to the other doses ($p<0.01$) .

Relative blood lead concentrations are dependent upon the dose administered, with increasing dose associated with decreasing values of the BCR retained in blood (Figure 9D). Significant age differences are noted only for the 4 mg/kg and carrier-free lead isotope doses ($p<0.01$). Both the adult carrier-free and 4 mg/kg doses had significantly different ($p<0.01$) BCR values compared to other adult dose groups. The mean BCR for the adult 19 mg/kg dose group is significantly greater than that of the 445 mg/kg group ($p<0.05$).

No significant differences were observed in BCR retained in femurs of adult compared to suckling mice, or among dose groups of either age (Figure 10). However, suckling pups retained significantly greater relative lead concentrations (BCR) in skull sections than did adult mice ($p<0.01$). Skulls of suckling (but not adult) mice retained a greater BCR than femurs for all doses. The concentrations of lead in bone samples from both suckling and adult mice were many-fold greater than the "average" concentrations of lead in whole body on day 6. This result is expected since bone is the principle long-term site of lead deposition.

Lead concentrations in organs tend to increase relative to the blood lead concentrations in both age groups with increasing initial doses of lead (Tables 3 and 4). This dose dependent relationship is

especially evident in the brain, and in skull and femur, where there are more than four-fold differences in the concentration ratios (organ to blood) in the lowest compared to the highest dose groups. In contrast, kidneys of both suckling and adult mice retained a relatively uniform concentration relative to blood concentration with increasing dose.

Discussion

Our results indicate that 6 days after lead doses ranging from 4 to 445 mg/kg (4 to 104 mg/kg for suckling pups) adult mice retained 0.011 FID and suckling pups retained 0.094 FID; retention (FID) was independent of the magnitude of the dose. However, in both suckling and adult mice much greater FID's were retained following administration of carrier-free ^{203}Pb (approximately 1×10^{-8} mg/kg). It should be noted that the carrier-free isotope probably traces the amount of lead present in the pelleted diet. If it is assumed that a 22 gram mouse consumes 4 grams of food per day which contains 0.9 µg Pb/g, and that food is cleared through the stomach and small intestine in 6 hours (so that the carrier-free isotope is mixed with 1 to 2 grams of food, depending on the feeding cycle of the mouse), then the estimated carrier-free dose is no more than 0.082 mg Pb/kg of body weight.

The constant FID's of lead retained after doses of 4 mg/kg and larger in suckling and adult mice do not support the absorption saturation hypothesis of Baltrop and Khoo (1975). However, the

Figure 7: Whole body retention (expressed as Fraction of Initial Dose) of lead in adult female mice (n=6/dose group) following per os administration (day 0) of: carrier-free ^{203}Pb (●), 4 mg/kg (▲), 19 mg/kg (■), 104 mg/kg (◆) or 445 mg/kg (▽). Vertical bars represent ± 1 SE. SE not shown when less than symbol dimension.

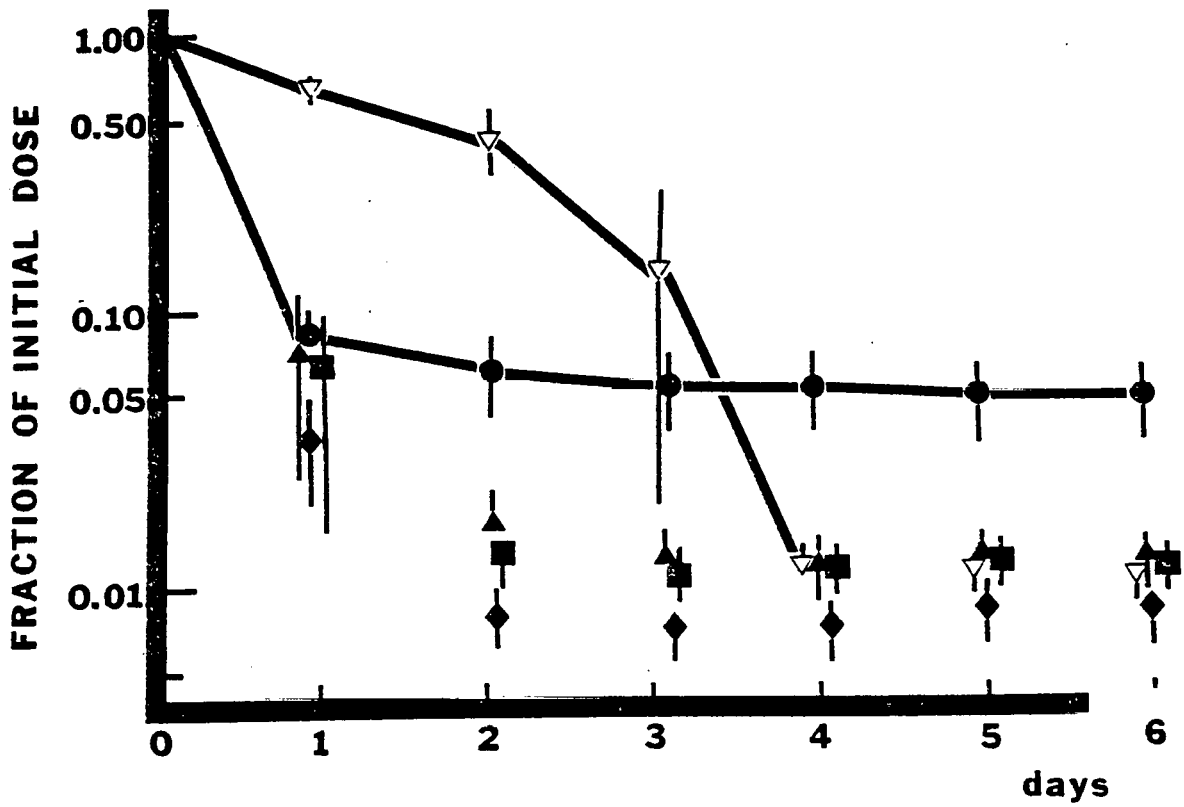

FRACTION OF INITIAL DOSE
1.00
0.50
0.10
0.05
0.01
0
1
2
3
4
5
6
days

Figure 8: Whole body retention (expressed as Fraction of Initial Dose) of lead in suckling female mice (n=5/dose group) following per os administration (day 0) of carrier-free ^{203}Pb (○), 4 mg/kg (△), 19 mg/kg (□) or 104 mg/kg (◇). Vertical bars (± 1 SE). SE not shown when less than symbol dimensions.

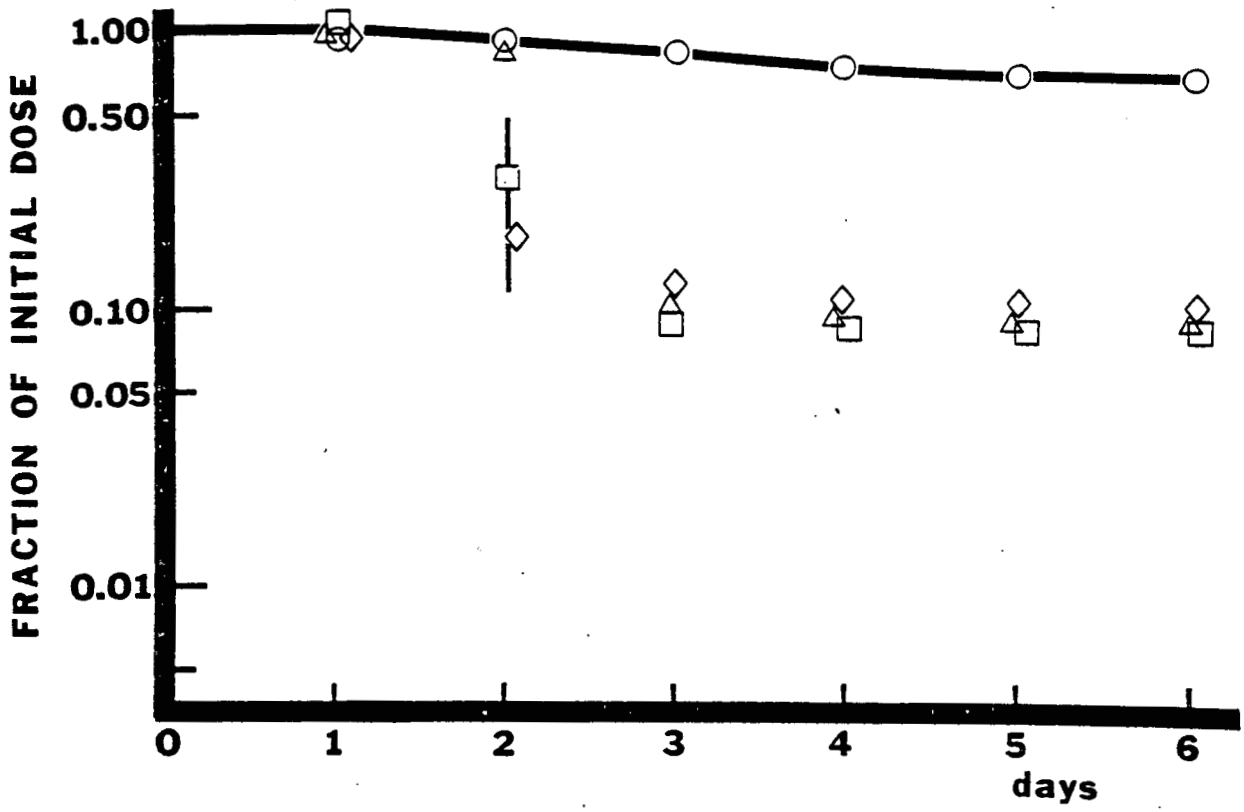
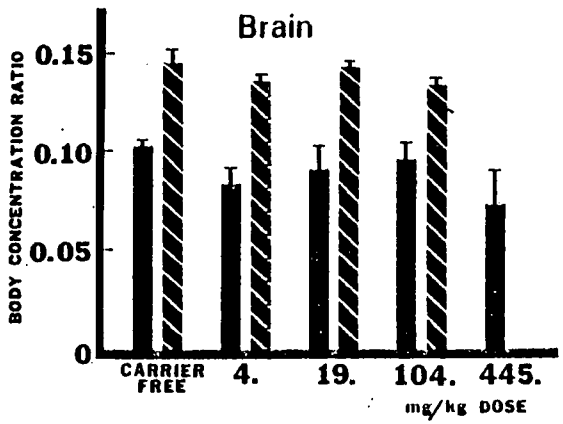
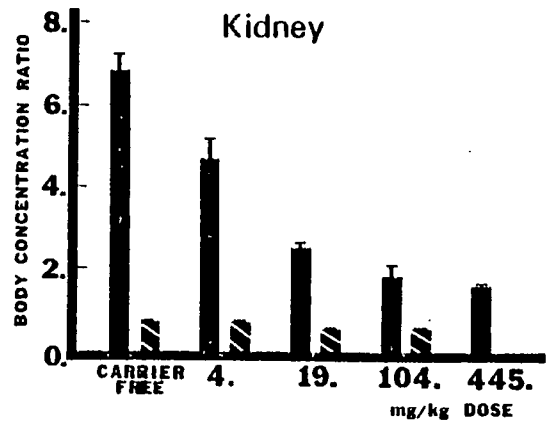
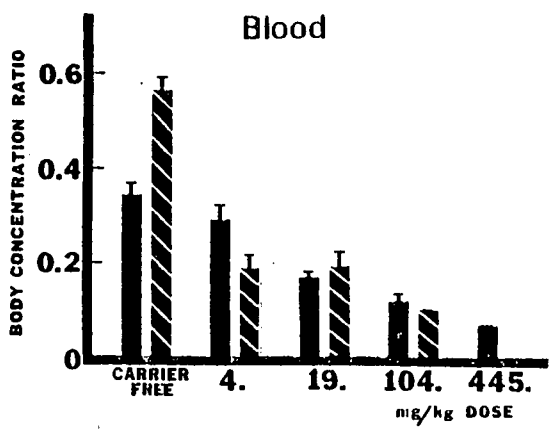

FRACTION OF INITIAL DOSE
1.00
0.50
0.10
0.05
0.01
0
1
2
3
4
5
6
days

Figure 9: Organ retention (expressed as Body Concentration Ratio) of lead in adult (n=6/group; solid bars) and suckling mice (n=5/group; hatched bars) 6 days after a single per os administration of various doses of lead. A) kidney, B) brain, C) liver and D) whole blood. See text for definition of Body Concentration Ratio (BCR). Vertical lines above bars represent + 1 SE.


Brain
BODY CONCENTRATION RATIO
0.15
0.10
0.05
0
CARRIER FREE
4.
19.
104.
445.
mg/kg DOSE


Kidney
BODY CONCENTRATION RATIO
8.
6.
4.
2.
0.
CARRIER FREE
4.
19.
104.
445.
mg/kg DOSE


Blood
BODY CONCENTRATION RATIO
0.6
0.4
0.2
0
CARRIER FREE
4.
19.
104.
445.
mg/kg DOSE

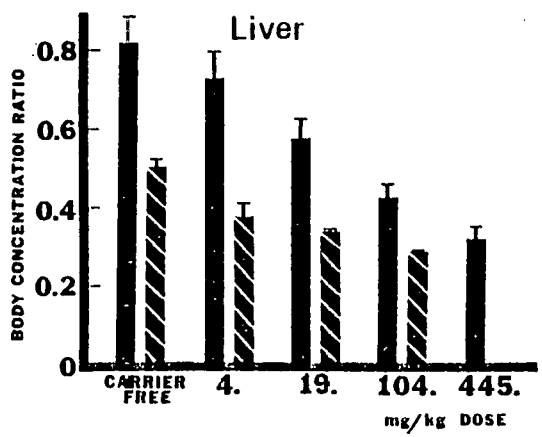

Liver
BODY CONCENTRATION RATIO
0.8
0.6
0.4
0.2
0
CARRIER FREE
4.
19.
104.
445.
mg/kg DOSE

Figure 10: Organ retention of lead in bone of adult (n=6/group) and suckling mice (n=5/group) 6 days after a single per os administration of various doses of lead. Adult skull (), adult femur (), pup skull (), and pup femur (). Vertical lines above bars represent + 1 SE.

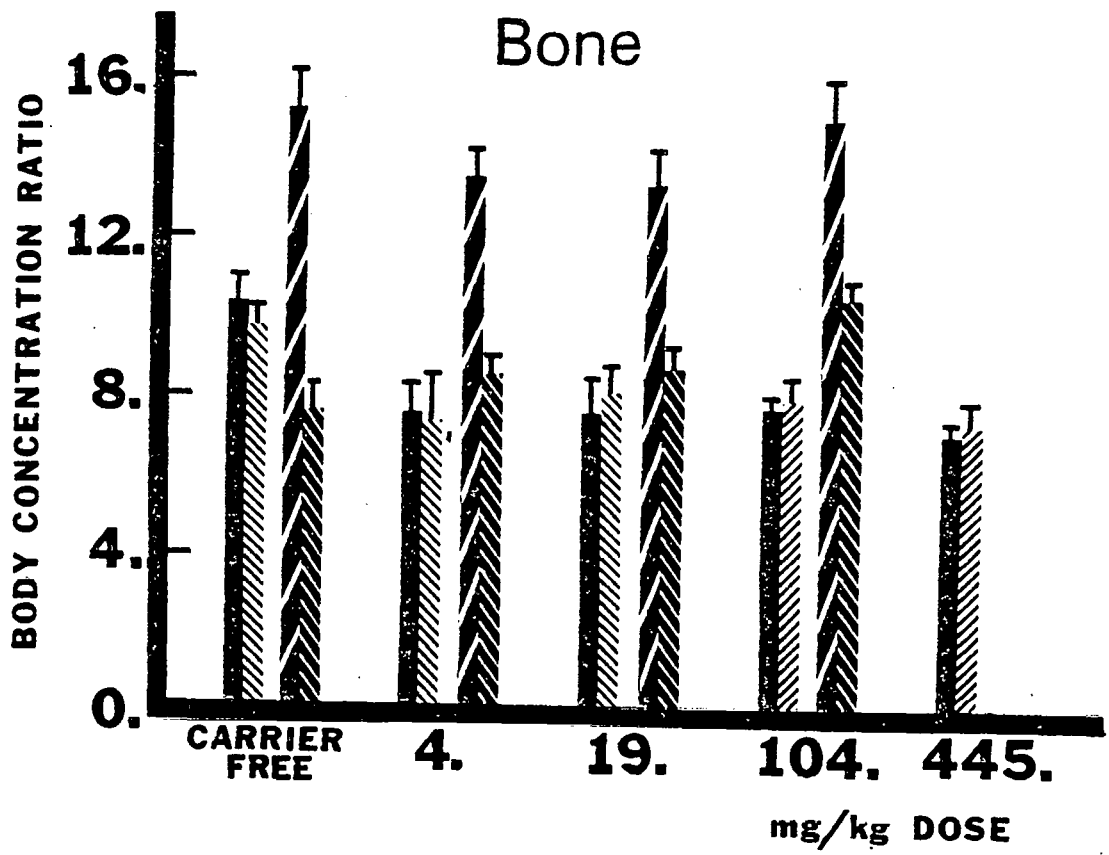

Bone
BODY CONCENTRATION RATIO
16.
12.
8.
4.
0.
CARRIER FREE
4.
19.
104.
445.
mg/kg DOSE

Table 2: Lead concentrations blood, organs, and bone of suckling pups (n=5/dose group and adult mice (n=6/dose group) 6 days after a single per os administration of various doses of lead.*

Tissue		Carrier-free	Dose 4 mg/kg	19 mg/kg	104 mg/kg	445 mg/kg
BLOOD (ng/ml)	Pups	3.2×10^{-4} ($\pm 0.1 \times 10^{-4}$)	54 (±10)	250 (±34)	1018 (±29)	—
	Adults	4.5×10^{-5} ($\pm 0.6 \times 10^{-5}$)	15.1 (± 3.7)	41 (±10)	85 (±16)	367 (±44)
BRAIN (ng/g)	Pups	8.3×10^{-5} ($\pm 0.4 \times 10^{-5}$)	38.2 (±3.5)	187 (±14)	1188 (±38)	—
	Adults	4.5×10^{-6} ($\pm 1.6 \times 10^{-6}$)	4.9 (±1.8)	25 (±10)	75 (±19)	383 (±49)
KIDNEY (ng/g)	Pups	4.0×10^{-4} ($\pm 0.3 \times 10^{-4}$)	201 (±16)	724 (±24)	4766 (±194)	—
	Adults	2.9×10^{-4} ($\pm 0.8 \times 10^{-4}$)	236 (±54)	579 (±132)	1325 (±271)	8275 (±618)
LIVER (ng/g)	Pups	2.9×10^{-4} ($\pm 0.1 \times 10^{-4}$)	107 (±11)	443 (±24)	2628 (±59)	—
	Adults	3.7×10^{-5} ($\pm 1.3 \times 10^{-5}$)	41 (±13)	145 (±42)	321 (±72)	1695 (±24)
SKULL (ng/g)	Pups	8.6×10^{-3} ($\pm 0.4 \times 10^{-3}$)	3820 (±310)	17100 (±900)	136000 (±3000)	—
	Adults	4.7×10^{-4} ($\pm 1.9 \times 10^{-4}$)	449 (±169)	2010 (±710)	6740 (±2030)	37700 (±5730)
FEMUR (ng/g)	Pups	4.4×10^{-3} ($\pm 0.4 \times 10^{-3}$)	2400 (±140)	11500 (±1300)	94600 (±2900)	—
	Adults	4.4×10^{-4} ($\pm 1.5 \times 10^{-4}$)	469 (±203)	2120 (±674)	7260 (±2530)	38600 (±6820)

* Results given in ng lead/g or ng/ml tissue (mean ± SE).

Table 3: Adult mice (N=6/dose group). Lead concentrations in organs relative to blood lead concentrations 6 days after a single per os administration of various doses of lead.* Horizontal lines beneath values connect cohorts of values which do not differ ($p<0.05$).

	Dose				
ORGAN	Carrier-free	4 mg/kg	19 mg/kg	104 mg/kg	445 mg/kg
BRAIN	0.23 (±.01)	0.29 (±.04)	0.53 (±.09)	0.82 (±.08)	1.04 (±.02)
LIVER	1.8 (±.1)	2.6 (±.2)	3.3 (±.2)	3.6 (±.3)	4.7 (±.4)
KIDNEY	15.3 (±.6)	16.1 (±.7)	14.7 (±.7)	15.6 (±.8)	23. (±1.)
SKULL	24. (±3.)	27. (±4.)	44. (±6.)	70. (±11.)	102. (±3.)
FEMUR	22. (±2.)	26. (±5.)	48. (±6.	74. (±15.)	106. (±14.)

* Results given as (ng of lead/g organ) ÷ (ng of lead/ml blood), (mean ± SE).

Table 4: Suckling mice (n=5/dose group). Lead concentration in organs relative to blood lead concentration 6 days after a single per os administration of various doses of lead.* Horizontal lines beneath values connect cohorts of values which do not differ at $p<0.05$. Values above dotted sections of lines are not members of the indicated cohort.

	Dose			
ORGAN	Carrier-free	4 mg/kg	19 mg/kg	104 mg/kg
BRAIN	0.26 (±.01)	0.78 (±.10)	0.80 (±.10)	1.2 (±.04)
LIVER	0.90 (±.02)	2.1 (±.2)	1.9 (±.2)	2.6 (±.1)
KIDNEY	1.3 (±.1)	4.1 (±.5)	3.1 (±.4)	4.7 (±.2)
SKULL	27. (±2.)	79. (±11.)	75. (±11.)	133. (±11.)
FEMUR	14. (±2.)	51. (±9.)	50. (±10.)	93. (±4.)

* Results given as (ng of lead/g organ) ÷ (ng of lead/ml of blood), (mean ± SE).

observed decrease in FID retained between the carrier-free and 4 mg/kg lead dose is consistent with the observations of Conrad et al (1978a) discussed above. The pattern of dose dependence of lead retention observed in this experiment and by Conrad et al in both adults and pups indicates the possible existence of two (or more) processes for lead absorption from the gastrointestinal tract. It is possible that the decrease in the fraction of lead dose absorbed which occurs between doses of approximately 0.05 mg/kg and 4 mg/kg is due to saturation of one (or more) absorptive process(es). The uniform FID's retained in the dose range of 4 to 445 mg/kg could be the result of lead absorption via a first-order (non-saturable) process. Conrad et al. (1978a, 1978b) report that lead competes for binding sites on intestinal mucosal proteins which are involved in absorption of calcium and iron, and that both calcium and iron inhibit lead absorption in a dose-related manner. Saturation by lead of these, or other, carrier-mediated transport processes could result in a decrease in the FID absorbed (or retained) following an oral dose of large quantities of lead.

Meredith et al (1977) also has suggested that there are two mechanisms for the absorption of lead from the G.I. tract. Increased concentrations of intraluminal calcium were found to inhibit lead absorption but calcium doses above 4 mmol did not further limit absorption, indicating that a second absorption mechanism was responsible for the residual amount of lead absorbed.

The results presented in Figures 7 and 8 extend previous observations concerning developmental differences in lead absorption and retention. Forbes and Reina (1972) administered essentially carrier-free ^{212}Pb to adults and to rat pups of 16 to 32 days of age. They found that in suckling pups (16 to 22 days old) 74 to 90% of the initial dose was retained in the carcass (minus G.I. tract) 8 hours after lead administration. Only 16% of the initial dose was retained in adults. Similar results have been published by Kostial et al. (1971). Human lead balance studies have also indicated that children retain a larger fraction of orally administered lead than do adults (Alexander et al., 1973; Rabinowitz et al., 1976; Zeigler et al., 1978). The mechanism responsible for increased lead absorption and lead retention in young animals has not yet been defined. Increased lead absorption could be due to very active pinocytotic activity known to occur in young animals. This mechanism has been shown to be operative in the uptake of ^{144}Ce and ^{95}Zr-^{95}Nb from the gastrointestinal tract of suckling rats (Shiraishi et al., 1972).

It has been reported that a much larger fraction of a single intraperitoneal lead dose is retained in the brains of suckling compared to adult rats (Momcilovic and Kostial, 1974). They also reported that kidneys of suckling mice retain a smaller fraction of the dose than do adult kidneys. Data presented in Figures 9A and 9B document similar results following dosing <u>per os</u>. Furthermore, while relative lead retention in adult kidneys was found to decrease with dose, retention (FID) in kidneys of suckling mice is linearly

related to dose. Increased relative brain lead concentration in suckling compared to adult mice could be due to increased initial distribution of lead to the brains of young animals rather than the result of reduced elimination of lead from the brain (Keller and Doherty, 1980b). Relative lead retention (BCR) in brain is apparently independent of the dose administered, i.e. similar brain BCR values are found for all lead doses. This indicates that the process(es) of lead accumulation in the brain allows ready influx of large amounts of lead following acute exposure to large doses. Apparently, rates of entry and distribution of lead to the brain are not saturable within the dose range studied and thus lead concentration is proportional to the dose administered rather than being limited by capacity for uptake or for retention by the brain.

Within the broad dose range studied relative retention (BCR) of lead in femur and in skull did not change with increasing dose (Figure 4). Sequestration of a constant fraction of the absorbed dose of lead would tend to provide a protective mechanism, reducing the likelihood of lead toxicity following an acute exposure, and perhaps after chronic intermittent exposure.

The relationships between the lead concentrations in organs relative to lead concentrations in blood were highly dependent upon the dose administered for brain, femur and skull section (Tables 2 and 3). However, if only the two lowest doses are considered the range of concentration ratios (organ to blood) is much narrower. In adult mice given the carrier-free and 4 mg/kg doses there were only

small differences in the organ to blood concentration ratios between the two dose groups. In pups of the 4 and 19 mg/kg dose groups there were no significant differences between the organ to blood concentration ratios for each of the organs examined. The ratios of kidney or liver lead concentrations to blood lead concentrations were relatively constant for both age groups in the dose range studied, though some statistically significant differences were noted. Kidney is a principal target organ for acute lead toxicity (Goyer, 1971). While age-related differences were observed in the organ:blood lead concentration ratios, the age-related differences for liver and bone were less than those observed in monkeys (Willes et al., 1977). However, a larger age difference in kidney:blood concentration and a similar age relationship of brain:blood concentration were observed in mice compared to monkeys.

Our results indicate that blood lead concentration provides a reliable indicator of renal lead concentration, and hence risk of renal toxicity, following an acute exposure to an unknown lead dose. However following a single dose, blood lead concentration is a poor indicator of lead concentrations in bone and brain due to the changing organ:blood concentration ratios. Therefore, following acute lead exposure caution should be exercised in direct application of blood lead concentration to estimation of exposure and potential toxicity.

II-3: Correlation between Lead Retention and Intestinal Pinocytosis in the Suckling Mouse

Abstract

Young animals absorb and retain a greater fraction of an oral dose of lead than do adult animals. It has been proposed that pinocytotic activity in young animals is partially responsible for the increased lead retention and absorption. Radiolabeled lead (5 mg/kg) and polyvinylpyrrolidone (PVP, 50 mg/kg) were administered orally to 12-day old suckling mice and to adult mice, and the uptake of lead and PVP was determined periodically during a six day interval. Small intestines were removed, flushed clear of intraluminal contents, then divided into 24 segments of equal length for analysis. Intestinal tissue from the distal jejunum and ileum were found to contain the greatest quantities of both lead and PVP. Pretreatment of suckling mice with cortisone acetate resulted in decreased content of lead and PVP within tissue of the intestine, and decreased whole body lead retention. Cortisone pretreatment produced lower lead concentrations in blood, brain, kidney and liver. Pretreatment with cortisone was also found to reduce lead absorption following intraluminal injection of lead into the ileum. Lead and PVP uptake into intestinal tissue of adult mice was much less than uptake in suckling pups. Cortisone pretreatment of adult mice had no effect on whole body lead retention or intestinal tissue content of lead or PVP. The correlation between pinocytotic activity and lead retention supports the hypothesis that pinocytosis is one of the mechanisms involved in lead absorption in suckling mice.

Introduction

The increased susceptibility of young children to the toxic effects of ingested lead may be in large part due to greater intestinal absorption of a given dose of lead in infants compared to adults. It has been repeatedly observed that a much larger fraction of an oral lead dose is retained in infants and young animals than in adults. Rabinowitz et al. (1976) reported that approximately 10% of an oral dose was absorbed in human adults. In contrast, absorption of an oral dose in children 3 to 5 years of age was approximately 42% (Ziegler et al., 1978). Similar results have been described in experimental animal studies. Pounds et al. (1978) and Willes et al. (1977) reported increased gastrointestinal lead absorption in neonatal and young monkeys compared to adults. Keller and Doherty (1980b) observed developmental differences in lead absorption over a wide dose range in mice. Forbes and Reina (1972) showed that an abrupt decrease in lead absorption occurred between 18 and 22 days of age in young rats.

A sharp decline in pinocytotic activity, as measured by uptake of ^{125}I-PVP, has been observed between 18 and 21 days of age in rats(Clarke and Hardy, 1969). The developmental sequence of decreasing pinocytotic activity ("gut closure") is closely correlated with the temporal pattern of decreasing lead absorption (Forbes and Reina, 1972). It was hypothesized that neonatal lead absorption could be due to increased pinocytotic activity. Daniels et al. (1973) demonstrated that pretreatment of young mice with cortisone

acetate resulted in precocious gut closure. A 5 mg dose of cortisone acetate administered to 5 day-old suckling rats reduced pinocytotic activity by 12 days of age to levels observed in non-treated rats of 22 days of age or older. Cortisone-induced closure was observed to be histologically similar to maturational closure. Furthermore, they reported that endogenous plasma corticosterone levels abruptly increased during the period of maturational gut closure (18-21 days of age).

In many mammalian species pinocytosis is an important developmental process which enables the absorption of protective immunoglobulins in mother's milk by suckling offspring (Patt, 1977). We report evidence which indicates that pinocytosis is one of the mechanisms involved in neonatal lead absorption in rodents. We have shown that lead and PVP uptake occurred in the same longitudinal segments of the small intestine, that lead and PVP intestinal tissue uptake was much greater in suckling (12 days old) than in adult mice, and that cortisone pretreatment of suckling mice reduced lead retention and intestinal tissue uptake of PVP. Furthermore, cortisone pretreatment reduced lead absorption from the ileum, but not from the jejunum, following intraluminal lead injection in 12 day-old suckling mice.

Methods

Female C129F_1 mice (BALB/c females x 129 males) were produced in our Inbred Mouse Unit, were maintained at 22°C with a light period between 5:00 a.m. and 7:00 p.m. (EST), and were fed a pelleted

diet (Agway RMH 2000) and tap water ad libitum. The diet contained 1.1% calcium, 0.8% phosphorous, 9.3% fat, 18% protein, 4% fiber and approximately 1 ppm lead. Litters of suckling mice (12 days of age at lead dosing) were reduced to 5 mice per litter and with a lactating mother were placed in stainless steel metabolism cages 1 day prior to lead administration. Adult female mice (21-24 weeks old) were housed 2 mice per stainless steel cage 1 day prior to lead administration.

Radioiodinated polyvinylpyrrolidone (^{131}I-PVP) was prepared according to the procedure of Gordon (1958) with the following modifications. Na-^{131}I (carrier-free; New England Nuclear) was mixed with 100 mg of PVP (40,000 MW; Sigma Chemical Co.) and following the iodination was held at room temperature overnight. Three hours prior to administration, free ^{131}I was separated from ^{131}I-PVP by G-25 Sephadex column chromatography. A solution of ^{210}Pb (Amersham) and lead acetate (Fisher, ACS) was mixed with ^{131}I-PVP in 150 mM Na acetate for per os administration. ^{131}I-PVP was stable in solution; less than 4% of the ^{131}I-PVP was released as free ^{131}I during an 8 day period (at room temperature). A single per os dose (stomach intubation, 5.0 ml/kg) of PVP and Pb acetate were administered to mice (see below). Mice were fasted for approximately 2 hours before ^{131}I-PVP and ^{210}Pb acetate administration. Initial dose and daily whole body retention of ^{131}I-PVP and ^{210}Pb were determined by placing individual mice in the 70 x 130 mm center well of a 135 mm diameter x 160 mm deep thallium-activated sodium iodide crystal, utilizing the 360 Kev gamma emission of ^{131}I and the 47 Kev gamma emission of

^{210}Pb. Content of ^{210}Pb and ^{131}I sealed standards were determined during each counting session to correct for radioactive decay and for changes in instrument efficiency, as well as to standardize counting between the two instruments used. Total cumulative excretion was collected at each necropsy. Mice were killed by CO_2 asphyxiation and blood was collected by orbital venous plexus bleeding into heparinized volumetric capillary tubes. The stomach, small intestine (pylorus to ileocaecal junction), caecum and colon were removed from all animals. The small intestine was flushed three times with either 5 ml (suckling pups) or 10 ml (adults) of 150 mM NaCl, and divided into 24 equal length longitudinal segments. This procedure had been used by Clarke and Hardy (1969). The final wash was free of intestinal debris and contained an insignificant (<5%) amount of ^{210}Pb or ^{131}I-PVP relative to the amount remaining in the flushed intestine. The high retention of ^{131}I-PVP indicates that the flushing procedure did not disrupt the intestinal mucosa. Total length of the small intestine was 24 to 29 cm in suckling pups and approximately 47 cm in adult mice. ^{210}Pb and ^{131}I-PVP retention in the intestinal segments, stomach, colon, and blood samples were determined in a Beckman 300 gamma spectrometer. ^{210}Pb and ^{131}I-PVP retention in the carcass (whole body minus G.I. tract) were also measured. All determinations of ^{210}Pb radioactivity were corrected for the low energy emission from ^{131}I which contributes to the radioactivity emission in the ^{210}Pb energy window when the two isotopes are determined simultaneously.

In the first experiment, 7 litters of 12 day-old pups (mean

weight 9.2 ± 0.4 grams, SD) were dosed per os on day 0 with a solution containing 4.9 ± 0.3 (SD) mg/kg of lead (approximately 1 μCi ^{210}Pb) and 52.5 ± 3.2 mg/kg of PVP (approximately 0.3 μCi ^{131}I-PVP). Single litters of suckling pups were necropsied 0.5, 3 and 6 hours, and 2, 3 and 6 days following administration. Small intestines were washed and sectioned as described above.

In the second experiment, 3 groups (4 litters each) of 6 day-old suckling mice were injected (s.c.) with 150 mM NaCl (saline control), 35 mg/kg cortisone acetate (Cortone, Merck, Sharpe and Dohme) or 370 mg/kg cortisone acetate. At 12 days of age all mice were given single oral doses of 4.8 ± 0.2 mg/kg of lead (approximately 1 μCi ^{210}Pb) and 50.5 ± 2.3 mg/kg of PVP (approximately 0.3 μCi ^{131}I-PVP). At 0.13, 1 and 6 days following lead administration, mice were necropsied as described above. In addition, ^{210}Pb contents of brain, kidneys and liver were determined 6 days after lead administration.

In order to assess the effect of growth retardation on lead retention two litters of 6 day-old pups were allowed limited access to a lactating mother. One litter was fed between 9:00 a.m. and 5:00 p.m. daily; the other litter, between 5:00 p.m. and 9:00 a.m. Both litters were suckled overnight prior to oral administration of lead and PVP at 12 days of age. Pups were treated as described above. One day after lead and PVP administration the two growth-retarded litters and the two litters of continuously-fed pups were sacrificed and lead retention in carcass, intestine and blood was determined. ^{131}I-PVP content of the intestines was also determined.

Since there were no differences in tissue lead content or weights, the two growth-retarded litters were pooled for statistical analysis.

The effect of a relatively low dose of cortisone on carcass lead retention was also studied. Six daily doses of 1 mg/kg of cortisone were administered (s.c.) to suckling pups (age 6 to 11 days). At 12 days of age single oral doses of lead (4.6 mg/kg) and PVP (48.8 mg/kg) were administered orally as described above to control and cortisone pretreated pups (8 pups in two litters/group). Pups were sacrificed one day following lead and PVP administration, and carcass lead retention measured.

Urinary lead excretion from 12 day old suckling pups was studied following an oral lead dose. Mice were anesthetized with ether, the bladder carefully emptied by application of slight pressure, and the urethra sealed with cyanoacrylate cement. Lead (5 mg/kg, labeled with ^{210}Pb) was administered orally to control and to 35 mg/kg cortisone pretreated mice (as described above), and pups were sacrificed 18 hours later. Urine was aspirated from the baldder using a 30 g. needle, the volume measured, and the total urine content of ^{210}Pb measured. Carcass ^{210}Pb content was also determined.

In the adult retention and distribution experiments, female mice were pretreated with 35 mg/kg of cortisone acetate (s.c.), or with saline. Six days later single oral doses of 4.75 ± 0.24 mg/kg of lead (approximately 1.5 μCi ^{210}Pb) and 48.2 ± 3.4 mg/kg of PVP (approximately 1.8 μCi ^{131}I-PVP) were administered to the mice (mean weight, 26.6 ± 0.2 g). Groups of cortisone and saline (control) pretreated adult mice were sacrificed 0.15 days (4 mice/group) and 6

days (6 mice/group) after lead and PVP administration. In addition, groups of 6 saline pretreated mice were sacrificed 0.03, 0.29, 1.2 and 2.2 days after lead and PVP administration. Blood was collected as previously described, the gastrointestinal tract was removed and radioactive lead content in the remaining carcass was measured. Gut flushing and longitudinal sectioning procedures were performed only on the 0.15 day necropsy group.

Lead absorption in 12 day-old suckling mice was also studied with in situ intestinal preparations. Pups were either pretreated with 35 mg/kg of cortisone as described before or left untreated. Pups were anesthetized with ether (for a total of 20 minutes) and opened with a midline incision. ^{210}Pb (as Pb-acetate, 5 µg/pup, 15 µl/pup, pH 6) was injected directly into the lumen of either the jejunum (4 cm distal to pylorus) or the ileum (13 cm distal to pylorus); the bile duct was ligated with silk suture; the urethra, sealed with cyanoacrylate cement; and the incision, closed by suturing. Mice were necropsied 1 hour after lead injection. After removal of intestine and colon, radioactivity in the remaining carcass was determined to assess lead absorption and retention.

Lead retention is expressed as Fraction of Initial Dose (FID) retained in the whole body, carcass (minus G.I. tract), or in sections of the small intestine.

$$FID = (\text{ng Pb in sample, time } t) \div (\text{ng Pb in whole body, time } 0)$$

Differences in organ lead retention were tested for statistical

significance using the Duncan multiple range test (Nie et al., 1973). All tests for significance were conducted on groups treated concurrently. Differences in the means of data presented in Table 2 were tested for statistical significance with Student's t-test.

Results

Time Course of in vivo ^{131}I-PVP Uptake and ^{210}Pb Retention in Suckling Mice

Figure 11 presents the FID of ^{131}I-PVP and ^{210}Pb retained in tissue segments of the small intestine of suckling mice in the first experiment. Total recovery (whole body retention at necropsy plus cumulated excretion) was 95.8 ± 8.9(SD)% for administered lead and 101.9 ± 2.7% for ^{131}I-PVP. ^{131}I-PVP uptake into flushed intestinal tissue segments increased between 30 minutes and 3 hours following oral administration. Only 0.030 ± 0.009(SD) FID remained in the stomach 3 hours after administration. One day following PVP administration, 0.92 ± 0.03 FID was retained in the 24 flushed intestinal tissue segments, indicating nearly complete uptake of administered PVP into the intestinal mucosa. This is consistent with ^{125}I-PVP pinocytosis observed in 14 to 16 day old rat pups given a similar initial dose of PVP (Clarke and Hardy, 1971). Loss of PVP from intestinal tissue occurred between 1 and 6 days after administration. On day 6 no PVP was found in the intestinal segments. This was presumably the result of exfoliation of epithelial cells of the intestinal mucosa. Between days 1 and 6 an apparent shift occurred in location of the intestinal segment exhibiting peak PVP retention toward the distal end of the small intestine.

A progressive small increase in retention of ^{131}I in the carcass (whole body minus G.I. tract) of suckling pups occurred between 0 and 6 days following ^{131}I-PVP administration. Mean retentions of ^{131}I (FID) were 0.006 (at 0.02 days), 0.013 (0.13 days), 0.014 (0.2 days), 0.030 (1 day), 0.044 (2 days), 0.050 (3 days) and 0.067 (6 days). The appearance of small amounts of ^{131}I in the carcass probably represented absorption of free ^{131}I released from ^{131}I-PVP, since a similar fraction of ^{131}I was found to be released from ^{131}I-PVP in solution during an 8 day period. The ^{131}I-PVP macromolecule probably was not absorbed into the body from the intestine. The small amount of free ^{131}I absorbed did not distort the pattern of ^{131}I-PVP uptake into intestinal segments. It was determined that less than 0.01 FID remained in the G.I. tract (including luminal contents) of adult mice 24 hours after per os administration of carrier-free $Na^{131}I$.

The results also indicate that lead did not interact with PVP in the G.I. tract. ^{131}I-PVP was retained for long periods (1-2 days) in the flushed intestine, while lead was absorbed rapidly (within hours) through the intestine into the carcass. Furthermore, differences were observed in the rates of emptying of ^{131}I-PVP and lead from the stomach, with lead being emptied more slowly. Finally, no interaction was found between ^{131}I-PVP and ^{210}Pb acetate in the administered solution, as indicated by complete separation by exclusion column chromatography (Sephadex G-25, 15 ml volume, 0.05 M Na acetate).

Greatest lead retention in flushed intestinal segments was

observed 30 minutes after oral administration (0.24 ± 0.09 FID; Figure 11b). One day following lead administration only 0.012 ± 0.001 FID of lead was found in the washed intestinal segments. While the magnitudes of intestinal uptake and the time courses of elimination of PVP and lead differed, the patterns of regional distribution of PVP and lead in the intestinal segments were very similar, with peak retention for both chemicals occurring in the distal half of the intestine. These observations indicate that the same processes may be involved in the uptake of ^{131}I-PVP and lead into the cells of the small intestine.

Effect of Cortisone Pretreatment on in vivo ^{131}I-PVP and ^{210}Pb Uptake in Suckling Mice

To assess the effect of cortisone pretreatment on lead retention and distribution, and ^{131}I-PVP intestinal pinocytosis in suckling mice, a second experiment was performed. Suckling mice (6 days old) were pretreated with saline or cortisone, and were dosed with ^{210}Pb and ^{131}I-PVP 6 days later. Total recovery of ^{210}Pb was 95.8 ± 10.5(SD)% and of ^{131}I-PVP, 107.3 ± 6.1%. Only 0.037 ± 0.005(SD) FID of administered ^{131}I was retained in the carcasses of mice necropsied on days 0, 1 and 6. As shown in Figure 12, the high cortisone dose (370 mg/kg) resulted in severe growth retardation. While there was no mortality in any of the other groups, 25% mortality (2/8) was observed in the 370 mg/kg cortisone pretreatment group. On day six, spleens in the 370 mg/kg cortisone pretreatment group were severely atrophied (mean weight, 0.025 g) compared to 0.085 g (35 mg/kg cortisone group) and 0.084 g (saline control group).

Lead retention (FID) in whole body and in carcass of suckling mice following saline or cortisone pretreatment is shown in Figure 13. The differences between whole body and carcass lead content on days 1 and 2 represent lead within the G.I. tract, nearly all of which is in the colon. Lead retention (FID) in carcass of the saline pretreatment group was 3 to 7-fold greater ($p<0.05$) than lead retention in the 370 mg/kg cortisone pretreatment group in mice necropsied at all time points (0.15, 1 and 6 days). Lead retention in carcass of the saline group was significantly greater ($p<0.05$) than in the 35 mg/kg cortisone group on days 1 and 6. On day 6 only, carcass lead retention was greater ($p<0.05$) in the 35 mg/kg cortisone group compared to the 370 mg/kg cortisone group. A cortisone dose-related decrease in lead retention is apparent.

The uptake and elimination of lead from blood following saline and cortisone treatment of suckling pups is shown in Figure 14. Control blood data were pooled from the two experiments. The maximum observed blood lead concentration (ng/ml) was measured 9 hours following oral lead administration. With the exception of the 1 day time point for the 35 mg/kg cortisone dose group, blood lead concentrations in suckling mice were decreased by cortisone treatment.

Cortisone pretreatment reduced lead concentrations in the brain, kidney and liver of suckling mice (Table 5). Six days after lead administration, brain, kidney and liver lead concentrations (ng Pb/g) were lower, and total lead content (ng Pb) of each organ was reduced by pretreatment with increasing dose of cortisone.

Figure 15 presents PVP retention in intestinal segments at 3 hours and at 1 day following an oral dose to suckling mice pre-treated with saline or cortisone. Significant differences ($p<0.01$) in ^{131}I-PVP retention in intestinal segments were observed among the three pretreatment groups at 3 hours and at 1 day following oral ^{131}I-PVP administration. At 3 hours, 0.91 ± 0.05 (SD) FID and at 1 day, 0.84 ± 0.06 FID of ^{131}I-PVP was retained in the 24 small intestine segments from the saline treated group. In the 35 mg/kg cortisone pretreatment group, 0.57 ± 0.20 FID (3 hours) and 0.57 ± 0.18 FID (1 day) of ^{131}I-PVP was retained. In the 370 mg/kg cortisone pretreatment group, only 0.10 ± 0.11 FID was retained at 3 hours, and less than 0.001 FID, at 1 day following PVP administration. Approximately 0.05 FID of PVP remained in the stomach in all pre-treatment groups 3 hours after oral PVP administration. Cortisone pre-treatment resulted in a dose-related decrease in PVP uptake and retention at both time points. A nearly constant FID and pattern of distribution of ^{131}I-PVP occurred in the small intestine between 3 hours and 1 day after administration in the saline pretreated (control) and the 35 mg/kg cortisone dose groups.

The retention of ^{210}Pb (FID) in intestinal segments of suckling mice pre-treated with saline or cortisone is presented in Figure 16. A decrease in lead retention in the small intestine (total FID retained in 24 segments) was observed 1 day following lead administration as a result of cortisone pretreatment. FID's retained in flushed intestines were 0.0184 ± 0.0084 (saline), 0.0098 ± 0.0064 (35 mg/kg cortisone), and 0.0064 ± 0.0017 (360 mg/kg cortisone).

Intestinal retention of ^{210}Pb was significantly greater ($p<0.05$) in the saline group than in the cortisone pretreatment groups. This is consistent with the observation that cortisone pretreatment reduced lead retention in carcass and whole body of suckling mice.

Cortisone pretreatment of suckling pups resulted in quantitatively similar decreases in pinocytotic activity and carcass lead retention. Pretreatment with 35 mg/kg cortisone decreased intestinal PVP uptake to 63% of the amount retained in the intestine of saline treated controls 3 hours after PVP administration. Pretreatment with 370 mg/kg cortisone resulted in a decrease to 11% of the PVP intestinal uptake of saline treated control animals. One day after administration of 35 mg/kg cortisone, retention of lead in the carcasses of suckling pups was reduced to 46% of lead retention in control pups. Pre-treatment with 370 mg/kg cortisone reduced lead retention in pup carcasses to 14% of control values. These data strongly support the hypothesis that the same mechanism(s) is involved in intestinal mucosal uptake of PVP and lead.

Growth retardation of suckling mice (by periodic removal of lactating mother) did not affect lead retention. Growth-retarded pups exhibited a mean weight increase of 24% between 6 and 12 days of age compared to 57% in control pups (which was similar to the growth rate difference between 35 mg/kg cortisone pretreated pups and controls). Lead retention (FID) in growth-retarded (0.18 ± 0.01, SE; $n=8$) versus control pups (0.20 ± 0.02; $n=8$) did not differ ($p>0.3$) 24 hours after p.o. lead administration. Blood lead concen-

trations and ^{131}I-PVP mucosal uptake were also not affected by growth retardation. These data indicate that the growth retardation of mice pretreated with cortisone was not a significant causative factor in the observed decrease in lead retention.

Lead retention in suckling pups could be diminished by much lower doses of cortisone if the dose was administered daily. A daily dose of cortisone (1 mg/kg, s.c.) administered from 6 through 11 days of age resulted in a 33% decrease in carcass lead retention following an oral lead dose of 4.6 mg/kg (0.19 ± .01 SE FID in cortisone pretreated pups vs. 0.29 ± .04 in controls; n=8/group; $p<0.05$). No differences were noted between the daily 1 mg/kg cortisone treated group and controls in body weight increase from 6 to 13 days of age (208 vs. 213% respectively) or in the relative weights of the spleens (0.0062 vs. 0.0064 fraction of final body weight, respectively). Diminished lead retention was noted in the absence of significant toxic effects of cortisone or growth.

^{210}Pb Absorption in situ in Suckling Mice

Ligation of the bile duct and blockage of the urethra enable reliable measurement of lead absorption following ^{210}Pb injection into the lumen of either jejunum or ileum since possible recycling into the intestine and urinary loss are prevented. It was determined that lead injected into the jejunum or the ileum remained at or near the site of intraluminal instillation during the one hour period. Table 6 shows that in control pups a greater FID was absorbed from the ileum than from the jejunum. In addition, cortisone pretreatment reduced lead absorption only in the ileum.

Urinary Lead Excretion in Suckling Mice

Following blockage of the urethra and an oral dose of 5 mg/kg of lead, lead excretion in urine during the subsequent 18 hour period accounted for only a small fraction of the administered dose. Only 0.0021 ± .0007 (SD) FID of lead was excreted over the 18 hour period in urine from control pups (n=4). A similar amount (0.0022 ± .003 (SD) FID) was excreted in the urine of pups pretreated with 35 mg/kg cortisone (n=3). This finding documents that urinary lead excretion is a minor route of lead elimination in suckling mice and that cortisone pre-treatment does not cause decreased carcass lead retention by increasing the rate or urinary lead excretion.

^{131}I-PVP Uptake and ^{210}Pb Retention in Control and in Cortisone Treated Adult Mice

PVP uptake and lead retention by the small intestine of adult female mice were greatly decreased relative to retention and uptake in suckling mice. Total recoveries (cumulated excretion in cages plus whole body retention at necropsy) of administered ^{210}Pb and ^{131}I-PVP were 84 ± 2(SD)% and 103 ± 1% respectively. Retention of ^{131}I in the carcass was constant over the time period of 3 hours through 6 days after ^{131}I-PVP administration; a mean ^{131}I carcass retention of 0.043 FID was observed which was similar to the retention found in suckling pups. This ^{131}I retention probably represents free ^{131}I which was not separated from the ^{131}I-PVP in the preparation of the labeled macromolecule, and/or that released by deiodination of the ^{131}I-PVP subsequent to administration.

Lead retention in the carcasses of adult mice, which represents absorbed lead minus lead absorbed but subsequently excreted, was not affected by prior cortisone administration (Figure 17). Furthermore, no differences were observed in blood lead concentrations among saline and cortisone pretreated adult groups (Figure 14).

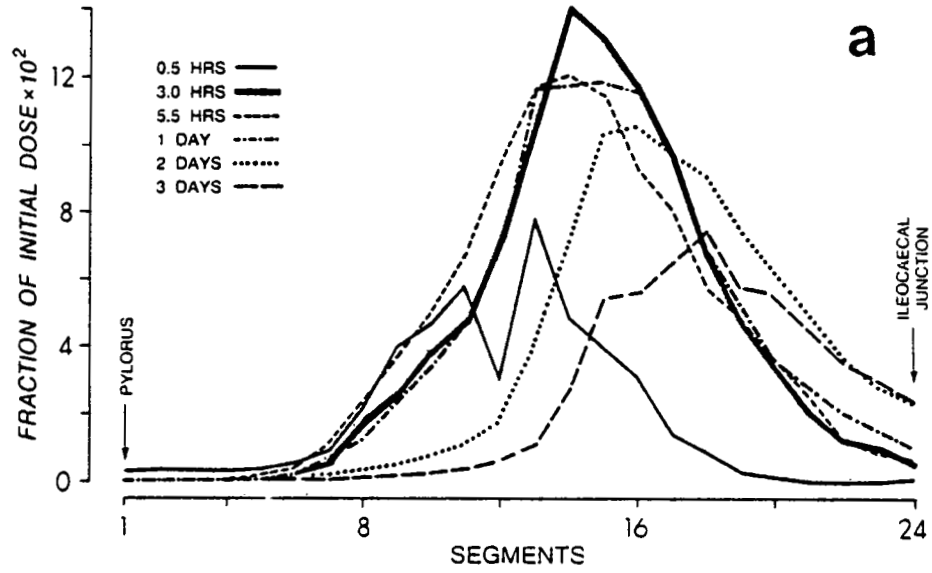
The ^{131}I-PVP and ^{210}Pb contents of the saline-flushed small intestine of adult mice were greatly reduced as compared to intestine of suckling mice. Only 0.0013 ± 0.0007(SD) FID of ^{131}I-PVP and 0.0047 ± 0.0017 FID of ^{210}Pb were retained in the 24 small intestine segments of adult mice 3 hours after lead administration. Furthermore, PVP uptake and lead retention in intestinal tissue segments was uniform along the length of the adult intestine, in contrast to the increased uptake and retention in the distal small intestine of suckling mice. Cortisone pretreatment of adults did not decrease intestinal uptake or retention of ^{131}I-PVP (0.0016 FID) or ^{210}Pb (0.007 FID) compared to uptake in control (saline pretreated) adult mice.

Figure 11: Retention (expressed as Fraction of Initial Dose) of ^{131}I-PVP (a) and ^{210}Pb (b) in saline flushed, consecutive small intestine segments of suckling mice (n=5/time point) at various times following p.o. administration of 53 mg/kg ^{131}I-PVP and 4.9 mg/kg ^{210}Pb acetate.

Duodenum: Segments 1-3

Jejunum: Segments 4-11

Ileum: Segments 12-24


a
FRACTION OF INITIAL DOSE × 10²
0.5 HRS
3.0 HRS
5.5 HRS
1 DAY
2 DAYS
3 DAYS
PYLORUS
ILEOCAECAL JUNCTION
0
4
8
12
1
8
16
24
SEGMENTS

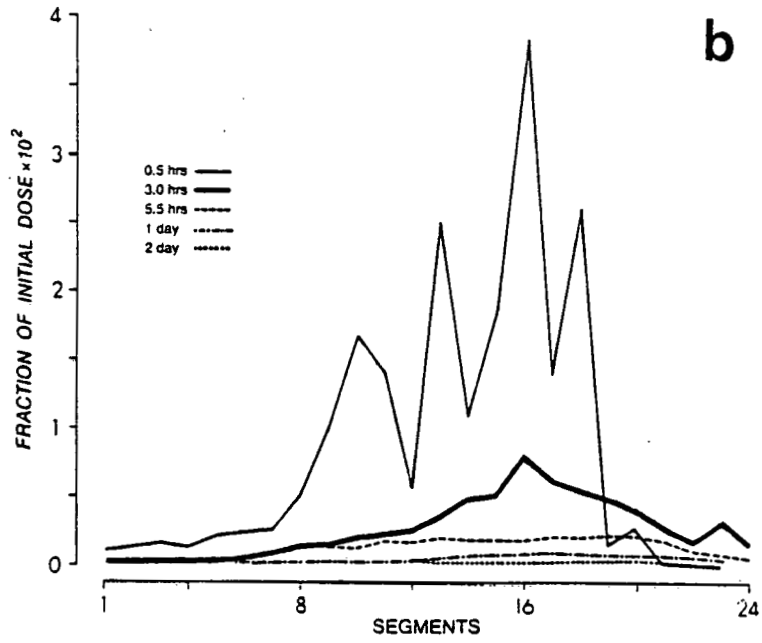

b
FRACTION OF INITIAL DOSE × 10²
0.5 hrs
3.0 hrs
5.5 hrs
1 day
2 day
0
1
2
3
4
1
8
16
24
SEGMENTS

Figure 12: Weights (g ± SE) of saline, 35 mg/kg cortisone, and 370 mg/kg cortisone pretreated suckling mice. Cortisone or saline pretreatment occurred on day (-6) when mice were 6 days old. Mice were 12 days of age on day 0 when ^{210}Pb acetate and ^{131}I-PVP were administered. Error bars not shown are smaller than symbol dimensions.

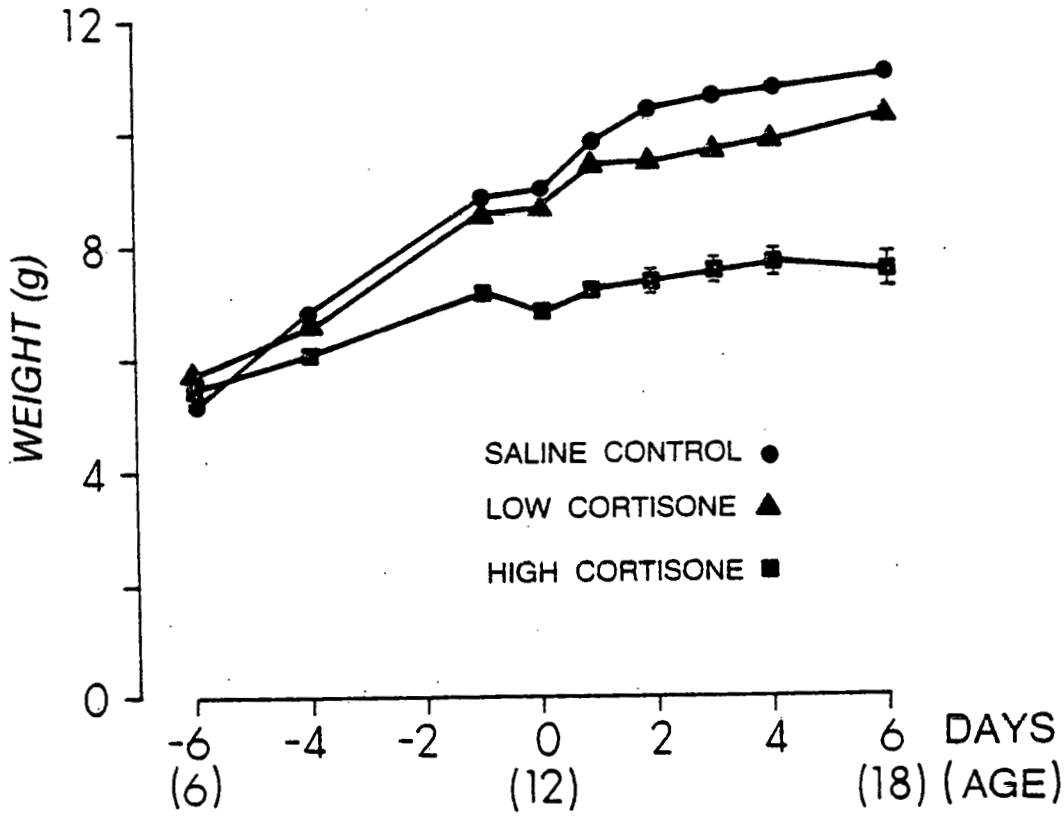

12
8
4
0
WEIGHT (g)
SALINE CONTROL
LOW CORTISONE
HIGH CORTISONE
-6
-4
-2
0
2
4
6
DAYS
(6)
(12)
(18) (AGE)

Figure 13: Lead retention (FID ± SE; n=5/data point) in the whole body (closed symbols) or carcass (whole body minus G.I. tract; open symbols) of suckling mice pretreated (day -6) with saline, 35 mg/kg cortisone, or 370 mg/kg cortisone, and administered 4.9 mg/kg ^{210}Pb acetate and 53 mg/kg ^{131}I-PVP *per os* on day 0. Error bars not shown are smaller than symbol dimensions.

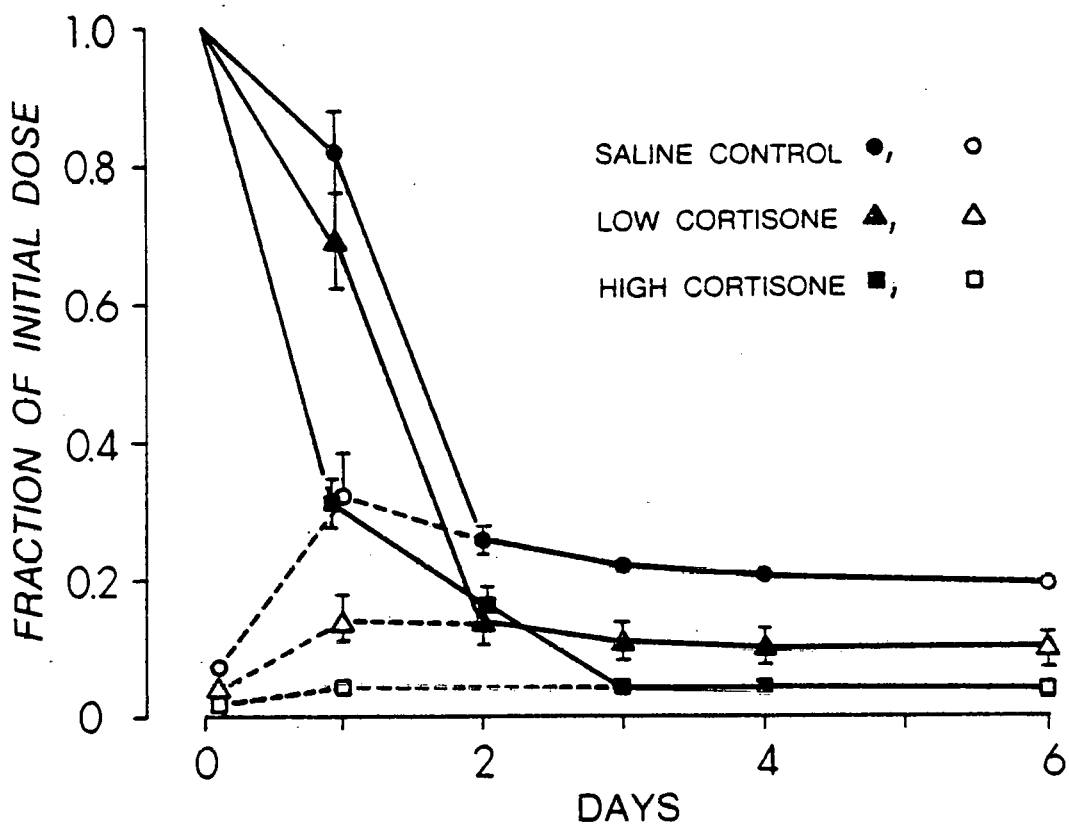

FRACTION OF INITIAL DOSE
1.0
0.8
0.6
0.4
0.2
0
0
2
4
6
DAYS
SALINE CONTROL ●, ○
LOW CORTISONE ▲, △
HIGH CORTISONE ■, □

Figure 14: Blood lead concentration (ng/ml ± SE) following saline (●), 35 mg/kg cortisone (▲) or 370 mg/kg cortisone (■) pretreatment of suckling pups; and saline (△) or 35 mg/kg cortisone (◑) pretreatment of adult mice. Mice received 4.8 mg/kg ^{210}Pb acetate p.o. on day 0. Error bars not shown are smaller than symbol dimensions.

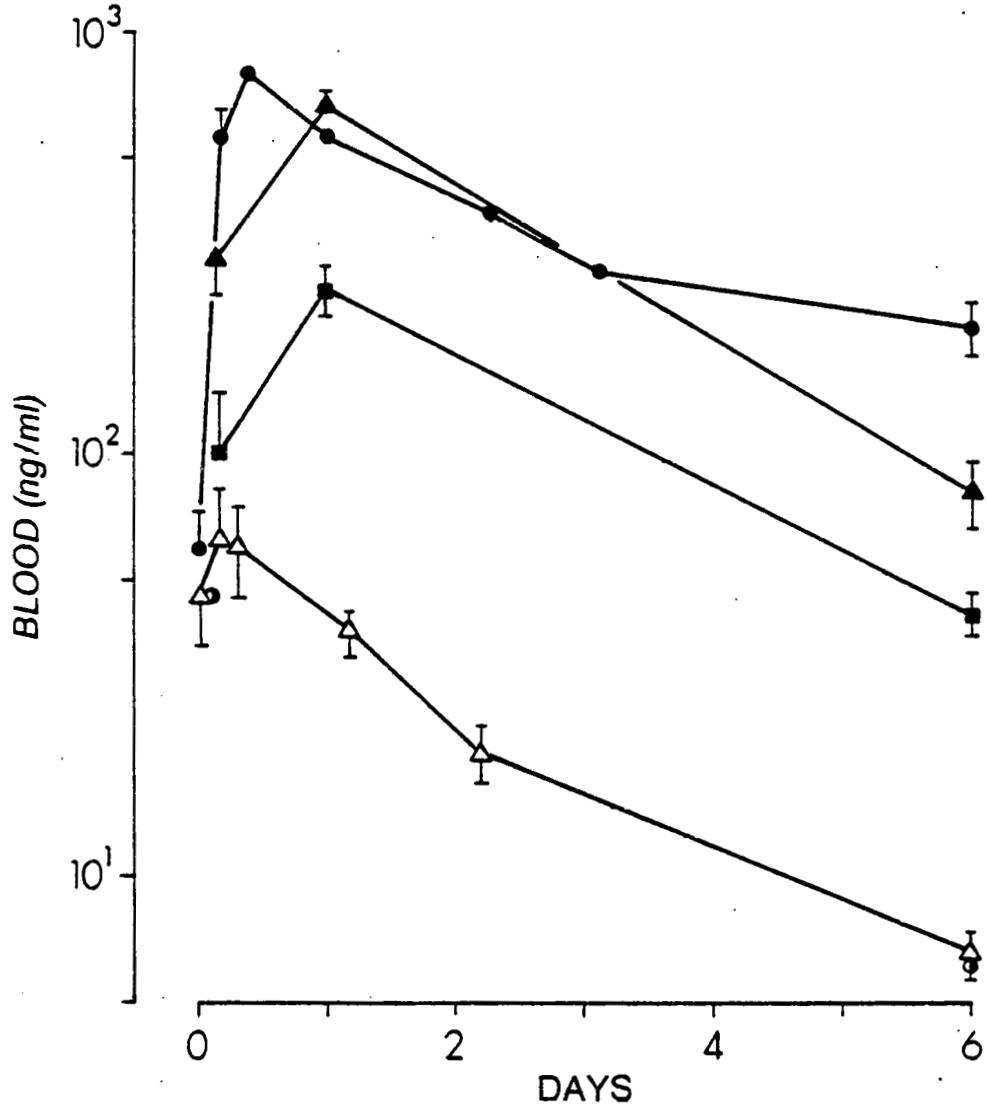
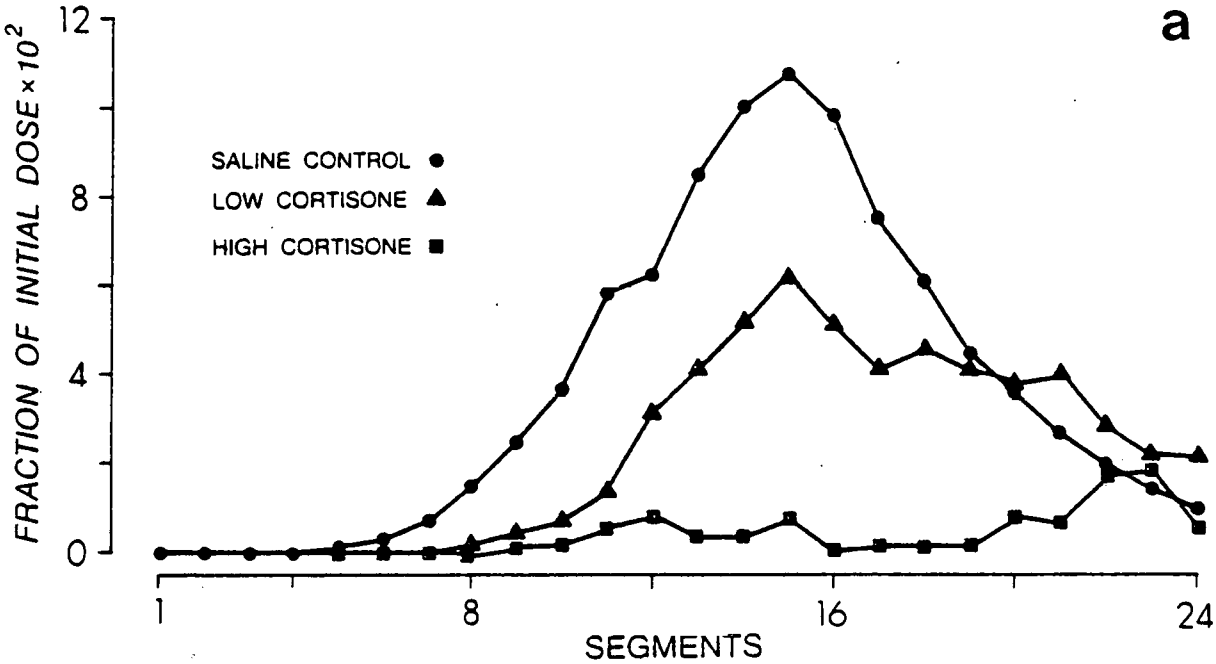

10^3
10^2
10^1
BLOOD (ng/ml)
0
2
4
6
DAYS

Figure 15: Retention (expressed as Fraction of Initial Dose) of ^{131}I-PVP in flushed, consecutive small intestine segments of suckling pups (n=5/group) 3 hours (a) and 1 day (b) following p.o. administration of 51 mg/kg of ^{131}I-PVP and 4.8 mg/kg ^{210}Pb acetate after pretreatment with saline, 35 mg/kg cortisone, or 370 mg/kg cortisone.

Duodenum: Segments 1-3

Jejunum: Segments 4-11

Ileum: Segments 12-24


a
FRACTION OF INITIAL DOSE x 10²
12
8
4
0
SALINE CONTROL
LOW CORTISONE
HIGH CORTISONE
1
8
16
24
SEGMENTS

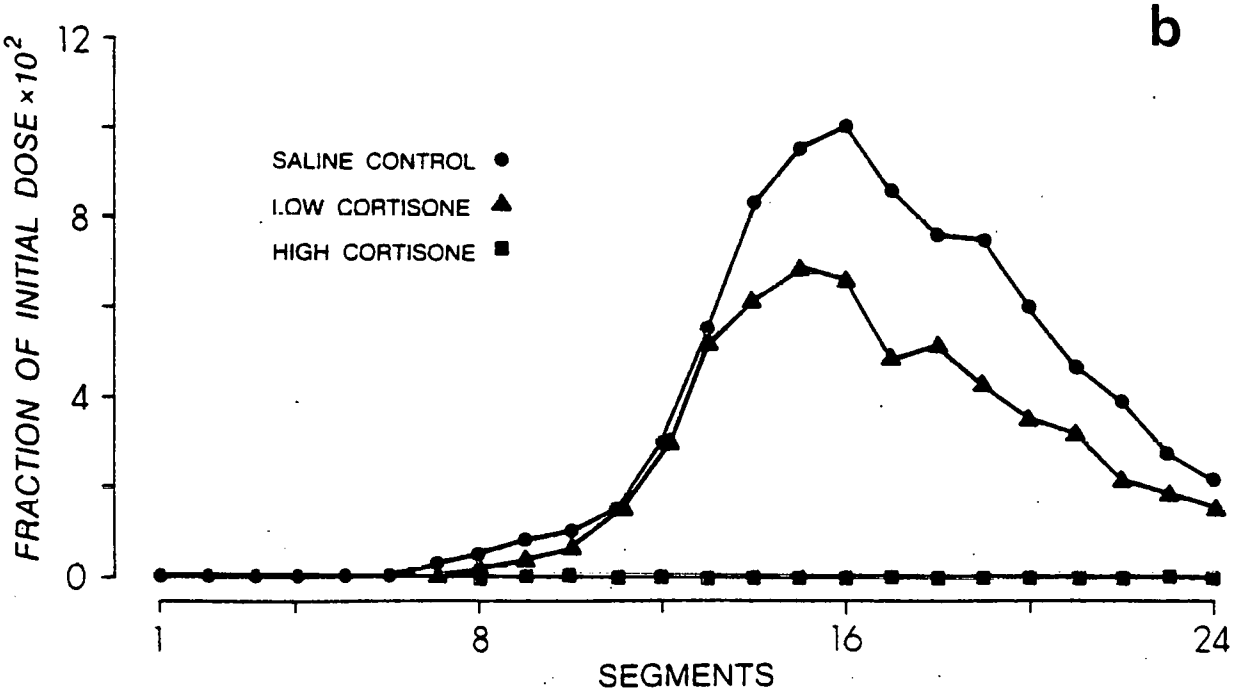

b
FRACTION OF INITIAL DOSE x 10²
12
8
4
0
SALINE CONTROL
LOW CORTISONE
HIGH CORTISONE
1
8
16
24
SEGMENTS

Figure 16: Retention (expressed as Fraction of Initial Dose) of ^{210}Pb in flushed, consecutive small intestine segments of suckling pups (n=5/group) 1 day after p.o. administration of 4.8 mg/kg ^{210}Pb acetate and 51 mg/kg ^{131}I-PVP, after pretreatment with saline, 35 mg/kg cortisone, or 370 mg/kg cortisone.

Duodenum: Segments 1-3

Jejunum: Segments 4-11

Ileum: Segments 12-24

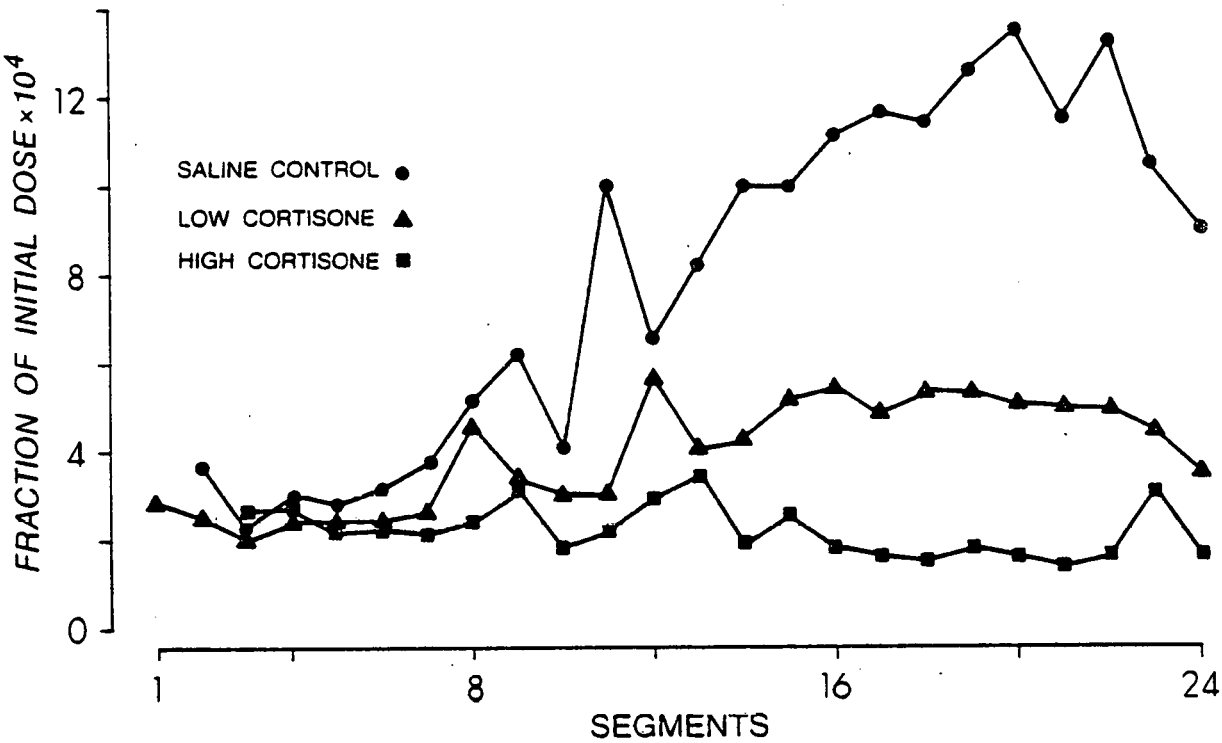

FRACTION OF INITIAL DOSE x 10^4
12
8
4
0
SALINE CONTROL
LOW CORTISONE
HIGH CORTISONE
1
8
16
24
SEGMENTS

Figure 17: Lead retention (expressed as Fraction of Initial Dose) in whole body (△) or in carcass (◑ ; whole body minus G.I. tract) of adult mice (n=6/time point) following p.o. administration of 4.8 mg/kg ^{210}Pb acetate, and 51 mg/kg ^{131}I-PVP. Error bars not shown are smaller than symbol dimensions.

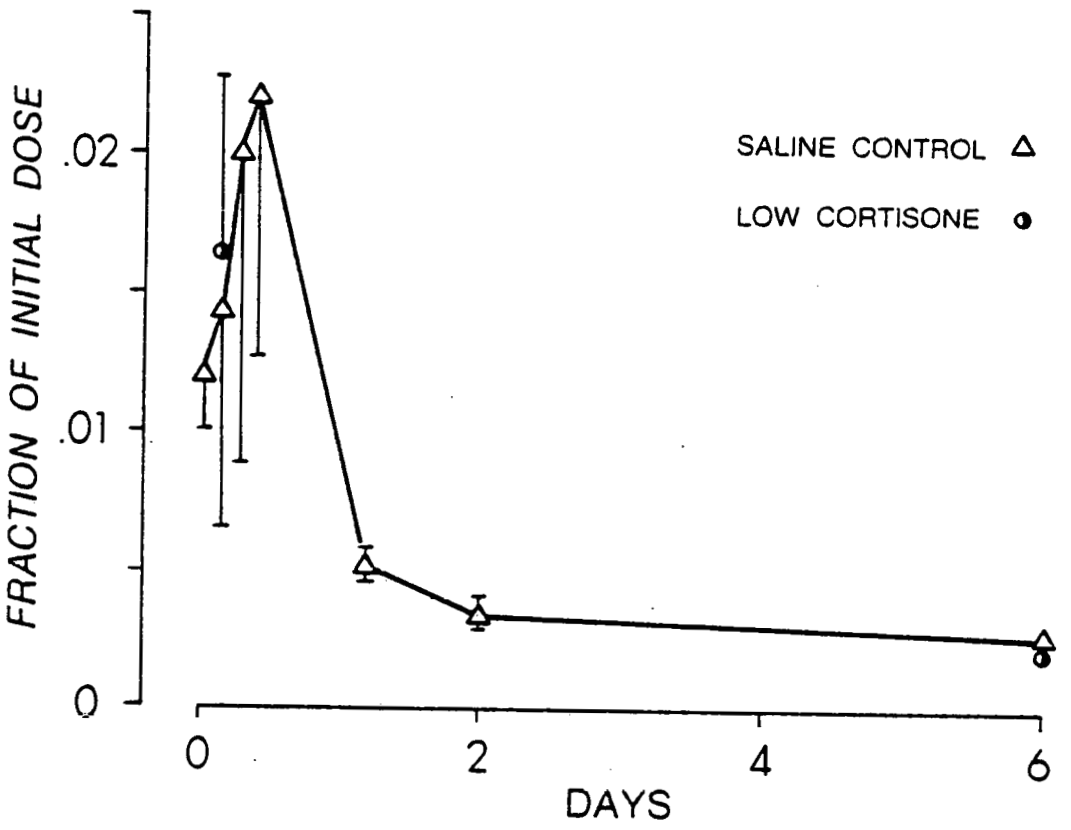

FRACTION OF INITIAL DOSE
.02
.01
0
SALINE CONTROL
LOW CORTISONE
0
2
4
6
DAYS

Table 5: Effect of Cortisone Pre-treatment on Lead Content of Organs.*

Organ	Pretreatment	ng Pb/g (±SE)	ng Pb (± SE)
Brain:	Saline (controls)	87(±3)†	38(±1)†
	Low Cortisone	47(±13)≠	19(±6)≠
	High Cortisone	16(±3)≠	5(±1)≠
Kidney:	Saline (controls)	555(±19)†	83(±5)†
	Low Cortisone	326(±74)	46(±11)
	High Cortisone	235(±30)	32(±5)
Liver:	Saline (controls)	199(±7)†	85(±2)†
	Low Cortisone	96(±26)	40(±12)
	High Cortisone	66(±15)	23(±5)

*Suckling mice were pre-treated by s.c. injection of saline (control, n=6), or cortisone acetate, 35 mg/kg (low cortisone, n=7) or 370 mg/kg (high cortisone, n=6) of cortisone acetate. Lead (5 mg/kg) was administered p.o. 6 days later (12 days of age) and necropsies were performed 6 days after administration.

†Significantly different ($P<0.01$) from other groups.

≠Significantly different ($P<0.05$) from other groups.

Table 6: Lead Absorption (FID±SE) *in situ* from Intestines of 12 day old Mice*

Pretreatment of 6 day olds	Fraction of Administered Dose	
	Jejunum	Ileum
Controls	0.23† (±.02)	0.33 (±.03)
Cortisone (35 mg/kg)	0.25 (±.03)	0.10†† (±.02)

*Suckling mice (n=7-9/group) were pretreated (6 days of age) by s.c. injection of 35 mg/kg of cortisone acetate, or left untreated (controls). Lead (5 µg) was injected directly into the lumen of either the proximal or distal small intestine (following ligature of bile duct and urethra) of 12 day old mice, and then necropsied 1 hr. later.

†Significantly different ($p<0.02$) from control ileum.

††Significantly different ($p<0.001$) from control ileum.

Discussion

Numerous investigators have reported increased gastrointestinal absorption of lead in suckling or young animals (Forbes and Reina, 1972; Kostial et al., 1971) and in children (Alexander et al., 1973; Zeigler et al., 1978). The mechanism(s) responsible for the age differences in lead absorption has not been identified. The suggestion that pinocytosis is involved in increased neonatal lead absorption was primarily based upon the age-specific decrease in PVP uptake and lead absorption occurring between 18 and 22 days of age in young rats (Forbes and Reina, 1972). The relatively sharp decrease in carcass lead retention noted by Forbes and Reina in this age period contrast with the results of Conrad and Barton (1978) which indicated a relatively constant decrease in lead absorption from just after weaning to one year of age. Their data indicated that lead absorption was a function of the growth rate of the rats. In young mice we have observed that six days after a single oral lead dose (5 mg/kg) a constant fraction of the dose is retained when the dose is administered prior to weaning (18 days of age). However, by 22 days of age only 0.033 ± 0.013 (SD) FID (n=10) is retained in the carcass, a value which approaches the carcass lead retention observed in adult mice.

Our observations provide substantial support for the hypothesis that pinocytotic capacity of suckling mice is directly related to increased lead absorption in suckling animals compared to that observed in adults. Our results indicate that maximal uptake of

^{131}I-PVP and ^{210}Pb by intestinal tissue occurs in the distal half of the small intestine of suckling mice. This finding is in agreement with the experimental results of Clarke and Hardy (1969) who reported that pinocytotic uptake of PVP occurs mainly in the distal small intestine, and that the intestinal tissue region exhibiting maximal pinocytotic activity shifts distally as the animal ages. The lead content of intestinal tissue from suckling mice is not a direct indicator of absorptive rate since tissue content of lead is dependent both upon internalization of lead into mucosal cells and subsequent transfer into the circulatory system. The observation that tissue lead content in the proximal intestine is very low, even 30 minutes after p.o. administration, indicates that pinocytosis of lead by the proximal small intestine does not occur, unless the rate of lead transfer to blood is much more rapid than that observed in the distal small intestine. In contrast, ^{210}Pb and ^{131}I-PVP contents of adult intestinal tissue were uniform throughout its length. The increased retention of lead (3 hours) in the intestinal tissue and carcass of suckling mice as compared to adults supports the hypothesis that an additional, or different, mechanism is involved in lead absorption in suckling and adult mice. In suckling mice the high correlation of retained lead and PVP contents of intestinal segments indicates that the same mechanism may be involved in the uptake of both chemicals.

Pretreatment of suckling rat pups with cortisone has been reported to decrease intestinal pinocytotic activity as indicated by

reduction of PVP uptake. In addition, increased levels of endogenous cortisone were shown to coincide with the developmental timing of cessation of pinocytotic activity (gut closure) (Daniels et al., 1973). We report a cortisone dose-related decrease in uptake of PVP and lead into intestinal tissue, in blood lead concentration, and in carcass (whole body minus G.I. tract) lead retention. Cortisone treatment reduced ^{131}I-PVP uptake into intestinal tissue and ^{210}Pb retention in carcass by nearly the same extent. Furthermore, in cortisone pretreated preweanling mice lead concentrations were reduced in target organs of lead toxicity. Although cortisone pretreatment reduced the growth rate of suckling mice growth, similar retardation produced by periodic fasting did not affect lead retention. This indicates that growth retardation was not a confounding variable in these experiments. Cortisone pretreatment of adult mice did not affect ^{210}Pb or ^{131}I-PVP content of intestinal tissue after flushing, whole body lead retention, or blood lead concentration.

Lead absorption following instillation of lead into the intestinal lumen was also determined (Table 6). Lead absorption in ileum, but not in jejunum, was strikingly decreased by cortisone pretreatment. This was probably the result of decreased pinocytotic activity in the ileum, as had been observed following oral administration of lead and PVP.

A large fraction of the lead dose was absorbed from the jejunal instillation site, a site where pinocytosis (^{131}I-PVP uptake) did not occur *in vivo*. While lead absorption from the ileum was decreased

by cortisone pretreatment, lead absorption from the jejunum was not altered by prior cortisone administration. These results indicate that at least one other absorptive mechanism is involved in lead absorption from the intestine of suckling mice. Studies in adult rats have indicated that iron (Barton et al., 1978b) and calcium (Barton et al., 1978a) carrier-mediated transport mechanisms are involved in lead absorption from the intestine of adult rats. In addition to the involvement of carrier-mediated mechanisms, it is probable that simple diffusion of lead is also involved in lead absorption by suckling mice. Our in situ results are compatible with diffusional and carrier-mediated lead absorption in the jejunum, but it is not possible to evaluate these processes in terms of ileal lead absorption. There are no published data with which to assess the quantitative contributions of diffusional and carrier-mediated absorption in young animals. However, the observation that pretreatment with even 360 mg/kg of cortisone (Fig. 13) failed to reduce lead retention in suckling pups to adult levels indicates that mechanisms other than pinocytosis are involved in lead absorption.

Our study documents that the cortisone-induced decrease in carcass lead retention following an oral lead dose is not the result of a cortisone-induced stimulation of lead excretion. The effect of cortisone pretreatment on ileal lead absorption provides a strong correlation between lead absorption and pinocytosis.

Previous reports have shown that there are large differences between young and adult animals in whole body lead elimination

following intraperitoneal lead administration (Momcilovic and Kostial, 1974; Quarterman and Morrison, 1978). Our results (Figures 13 and 17) indicate that differences in the rates of lead elimination from the carcasses of suckling and adult mice are not substantial following oral lead administration, the principal route of human lead exposure. This is supported by our observation of similar rates of lead elimination from blood of suckling and adult mice (Figure 14). However, large differences were observed in the fractions of the initial doses absorbed, with suckling mice absorbing a much larger FID.

Shiraishi and Ichikawa (1972) hypothesized that pinocytosis may be involved in the gastrointestinal absorption of ^{144}Ce and ^{95}Zr-^{95}Nb radioisotopes Sasser and Jarboe (1977) reported increased ^{115}Cd absorption in newborn compared to 6 week-old rats. In addition, they found that nearly all of the p.o. administered Cd remained bound to the small intestine until 12 to 15 days of age, when loss presumably occurred by intestinal mucosal exfoliation. Landry (personal communication) has observed a pattern of PVP and mercuric mercury uptake in intestinal segments of 14 day-old mice similar to uptake of ^{131}I-PVP and ^{210}Pb . Forbes and Reina (1972) demonstrated age-specific absorption of ^{59}Fe and ^{85}Sr as well as ^{212}Pb in rats. Kostial et al. (1978)reported increased absorption of radioisotopes of cadmium, inorganic mercury, and manganese, as well as lead, in 1 week-old suckling rats compared to adult rats.

It has not yet been possible to establish whether pinocytosis is responsible for increased lead absorption in infants and young

children. Species variation in pinocytotic activity and timing of closure are known to exist (Kraehenbuhl and Campiche, 1969). However, indirect evidence suggests that newborn infants can absorb intact macromolecular antigens which have been ingested (Kletter et al., 1971; Rothberg, 1969). Absorption of intact macromolecules requires that the macromolecule be internalized by pinocytosis, and then transferred from the pinocytotic vesicle into the circulatory system without degradation. It is probable that only the first step (cellular internalization) is required for lead to be absorbed. Therefore, absorption of antigenic macromolecules by infants might not be an reliable indicator of potential pinocytotic lead absorption. Pinocytotic internalization of macromolecules (such as PVP) has not been reported in humans. While pinocytotic uptake of metals may be a general phenomena in rodents, further investigation will be required to establish whether this mechanism of absorption occurs in young children.

II-4: Lead and Calcium Distributions in Blood, Plasma and Milk of the Lactating Mouse

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Abstract

Though it has been established that lead is transferred in milk from mother to suckling offspring, the physiological processes and parameters involved are not well understood. Single i.v. (0.2 mg/kg) or p.o. (2 mg/kg) doses of radiolabeled lead were administered to lactating and non-lactating female mice, and lead concentrations in blood, plasma and milk were determined during a 21 days period. Large differences in lead elimination were observed between lactating and non-lactating mice. A significant fraction (25%) of the initial maternal dose ("absorbed" dose) was transferred to the suckling pups. The ratio of lead concentration in milk to lead concentration in plasma was found to be nearly constant over time. However, the milk to blood concentration ratios decreased during the same period. Furthermore, the concentration of lead in milk exceeded the plasma concentration by a factor of approximately 25, indicating that there is a physiological process(es) which establishes a large milk to plasma concentration ratio. A similar calcium concentration ratio was also observed. It is concluded that plasma lead concentration is a more accurate index for the estimation of milk lead concentration than is whole blood lead concentration.

Introduction

The transport of lead from lead exposed mothers to suckling offspring has been reported in humans and in experimental animals (Green and Gruener, 1974; Bornschein et al., 1977; Kostial and Momcilovic, 1972). Human studies have generally been limited to the measurement of lead concentration in milk, without measurement of blood lead concentration or estimation of maternal body burden. Human milk from urban mothers (presumably not exposed to lead occupationally) has been found to have a mean lead concentration of 0.026 μg/ml ± 0.011 (SD) (Dillon et al., 1974). Blood lead concentrations of the mothers were not reported. However, this is approximately five to ten fold lower than blood lead concentrations observed in "unexposed" individuals (0.10 to 0.30 μg/ml)(World Health Organization, 1977). Ryu et al. (1978) reported that milk lead concentration was only a small fraction of blood lead concentrations in an individual woman previously employed in a lead industry. Lorenzo et al. (1977) measured milk lead concentrations much higher than blood lead concentrations following administration of an intravenous dose of lead to lactating rabbits. With increasing influx of women into the workplace, and resulting possible occupational exposure of lactating mothers, it is important that the relationship between blood (or plasma) lead concentration and milk lead concentration be quantified. Human milk could be a significant source of neonatal lead exposure in occupationally exposed women, or in women with large lead body burdens if lead is mobilized from bone during lactation.

Kostial and Momcilovic (1974) and Momcilovic (1978) reported that following an intraperitoneal or an intravenous dose of radio-labeled lead, whole body lead retention was reduced in lactating compared to non-lactating female rats. They also found that a large fraction of the maternal dose (10-19%) was transferred via milk to suckling pups during the 2 days immediately following lead administration. We have extended these observations and have studied the elimination of lead from the whole body of lactating and control mice following a single intravenous or oral dose, and have measured the uptake of lead by suckling pups during a 3 week period. In addition we describe the elimination of lead from the blood of lactating and non-lactating mice. The ratios of milk to plasma lead concentrations were found to be relatively constant during the 4 day period following both oral and i.v. lead administration. Furthermore, milk lead concentrations were much higher than plasma lead concentrations (approximately 25-fold) indicating that a mechanism must exist to maintain a large lead concentration difference between plasma and milk. A similar calcium concentration ratio was observed, suggesting that lead and calcium concentration gradients may be the result of a common physiological process.

Materials and Methods

Adult female BALB/c mice (24 weeks of age) produced in our Inbred Mouse Unit were mated to strain 129 males to obtain litters of C129F_1 suckling pups with lactating mothers. Individual lactating

mothers and their litters were housed in stainless steel metabolism cages, and fed a pelleted diet (Agway RMH 2000) and tap water ad libitum. The diet contained 1.1% calcium, 0.8% phosphorous, 9.3% fat, 18% protein, 4% fiber and approximately 1 μg of lead/g. In addition, groups of non-pregnant, non-lactating BALB/c female mice (21 weeks of age) were housed two per cage. Animals were maintained at 22°C with a light period between 5:00 a.m. and 7:00 p.m. (EST). Mice were not fasted prior to lead administration.

Two solutions of lead were obtained by mixing radiolabeled lead (^{203}Pb, as lead acetate, New England Nuclear) with lead acetate (Fisher Scientific Co.) in 150 mM sodium acetate and adjusting the pH to 6.2 with NaOH. A single dose of 0.22 ± 0.01(SD) mg/kg (5.3 mCi/mg Pb) was administered by tail vein injection (5 ml/kg) to 10 lactating mice (mean body weight = 33.9 ± 1.7g, SD) and to 12 non-lactating female mice (27.9 ± 2.7g) on day 0 (between 10 a.m. and 3 p.m.). In addition, a single per os dose of 1.93 ± 0.12 mg/kg of lead (0.41 mCi/mg Pb; 5.0 ml/kg) was administered to a group of 5 lactating female mice (36.9 ± 6.4g). All litters of 5 suckling pups were 6 days old on day 0. Initial lead dose and daily whole body retention of ^{203}Pb were determined in each mouse in the 70 x 130 mm center well of a 135 mm diameter x 160 mm deep thallium activated sodium iodide crystal utilizing the 279 Kev gamma emission of ^{203}Pb. Accumulated feces and urine were collected from the cages on days 3 and 11, and at necropsy. Blood, plasma, and milk samples were collected periodically in heparinized volumetric capillary tubes.

Samples of 0.080 ml blood were drawn no more frequently than once every 48 hours from each mouse by insertion of the capillary tube into the orbital venous plexus. Milk collection was aided by subcutaneous injection of 0.3 units of synthetic oxytocin 15 minutes before hand expression of milk into capillary tubes. Orally dosed lactating mice were sacrificed on day 8. Intravenously dosed mice were sacrificed on days 14, 16 and 21 to allow for collections of larger quantities of blood. Lead retention of the collected fluids was calculated from the decay-corrected specific activity (cpm/ng Pb) of the administered lead and the ^{203}Pb content of the fluids determined with a Beckman 300 gamma spectrometer. ^{203}Pb standards were counted at 6 hour intervals (or more frequently when indicated) to allow corrections to be made for radioactive decay and for changes in instrument efficiency, as well as to standardize radioactivity determination between the two instruments used.

To determine the relationship between plasma and milk calcium concentrations a second experiment was performed. The experimental protocol was identical to that of the lead experiment, with the following modifications. Eight lactating mice were injected i.v. with ^{45}Ca (24 mCi/mg, New England Nuclear) in 155 mM NaCl. Samples of plasma and milk were applied directly to glass fiber filters (Whatman GF/B), the capillary tubes were rinsed twice with distilled water, and the filters were placed at the bottoms of 20 ml glass scintillation vials containing 15 ml of Bray's solution (National Diagnostics, Parsippany, NJ). Samples were counted in a Beckman LS 350 scintillation counter.

Whole body lead retention is expressed as Fraction of Initial Dose (FID) retained during the 21 days following lead administration. Lead content of blood, plasma, and milk is expressed as Multiple of Initial Body Concentration (MIBC).

FID = (ng of Pb, time t) ÷ (ng of Pb in whole body, day 0)

FID_m = (ng of Pb in litter) ÷ (ng of Pb in mother, day 0)

MIBC = (ng of Pb/ml fluid, time t) ÷ (ng of Pb/g whole body, day 0)

MIBC is a data transformation which normalizes fluid lead concentrations for variance in the initial lead dose. FID_m designates the fraction of the initial maternal dose which is transferred in milk to a suckled litter of 5 offspring. Exponential equations describing whole body lead retentions and blood lead concentrations were fitted using a computer program which provides a "best-fit" by minimizing the sum of the squared deviations (Knott, 1979). Least-squares linear regressions were calculated using a computer program which force-fitted the regression line through the origin (Ryan et al., 1976).

Results

Whole body retention (FID) of lead following a single i.v administration to lactating mothers and non-lactating (control) female mice is presented in Figure 18. Figure 18 also shows lead retention in pups suckled by the i.v. injected lactating females. Total recovery of the administered lead (sum of whole body retention, cumulative lead in urine and feces, and lead in suckling pups, if any) was 103.1 ± 5.5% (SD) in cages housing lactating mothers and

101.4 ± 4.0% in cages housing control females (mean recovery at each excretion collection). Whole body retention of lead in lactating and in control mice during the 21 day experimental period was fitted mathematically by three component exponential equations (where t = days):

lactating females: $FID = 0.47\ e^{-0.95t} + 0.22\ e^{-0.27t} + 0.32\ e^{-0.0043t}$

control females: $FID = 0.28\ e^{-0.83t} + 0.25\ e^{-0.25t} + 0.47\ e^{-0.0061t}$

Elimination of lead from non-lactating control mice is similar to that reported following a larger (2.2 mg/kg) i.v. dose (Keller and Doherty, 1980a). A large fraction of the maternal dose is transferred in milk to the suckling pups. Uptake and excretion of lead from litters of suckling pups following an i.v. maternal dose was fitted mathematically by a two-component exponential equation:

$$FID_m = 0.24\ e^{-0.0020t} - 0.24\ e^{-1.21t}$$

The estimated mean half-time of clearance of lead from the suckling pups is 347 days. By day four 0.25 of the i.v. maternal dose (FID_m) had been transferred to the litters of suckling pups (Figure 18). Whole body retention of orally administered lead in lactating mice was variable (range, 0.001 to 0.047 FID retained on day 8). However, constant fractions of the maternally absorbed oral doses were transferred to the suckling pups. By day 8, litters of both i.v. and orally dosed mothers contained nearly as much lead as was retained in the mother. Litters of orally dosed mothers contained 75 ± 11% (SD) and litters of i.v. dosed mothers contained 74 ± 10% of the amount of lead retained in the mothers. These data indicate

that the process(es) of lead transfer to suckling pups is independent of route of maternal lead administration.

The relative concentrations (MIBC) of lead in blood, plasma and milk of female mice following i.v. and p.o. administration of lead are shown in Figure 19. Following lead administration by both routes the initial lead concentrations in milk exceed the lead concentrations in whole blood. A similar pattern of lead elimination from whole blood is evident following p.o. and i.v. lead administration. Values for blood and milk MIBC in orally dosed mice are reduced (by approximately 10-fold) because of limited gastrointestinal absorption of lead. Large standard errors of blood and milk MIBC's of orally dosed mice are due to large variations in gastrointestinal absorption of lead. The elimination rate of lead from the blood of lactating females was increased compared to control females following an i.v. dose. Lead elimination from the blood of i.v. dosed mice can be described by three component exponential equations:

Lactating: $MIBC = 0.72\ e^{-0.94t} + 0.55\ e^{-0.31t} + 0.04\ e^{-0.028t}$

Control: $MIBC = 1.04\ e^{-0.84t} + 0.41\ e^{-0.21t} + 0.16\ e^{-0.070t}$

The increased rate of lead elimination from the blood of lactating mice is due to an additional route of lead elimination (milk). This is consistent with the observed differences in whole body retention (Figure 18).

The lead concentration ratios (milk:plasma; milk:blood) during the experimental period are presented in Table 7. It was not possible to accurately determine plasma lead concentrations more than four

days after lead administration as radioactivity in plasma samples approached background levels. Similarly the low radioactivity and the gradual decrease in the milk supply during weaning prevented determination of milk lead concentrations for longer than 11 days after dosing. During the period 0.06 to 7 days after dosing the milk:blood lead ratios decreased by a factor of approximately 5 for i.v. dosed females. However, between 7 and 11 days the milk:blood lead ratios became relatively stable (0.31 to 0.38). A milk:blood concentration ratio of 0.344 was obtained by least squares regression analysis of milk vs. blood concentration for time points between 7 and 11 days ($r^2 = 0.92$).

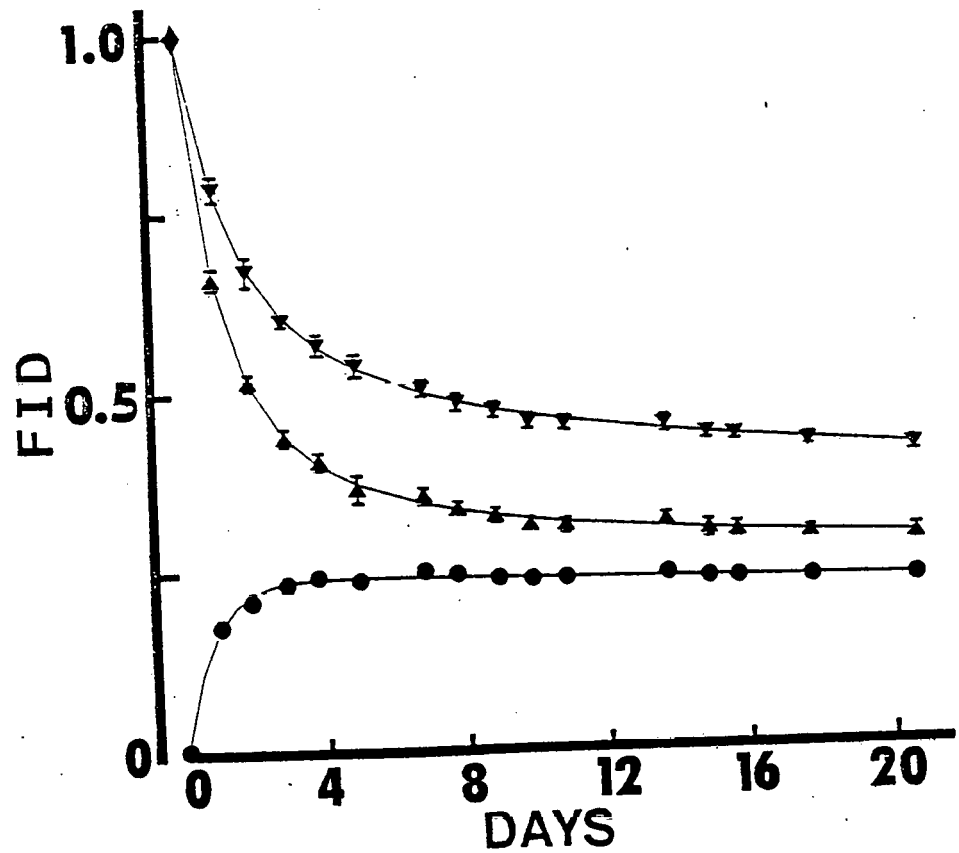
The ratios of milk to plasma lead concentrations (Table 7) were relatively constant throughout the period of measurement (0.06 to 4.2 days). Although the number of available data points was limited, similar patterns of milk:plasma and milk:blood concentration ratios occurred following oral administration of lead to lactating female mice. Data presented in Figure 20 indicate that the milk:plasma lead concentration ratio (between 0.2 and 4 days after lead administration) is independent of lead concentration in plasma. Least-squares linear regression analysis yields a milk:plasma lead concentration ratio of 22.5 ($r^2 = 0.94$).

The milk:plasma ^{45}Ca gradient following i.v. ^{45}Ca administration is presented in Table 7. The gradient rises to a peak value 1 day after ^{45}Ca administration, but stabilizes between 6 and 10 days after administration. The stabilization of the ^{45}Ca gradient is

probably the result of ^{45}Ca equilibrating with the exchangeable calcium pool in the body. It is interpreted that the actual milk:plasma calcium ratio in lactating mice is approximately 30:1.

Plasma:blood lead concentration ratios for lactating and control mice following i.v. and p.o. lead administration are listed in Table 8. The data indicate that plasma:blood ratios decrease with time following i.v. lead administration. The only two time points available for plasma:blood concentration ratios following oral lead administration are consistent with the results observed in the i.v. dosed mice. The decrease in plasma:blood ratios with time is consistent with the constancy of the milk:plasma concentration ratios and the time related decrease in the milk:blood ratios (Table 7). The constant milk:blood ratios observed between 7 and 11 days after i.v. lead administration (Table 7) are also consistent with the milk:plasma ratios observed between 0 and 4 days if it is assumed that the plasma:blood ratio decreases to a stable value of approximately 0.008 to 0.010 during the 7 to 11 day period. While the plasma:blood concentration ratio could not be measured directly, a concentration ratio of 0.008 to 0.010 between 7 and 11 days would be in relatively close agreement with the plasma:blood concentration ratios presented in Table 8. It is expected that a constant milk:blood concentration ratio would occur when plasma and blood lead are in equilibrium.

Figure 18: Whole body retention (Fraction of Initial Dose, FID) of lead in lactating (▲) and non-lactating (▼) female mice following i.v. lead administration, and transfer of maternal lead to suckling pups (●). Vertical bars represent ± 1 SE. Bars not shown when smaller than symbol dimensions.


1.0
0.5
0
FID
0
4
8
12
16
20
DAYS

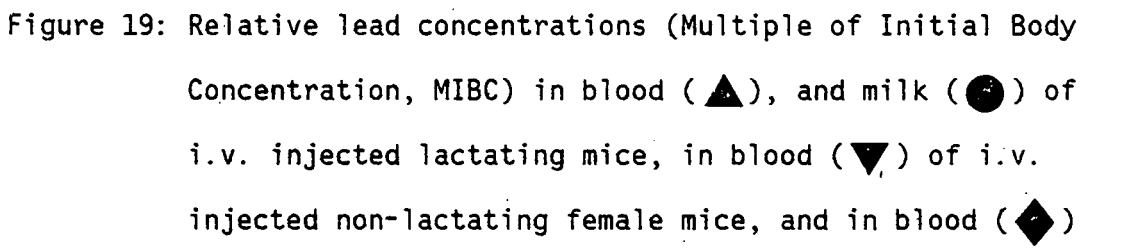


Figure 19: Relative lead concentrations (Multiple of Initial Body Concentration, MIBC) in blood (▲), and milk (●) of i.v. injected lactating mice, in blood (▼) of i.v. injected non-lactating female mice, and in blood (◆) and milk (■) of lactating mice administered lead orally. Vertical bars represent ± 1 SE.

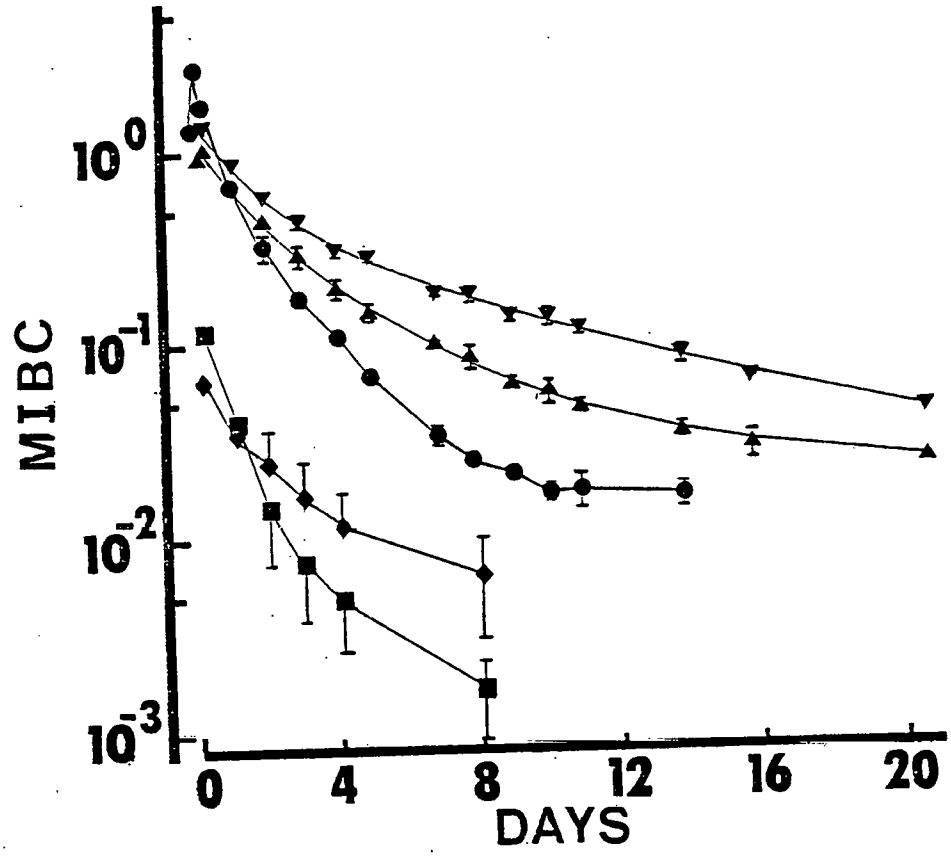

MIBC
10^{0}
10^{-1}
10^{-2}
10^{-3}
0
4
8
12
16
20
DAYS

Figure 20: Relationship between lead concentrations in milk and plasma following an oral (●) or i.v. (▲) lead dose. Least squares linear regression, $y = 22.5x$, ($r^2 = 0.94$).

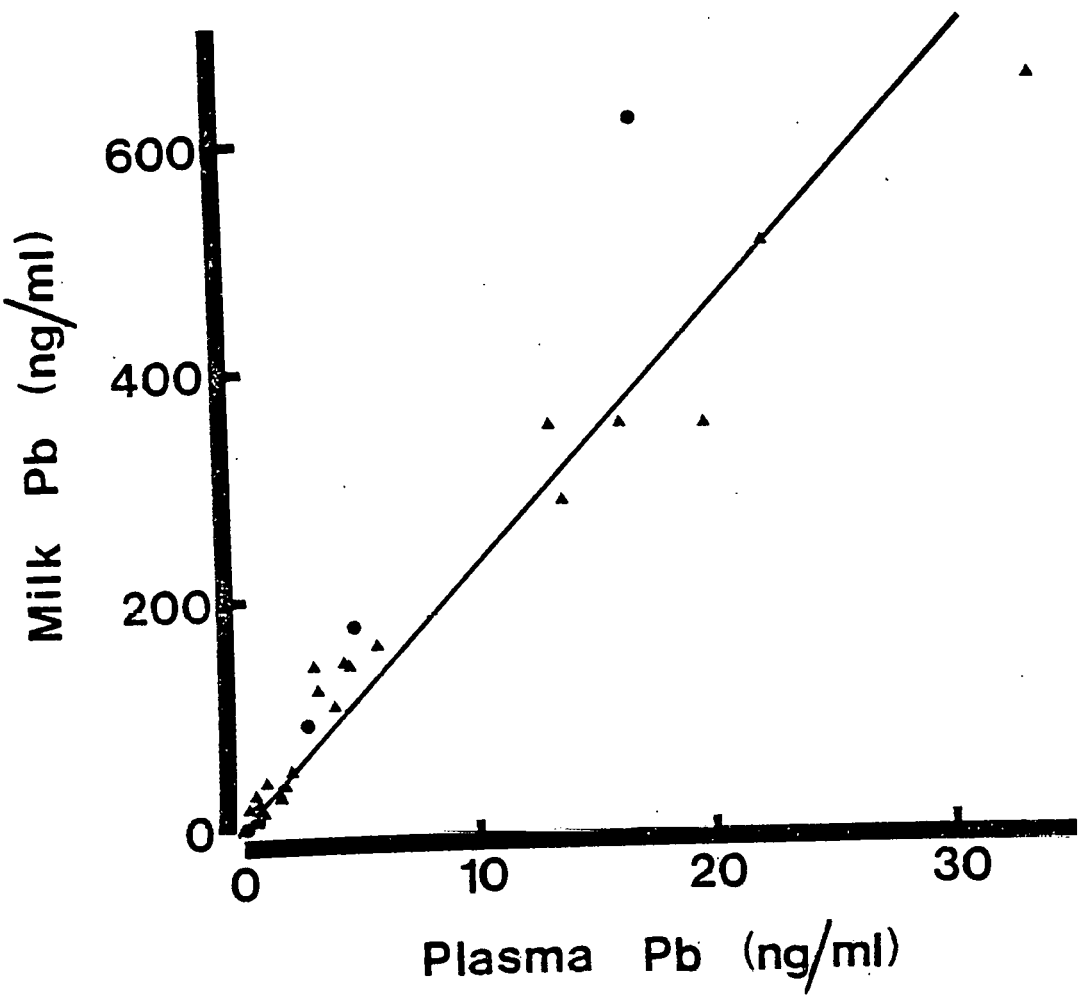

600
400
200
0
Milk Pb (ng/ml)
0
10
20
30
Plasma Pb (ng/ml)

Table 7: Lead or calcium concentrations in milk relative to plasma or whole blood at various times after a single radiolabeled lead dose of 0.2 (i.v.) or 2.0 (p.o.) mg/kg.

	Lead $\frac{\text{ng/ml milk}}{\text{ng/ml plasma (or blood)}}$ (±SE)				Calcium $\frac{\text{cpm/ml milk}}{\text{cpm/ml plasma}}$ (±SE)
	milk:blood		milk:plasma		
days	i.v.	p.o.	i.v.	p.o.	i.v.
0.06	1.1 ± 0.4		18 ± 9		12 ± 5
0.20	2.1 ± 0.3		21 ± 2		
0.3	1.9 ± 0.3	1.2 ± 0.5	26 ± 11	25 ± 6	46 ± 3
0.4	1.6 ± 0.1		22 ± 3		
1	0.93 ± 0.05	0.89 ± 0.13	33 ± 3	27 ± 4	75 ± 8
2	0.73 ± 0.14	0.55 ± 0.07			68 ± 10
3	0.61 ± 0.03	0.49 ± 0.08	27 ± 5		
4	0.60 ± 0.07	0.47 ± 0.08	34 ± 3		44 ± 8
5	0.48 ± 0.04				
6					37 ± 6
7	0.35 ± 0.05				31 ± 5
8	0.31 ± 0.03	0.44 ± 0.13			
9	0.36 ± 0.06				32 ± 4
10	0.31 ± 0.02				30 ± 1
11	0.39 ± 0.08				

Table 8: Lead concentrations in plasma relative to whole blood at various times after a single i.v. or p.o. lead dose.

	$\frac{\text{ng/ml plasma}}{\text{ng/ml whole blood}}$ (±SE)		
Days	i.v. lactating	i.v. control	p.o. lactating
0.06	0.075 (± 0.018)		
0.2	0.093 (± 0.018)	0.093 (± 0.008)	
0.4	0.072 (± 0.009)	0.065 (± 0.003)	0.049 (± 0.006)
1	0.029 (± 0.002)	0.039 (± 0.003)	0.025 (± 0.004)
2		0.026 (± 0.002)	
3	0.026 (± 0.004)	0.030 (± 0.001)	
4	0.019 (± 0.004)	0.017 (± 0.004)	
5	0.012 (± 0.002)	0.011 (± 0.002)	

Discussion

Our results indicate that there is a constant relationship between lead concentrations in milk and plasma following a single lead dose. However, the relationship between milk and whole blood lead concentrations was found to change with time after lead administration. Between 0 and 7 days the milk:blood concentration ratio decreased by 5-fold, but then stabilized between 7 and 11 days. We conclude that plasma lead concentration is an accurate indicator of milk lead concentration, and therefore, is a reliable index of potential lead transfer from exposed mother to suckling offspring. It appears that blood lead concentration would be a reliable indicator of milk lead concentration when plasma and erythrocyte lead distributions are in equilibrium.

Our data indicate that lead concentration ratios (milk:blood; milk:plasma), and fractional transfer of maternally absorbed lead to suckling pups are independent of route of lead administration. Furthermore, following the administration of a much larger i.v. lead dose (2.6 mg/kg) a similar pattern of whole body lead elimination and milk:plasma lead concentration has been observed in lactating mice (unpublished observations).

The rate of lead elimination from the whole body and whole blood of lactating mice was markedly increased compared to non-lactating mice. This difference has been shown to be due to secretion of significant amounts of lead in milk. An increased rate of methylmercury elimination following methylmercury exposure has also been

observed in lactating women and mice compared to nonlactating individuals (Greenwood et al., 1978). However, in contrast to the observations we report for lead, secretion of methylmercury in milk accounts for only a small fraction of the difference in whole body elimination rate. Lead transmission by suckling has toxicological significance since lead encephalopathy has been observed in suckling pups exposed to lead in milk from mothers not adversely affected (Pentschew and Garro, 1966).

Blood lead is tightly bound to erythrocytes, and only a small fraction of total blood lead is in plasma (Barltrop and Smith, 1971; 1972). Furthermore, Vander et al. (1977) has report that only 11% of plasma lead is ultrafilterable. Data presented in Table 7 indicate that there is a large lead concentration difference between milk and plasma. However, this observed difference is based upon measurement of total lead concentrations in the fluids. Chemical species of lead in milk and plasma were not determined. We observed a milk:plasma calcium ratio which was similar but larger than the lead concentration ratio. A stable milk:plasma lead concentration ratio of approximately 25:1 (days 1-4) was observed while the stable calcium ratio (days 6-10) was approximately 30:1. This could indicate that a common mechanism or pathway is involved in the accumulation of lead and calcium in milk. Among different species large differences have been found in milk calcium concentrations (O'Connor and Fox, 1977), while only small interspecies differences have been observed in plasma calcium concentrations. Calcium phosphate complexes are

known to occur in cow's milk (Boulet et al, 1970), and lead phosphate complexes have been demonstrated in rat blood (Clarkson and Kench, 1958). It is possible that the observed milk:plasma lead concentration ratio was due to the formation of highly insoluble lead phosphate complexes. It has not been established whether a similar situation occurs in humans. However, human milk:serum calcium concentration ratios are only about 3:1 (Barltrop and Hillier, 1974; O'Kell and Elliott, 1970).

Twardock and Comar (1961) reported that the concentrations of radiostrontium in goat's milk exceeded blood concentrations by approximately 7-fold. However, a large milk:plasma concentration gradient is not found for all metals. In women exposed to methylmercury, milk methylmercury concentrations were determined to be only one-thirtieth blood methylmercury concentrations (Bakir et al., 1973).

In women with large body burdens of lead (sequestered in bone), blood and plasma lead concentrations could increase during lactation concurrent with bone calcium mobilization. This possibility is compatible with data presented by Ryu et al (1978). In rats which chronically ingested lead throughout gestation and lactation blood lead concentrations at the end of the period of lactation were observed to be higher than concentrations prior to mating (Buchet et al., 1977). Since blood lead concentration would be expected to decrease following termination of exposure, (Rabinowitz) the reported results could have been due to mobilization of lead from bone during lactation.

Calcium and lead have been shown to interact in a variety of metabolic processes (Goyer, 1973). Lactation results in a large calcium efflux from the mother, and mobilization of calcium from bone is the principle homeostatic mechanism (Rasmussen, 1977; Lamke et al., 1977; Goldsmith and Johnston, 1975). In bone organ cultures low concentrations of calcium in the medium have been shown to increase lead mobilization from bone (Rosen and Wexler, 1977). A low dietary calcium intake in lactating mothers could increase lead mobilization from bone, and could produce higher lead concentrations in milk. This possiblity warrants further investigation relevant to possible detrimental effects due to occupational exposures of women of child-bearing age.

II-5: Bone Lead Mobilization in Lactating Mice and Lead Transfer to Suckling Offspring

Abstract

Drinking water containing 200 µg/ml of lead (labeled with ^{210}Pb) was provided to adult female mice for 100 days prior to the date of mating or for 100 days prior to mating and during the periods of gestation and lactation (160 days total exposure). During lactation, but not gestation, there was an increased rate of elimination of lead from adult mice exposed prior to mating compared to nonpregnant female mice. The magnitude of the additional lead elimination during lactation was similar to the magnitude of the decrease in femur ash weights during lactation. These data support the hypothesis that lead mobilization during lactation is the result of bone mineral mobilization. Approximately 3% of the lead body burden of mothers exposed to lead prior to mating was retained in litters of suckling pups as a result of lead transferred in milk. The magnitude of lead mobilized from maternal bone during lactation was not increased by a diet which was moderately calcium deficient. Lactation also increased the extent of lead retention in adult female mice exposed to lead throughout lactation. Continuous exposure of the lactating mother to lead during lactation significantly increased the net transfer of lead to suckling pups. Lead transferred from mother to offspring during lactation greatly exceeded transfer during gestation for all treatment groups. These data indicate that both prior and current maternal lead exposure should be considered in assessing potential lead exposure of suckling infants.

INTRODUCTION

Occupational exposure of women of child-bearing age to lead could represent a toxicological hazard to their suckling infants. Occupational exposure can result in a considerable increase in the body burden of lead, which is almost completely sequestered in bone (Barry and Mossman, 1970). The transfer of chemicals to suckling offspring to which the mother has been previously exposed is of toxicologic concern. The relationships between prior maternal exposure and subsequent toxicological manifestations in offpsring may be difficult to demonstrate in human populations, thus development and utilization of animal model systems are critically important to the identification and definition of potentially hazardous situations. This paper presents data demonstrating that lead is mobilized from maternal bone during lactation and transferred in milk to suckling pups.

Lactation represents a calcium demand on the mother, so that measurable bone calcium mobilization occurs. Furthermore, lead and calcium are known to interact in many metabolic processes. In bone organ cultures low concentrations of calcium in the medium have been shown to increase lead mobilization from bone (Rosen and Wexler, 1977). Data presented by Ryu et al (1978) demonstrated that maternal blood lead concentrations increased during lactation, indicating probable bone lead mobilization. In rats, blood lead concentrations at the end of the period of lactation were observed to be higher than concentrations prior to mating (Buchet et al, 1977). Very

little information is available concerning the physiologic mechanism of lead transfer from lactating mothers to suckling pups. Keller and Doherty (1980c; Section II-4) have determined that the milk:plasma lead concentration ratio in lactating mice is approximately 23:1. This was similar to the observed milk:plasma calcium concentration ratio. In this report lactating female mice and their litters were used to assess the extent of bone lead mobilization and net lead transfer to suckling pups, the effect of reduced calcium intake on lead mobilization and transfer, and the extent of lead transfer from mothers exposed to lead during lactation.

METHODS

Adult (16 weeks of age) virgin BALB/c female mice produced in our inbred mouse unit were housed 6 mice per cage in plastic cages with stainless steel floors. Mice were fed a pelleted diet (Agway RMH 3000) which contains approximately 1 µg of lead/g, 22% protein, 1.0% calcium, 0.9% phosphorous and 5% fat. Lead contents of the diets and tap water were determined by flameless atomic absorption spectroscopy. Drinking water was supplied as either tap water (n=18 mice) containing less than 5 ng/ml of lead, or as a 200 µg/ml solution of lead (lead acetate; Fisher ACS) in distilled H_2O (n=54). The lead solution also contained 0.1 µCi of ^{210}Pb/ml (Amersham). Mean initial weight of control mice was 26.8 (± 0.08,SD) and of lead fed mice, 27.0 (± 1.0). Water consumption during the first six weeks of lead feeding was determined by weighing of bottles at weekly intervals.

Lead retentions in the mice were determined periodically by measuring the ^{210}Pb content of each mouse with a sodium-iodide well crystal (Bicron Corp.) and Canberra Series 30 multichannel analyzer. ^{210}Pb content of sealed standards were determined in each counting session to correct for radioactive decay and changes in instrument efficiency. ^{210}Pb feeding solutions were removed from the cages and were replaced with tap water 24 hours prior to whole body counting to ensure that no unabsorbed ^{210}Pb was present in the gastrointestinal tract.

After 105 days of 200 µg/ml lead (^{210}Pb labeled) ingestion, 10 mice were necropsied (Group I). The ^{210}Pb contents of the femurs (both sides), skull section (containing parts of parietal, interparietal

and frontal bones), kidneys, liver, and brain from each mouse were determined using a Beckman 300 gamma spectrometer. Organs were weighed immediately following dissection. At this point nine of the mice were continued on the 200 µg/ml lead solution but the remainder were provided lead-free tap water. All mice were mated to strain 129 males. Copulation was confirmed by the presence of a vaginal plug the morning after mating. Stainless steel floors were removed at the time of mating to minimize stress during pregnancy. After mating, females without plugs (nonpregnant) were housed separately. At this time the pelleted diet was changed to a diet (NIH-103; Zeigler Bros. Inc.) containing 18% protein, 9.0% fat, and 1.1% calcium (normal calcium diet) or to a modification of this diet which contained only 0.3% calcium (low calcium diet). All diets were determined to contain less than 1 µg/g of lead. Some adult female mice were sacrificed because of obstetrical problems experienced at the time of birth. These problems were unrelated to treatment.

Following parturition litters were culled to 6 pups each, and the ^{210}Pb contents of the litters were measured periodically during a 24 day period of suckling. Lead transfer (in milk) from lactating mother to suckling pups was determined in three groups: 1) normal calcium diet, continued maternal ingestion of ^{210}Pb-labeled lead in drinking water during gestation and lactation (Group IIa, n = 4), 2) normal calcium diet, no maternal lead ingestion after conception (Group IIIa, n = 6 litters) and, 3) reduced calcium diet, no additional maternal lead ingestion after conception (Group IVa, n = 6). ^{210}Pb retentions in the mothers were also measured during lactation. In

addition, ^{210}Pb retentions were measured in three groups of nonpregnant, nonlactating female mice which received the same dietary regimens as the lactating mothers (Groups IIb, IIIb and IVb). Mothers, suckling pups, and the nonpregnant, nonlactating female mice which had ingested lead in the drinking water were all necropsied when the pups reached 24 days of age. Skin and hair were removed from the carcasses, and the ^{210}Pb content of the skin and hair was measured. Organs were collected as described before. Femur and skull sections were dried overnight at 110°C, weighed, then dry ashed overnight at 600°C in preweighed containers, and weighed again. The grouping of adult mice and their treatments are summarized in Table 9.

Lead retention in lactating adults following termination of ^{210}Pb ingestion is expressed as percentage of initial body burden retained. The initial ^{210}Pb body burden was determined prior to mating of the female mice (at 114 days following initiation of ^{210}Pb intake). Lead transfer to suckling offspring is expressed as percentage of the mother's initial body burden (at time of mating) transferred to the pups. These measurements were used to normalize the large variations in adult lead uptake during the period of ^{210}Pb feeding. Differences between the means of data presented in Tables 10, 11 and 13 were tested for statistical significance with Student's t-test. Data in Table 12 was tested for statistical significance using an unbalanced two-way analysis of variance.

RESULTS

Retention of ^{210}Pb in adult female mice fed a 200 µg/ml lead in water is shown in Figure 21. Ingestion of this concentration of lead in drinking water did not produce observable signs of toxicity. There were no differences in weight gains between lead fed and control mice (107 and 102% of initial weights respectively). No deaths occurred in either lead-fed or control groups. Mean drinking water consumption was 3.70 ml/day/mouse for lead fed mice and 3.85 ml/day/mouse mice ingesting tap water. The rate of increase in lead retention (Figure 21) was much greater in lactating females (Group IIa) than in non-lactating mice (Group IIb). During the period of gestation and lactation (46 days) female mice retained as much additional lead as had been retained during the previous 100 day period. Soft-tissue lead concentrations (Table 10) were greatly increased in the lactating adult females, reflecting the increased body burden of lead. In these lactating mice 8% of the final lead body burden was contained in the skin and hair. In the nonlactating controls 13% of the final lead body burden was found in skin and hair. Much of this difference probably represents contamination from the fecal and urinary excretion of lead which could have occurred after removal of the steel cage floors, although some may be the result of incorporation of lead into growing hair.

Following cessation of lead exposure, the rate of whole body lead elimination was observed to increase during lactation (Figure

22). In contrast, gestation did not produce an increased rate of lead elimination. By the end of the lactation period, lactating female mice (Group IIIa) exhibited 21% lower (15% of Initial Body Burden) whole body lead retention than nonlactating female adult mice (Group IIIb). Identical results were obtained when female mice were fed a 0.3% calcium diet during gestation and lactation. Only a small fraction (less than 2%) of the lead body burden at the time of necropsy was determined to be in the skin and hair of adult mice when lead exposure was terminated 50 days before necropsy.

Lead was transferred in maternal milk to suckling offspring subsequent to maternal exposure. In lactating mice (Groups IIIa and IVa) which had been exposed to lead until conception, but not exposed during gestation and lactation, approximately 3% of the initial maternal lead body burden was retained in litters as a result of lead transfer in milk (Figure 23). Less than 5% of the lead body burden of these pups was contained in skin and hair, indicating that contamination from maternal urinary and fecal excretion was minimal. There were no significant differences in the extent of litter lead retention between litters suckled by mothers fed 1.1% compared to those fed 0.3% calcium diets. Cumulative lead retention in litters was greatest at 17 days of age and declined thereafter. This was probably the result of weaning (decrease in milk transfer) and elimination of lead from the pups.

When lactating mothers (Group IIa) continuously ingested water containing 200 µg/ml lead during lactation the amount of lead

transferred to suckling litters of pups was greatly increased (Figure 24). Less than 5 μg of lead had been retained in each litter during gestation, but total retention in suckled pups during lactation exceeded 100 μg. Less than 1% of the final lead body burden of these pups was contained in the skin and hair. Thus within a 17 day period the litter of pups received (via maternal milk) an amount of lead equal to the amount adult female mice retained over a 100 day period. The transfer of lead in milk to the litters (100 μg) was greater than the additional lead (70 μg) retained by lactating mothers during lactation (Figure 21).

Lead concentrations in the femurs and skull sections of continuously exposed lactating mice were much greater than in non-lactating mice exposed over the same 160 day period (Table 11). The relationship between the lead concentrations in bone of the two groups is similar to the relationship between their lead body burdens (Figure 21). This is consistent with the sequestration of nearly the entire body burden of lead in bone, and with the increased extent of whole body lead retention observed in lactating mice.

Relative lead retention in the femurs of lactating females exposed to lead prior to mating were reduced as compared to non-lactating controls (Table 12). Statistical analysis indicates that lactation results in a large decrease in relative lead content of the femur. Smaller effects can be attributed to the reduced calcium diet and to an interaction of diet and lactation. The differences

are consistent with the observed increase in whole body lead elimination rate in lactating as compared to nonlactating mice.

Lactation results in a relatively large calcium demand on the lactating mother. This is indicated by the decreases in dry weights and ash weights of femurs from mice after lactation (Table 13). Dry weights were found to decrease by 11% as a result of lactation. There was a parallel 16% decrease in the mean ash weights of the femurs. The decreases in dry and ash weights of the femurs were similar to the observed difference (21%) between lead retention in lactating compared to non-lactating mice. Since nearly the entire body burden of lead is known to be sequestered in bone, it is possible that bone mineral mobilization during lactation could account for increased mobilization of lead during lactation. However, while an additional 15% of the initial maternal body burden was eliminated during the period of lactation, only 3% of the initial maternal body burden was transferred via milk to suckling litters of pups. The remainder of the lead (12%) excreted by lactating mice could have occurred via fecal and urinary excretion, though these were not measured. While the results indicate that lead may be mobilized to the same extent as bone minerals during lactation, physiological discrimination occurs reducing the subsequent transfer of the entire amount of mobilized lead into the milk.

Figures 21: Lead retention (μg ± SD) in female mice ingesting 200 μg/ml (in drinking water) of lead. (Non-lactating mice days 0-105 n=54, days 112-160 n=5; lactating mice days 112-160 n=4).

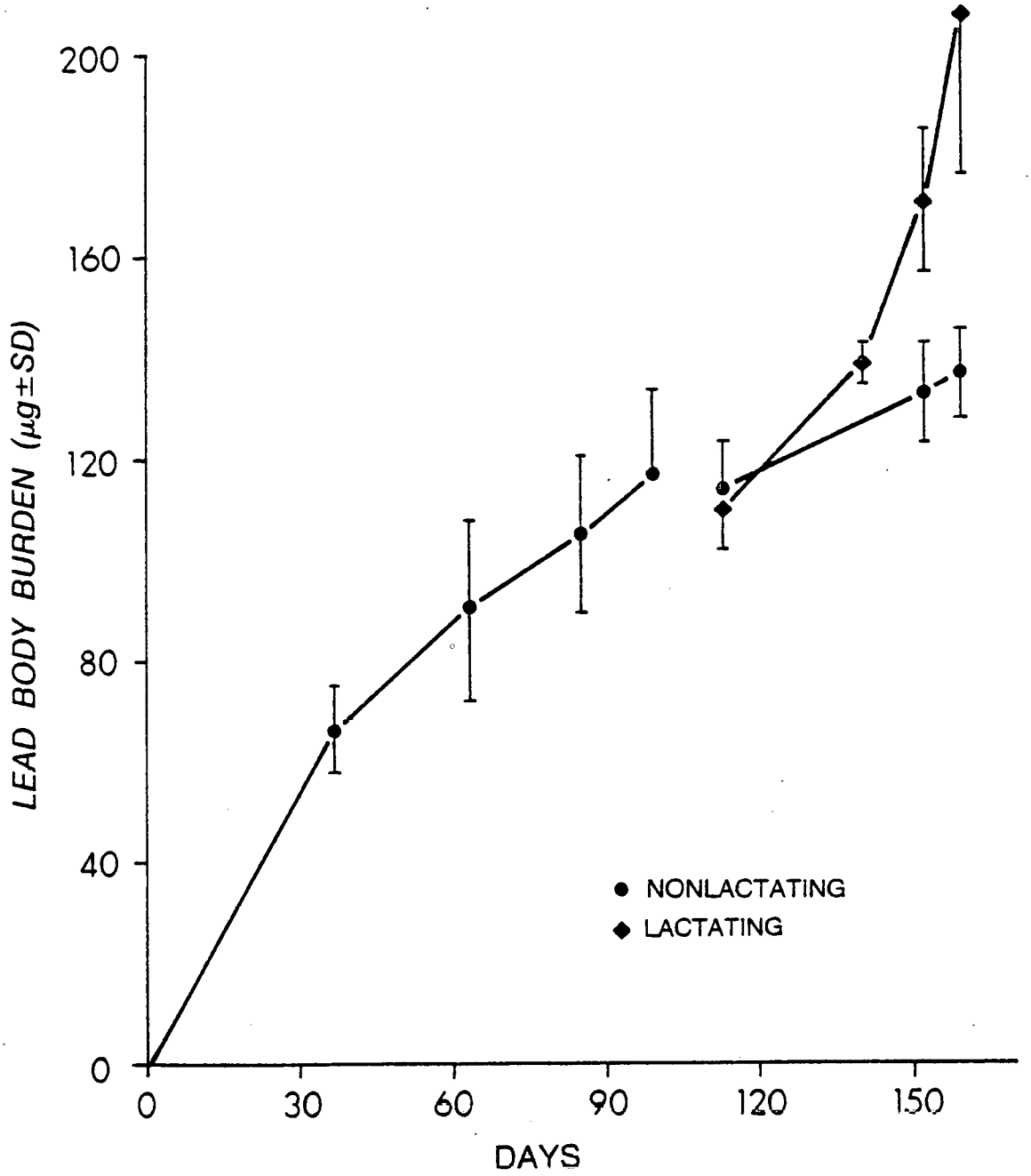

200
160
120
80
40
0
LEAD BODY BURDEN (μg±SD)
0
30
60
90
120
150
DAYS
NONLACTATING
LACTATING

Figure 22: Lead retention (Percentage of Initial Body Burden ± SE) in lactating (Group IIIa, n=6) and non-lactating (Group IIIb, n=8) mice following cessation of lead ingestion (200 µg/ml in drinking water) on day 0. Error bars not shown are smaller than symbol dimensions.

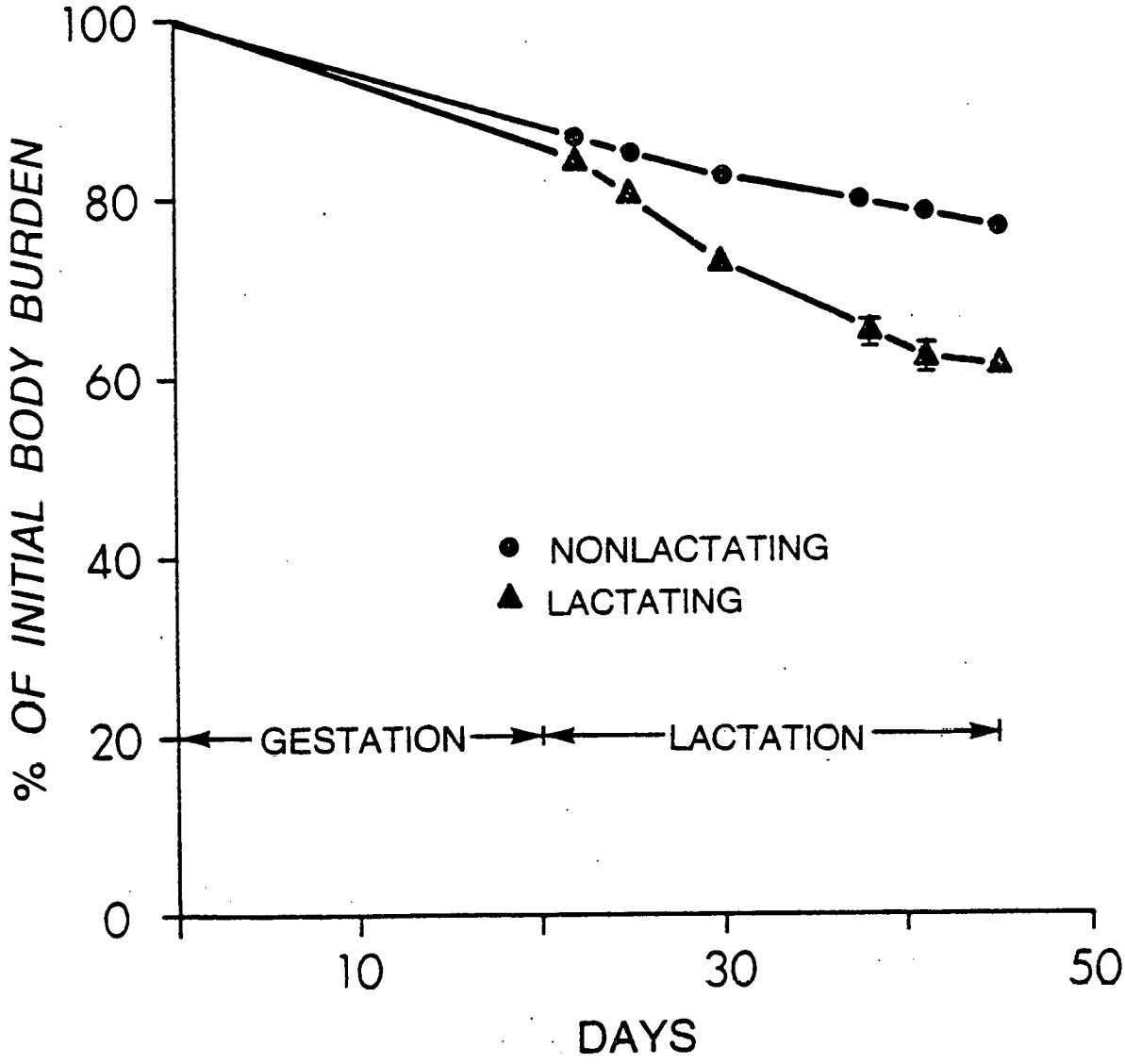

100
80
60
40
20
0
% OF INITIAL BODY BURDEN
NONLACTATING
LACTATING
GESTATION
LACTATION
10
30
50
DAYS

Figure 23: Lead retention (Percentage of Initial Maternal Lead Body Burden ± SE) in litters (6 pups/litter) of mice suckled by mothers exposed for 105 days prior to conception. Mothers received diets containing either 1.1% (Group IIIa; ▲) or 0.3% (Group IVa; △) calcium.

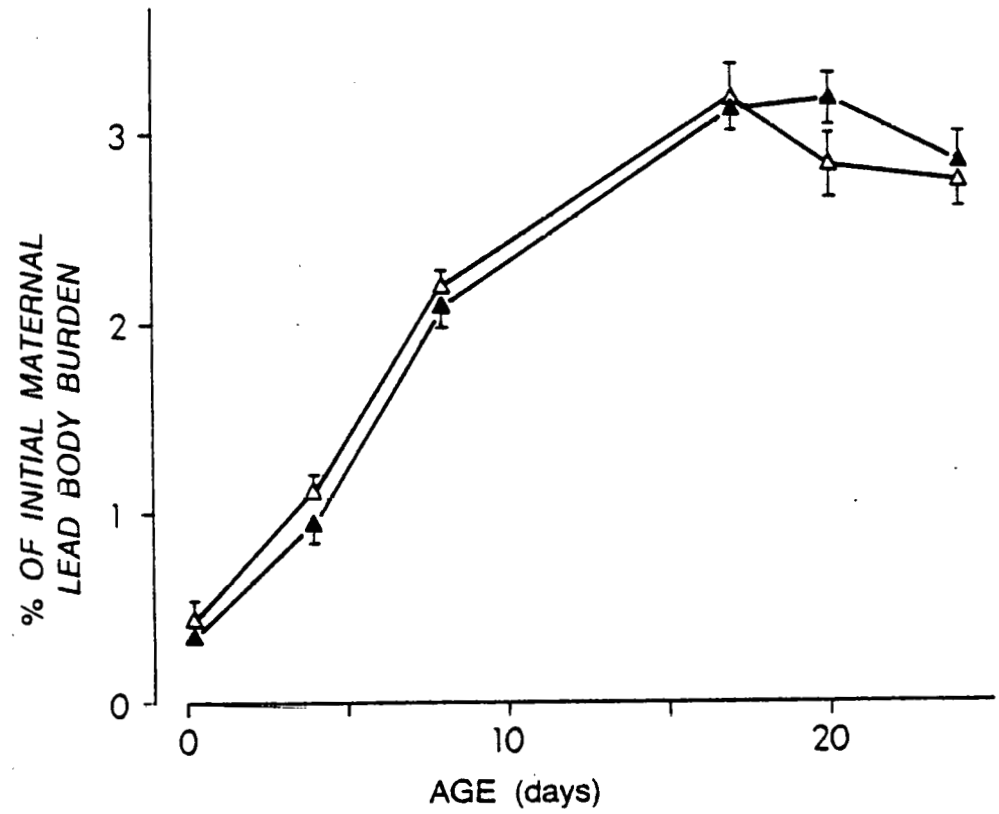

% OF INITIAL MATERNAL
LEAD BODY BURDEN
0
1
2
3
0
10
20
AGE (days)

Figure 24: Lead retention (µg ± SD) in litters suckled by lactating females (Group IIa) continuously ingesting 200 µg/ml lead for 105 days prior to conception, and during pregnancy and lactation (n=4 except for age 16 days when n=2).

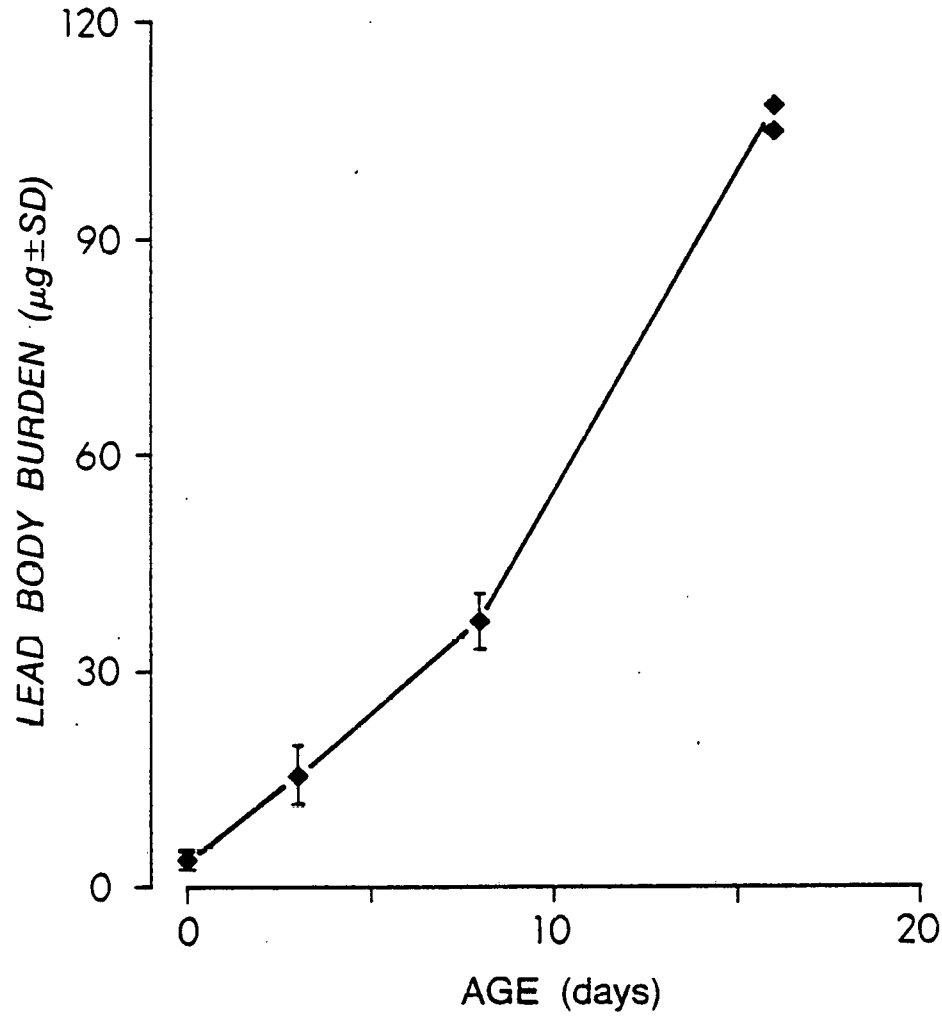

120
90
60
30
0
LEAD BODY BURDEN (μg±SD)
0
10
20
AGE (days)

TABLE 9: Outline of Animal Groupings

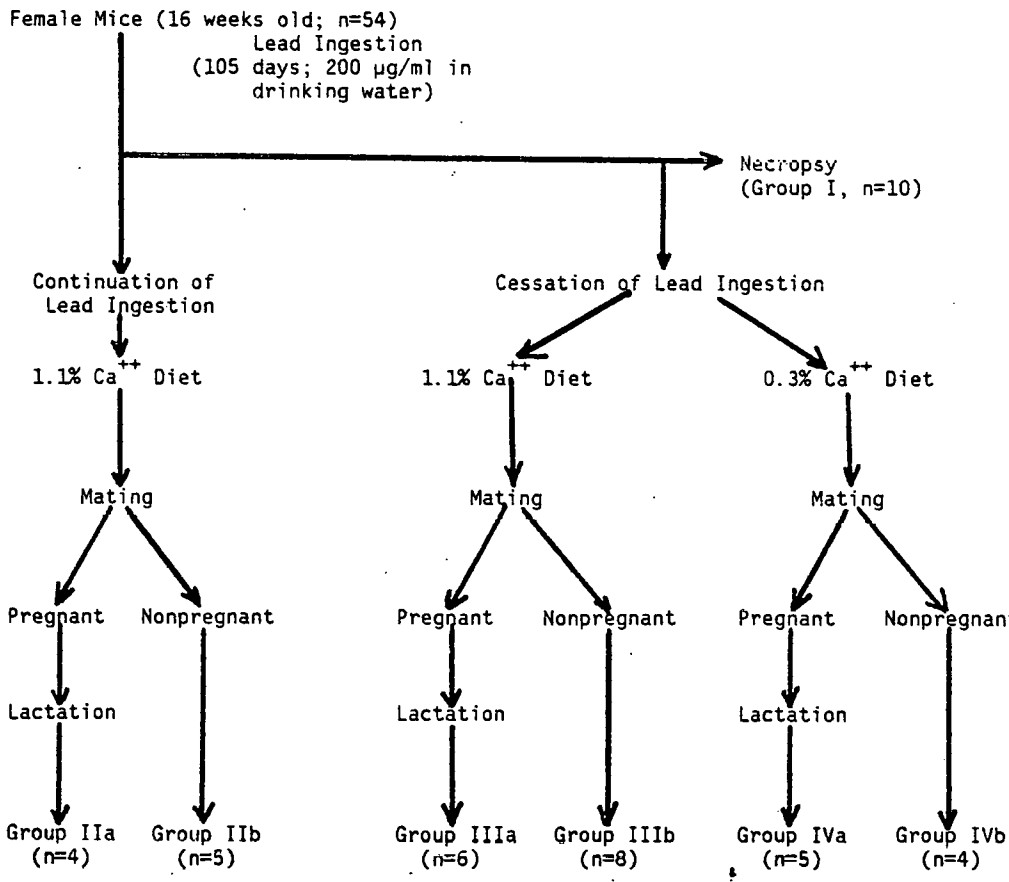


TABLE 10: Lead Concentration in Organs of Lactating (n=4) and Nonlactating (n=5) Female Mice Following 160 Days Intake of Lead (200 µg/ml) in Drinking Water.

µg Pb/g (±SE)

ORGAN	LACTATING (Group IIA)	NONLACTATING (Group IIb)
Brain	0.40 (±.03)[b]	0.25 (±.02)
Liver	0.92 (±.14)[a]	0.48 (±.04)
Kidney	2.75 (±.12)[c]	1.77 (±.09)

Difference between organ concentrations of lactating vs non-lactating mice:

a: $p<0.02$; b: $p<0.01$; c: $p<0.001$.

TABLE 11: Lead Concentration in Bones of Adult Female Mice[a]

GROUP	µg of Pb/g dry weight (±SE) FEMUR	SKULL
Controls (Group I, n=10), Sacrificed Prior to Mating (105 days Pb exposure)	63.1 (±2.8)	69.1 (±3.4)
Lactating (Group IIa, n=4) (160 days Pb exposure)	96.4[b] (±1.9)	126.6[b] (±2.1)
Nonlactating (Group IIb, n=5) (160 days Pb exposure)	67.6 (±3.9)	81.4 (±4.9)

[a] Group I sacrificed after 105 days of 200 µg/ml lead ingestion; Groups IIa and IIb sacrificed after 160 days of lead feeding.

[b] Different from non-lactating mice and controls, $p<0.001$.

TABLE 12: Relative Lead Concentrations in Femurs (Both Sides) of Lactating and Non-Lactating Mice.[a]

Percentage of Initial Lead Body Burden (±SE)

GROUP	DIET: 1.1% Ca^{++}	DIET: 0.3% Ca^{++}
Lactating[b]	3.9 (±0.1) Group IIIa, n=6	3.9 (±0.1) Group IVa, n=5
Non-Lactating	5.1 (±0.1) Group IIIb, n=8	4.6 (±0.1) Group IVb, n=4

[a] Ingestion of lead (200 µg/ml) was terminated on Day 105 when mice were mated. Mice were necropsied on Day 146.

b Results of Two-Way Analysis of Variance:
predicted value = 4.38 + [Ca^{++} effect] - [lactation effect] - [Interactions of effects]
predicted values for Group: IIIa = 3.90, IIIb = 5.14, IVa = 3.88, IVb = 4.60

TABLE 13: Femur (both sides) Weights Following Lactation Compared to Nonlactating Female Mice. (g ± SE)

	DRY WEIGHT	ASH WEIGHT
Nonlactating Mice (Groups I, IIb, IIIb and IVb, n=27)	0.109 (±.001)	0.0679 (±.0007)
Following Lactation (Groups IIa, IIIa and IVa, n=16)	0.097* (±.001)	0.0571* (±.0006)

* Different from control weights, $p<0.001$.

DISCUSSION

The results (Figure 23) provide evidence that lead which is sequestered in maternal bone is mobilized during lactation and is subsequently transferred in milk to suckling pups. The amount of lead transferred via milk to suckling pups was not increased by a moderate deficency of dietary calcium intake. However, continuous maternal ingestion of lead during lactation (Figure 24) greatly increased the amount of lead transferred from mothers to suckling pups. These data also indicate that very small amounts of lead were transferred from mother to fetus during gestation. It is clear the developing mouse is at much greater risk in terms of maternally mediated lead exposure during suckling than during prenatal development.

Kostial and Momcilovic (1974) quantitated maternal to offspring lead transfer during gestation and lactation following a single intravenous carrier-free ^{203}Pb dose. This was confirmed in a later report (Momcilovic, 1978) in which a lead carrier was added to the injection solution. They found that lead transfer during lactation was greater than lead transfer during gestation. Furthermore, they observed a parallel increase in lead and calcium transfer to suckled offspring when comparing transfer during early and late lactation. Keller and Doherty (1980) determined that milk lead concentrations exceeded plasma lead concentrations by approximately 25-fold. This was similar to the observed milk:plasma calcium concentration ratio of 30:1. Bornschein et al (1977) determined the rate of lead transfer

to suckling rats when the dam was exposed to lead in the drinking water. Buchet et al (1977) suggested that lead was mobilized from bone during pregnancy (and/or lactation, which they failed to note), but inconsistent observations among various treatment groups seriously compromised their interpretation.

Results presented in Figure 23 indicate that the magnitude of lead transfer from mothers continuously ingesting lead (in drinking water) greatly exceeded lead transfer when exposure was terminated prior to mating. Furthermore, the amount of lead transferred in milk to suckling pups (100 µg during days 0-17 of lactation) was greater than the net retention of lead in the lactating mother during this same period (70 µg). The lactating mouse is therefore a very efficent means of transferring maternal lead exposure to the suckling offspring. Lead retention in continuously exposed lactating mothers exceeded that of non-lactating controls even though a large fraction of the maternal dose absorbed during lactation was transferred to pups in milk. The increased retention of lead by lactating mothers could be due to increased consumption of the 200 µg/ml lead solution and to the increased rate of lead absorption noted in lactating animals (Kostial and Momcilovic, 1972).

Occupational lead exposure can result in large body burdens of lead, most of which is sequestered in the skeleton. Schroeder and Tipton (1968) estimated from autopsy samples a mean skeletal lead burden of 110 mg in the general population. Barry and Mossman (1970) reported a similar value for non-occupationally exposed adult females,

but mean bone lead burden in occupationally exposed males was 475 mg. Sorenson and Cameron (1967) determined that a 6% loss of cortical bone occurred during the ninth month of gestation and the first five months of lactation in a young woman. Goldsmith and Johnston (1975) found that approximately 3% of the distal radius mineralization was lost in a large number of previously lactating women. Atkinson and West (1970) calculated a rate of bone mineral loss from femur of 2.2% per 100 days of lactation. Thus there are large differences between man and mouse in the extent of mineral mobilization during lactation. The high relative output of mouse milk is probably responsible for the relatively large degree of mineral loss from bone. If a bone lead burden (in occupationally exposed women) of 500 mg is assumed, and there is 5% bone mineral mobilization then a maximum of 25 mg would be transferred through maternal milk to the offspring. Results with lactating mice suggest that only 20% of the mobilized bone lead is actually transferred to (or retained in) in the suckling pups. If this were also the case in humans then transfer of lead in maternal milk could be as much as 5 mg during the period of lactation. If lactation occurs over a 5 month period and 1 liter of milk is produced per day then the milk lead concentration would be 33 µg/l. This value approximates the milk lead concentration found in "non-exposed" mothers (Dillon et al, 1974; Lamm and Rosen, 1974) and in an occupationally exposed mother (Ryu et al, 1978). Lead mobilized from bone would be expected to add to current maternal lead exposure in contributing to a final lead concentration in milk.

In view of the fact that this additional lead intake could result in lead exposure exceeding the tolerable level of lead ingestion (Mahaffey, 1977) it is apparent that some caution should be exercised until lead transfer from lead exposed women to infants can be quantitatively assessed.

In the lactating mouse model continuous maternal lead ingestion during lactation results in large quantities of lead passing through the mother to suckled offspring. If we ignore the apparent species difference in adult gastrointestinal lead absorption and consider only the blood or plasma lead concentration then an extrapolation to humans may be possible. Previous results (Section II-4) indicate that plasma lead concentration (and blood lead concentration when in a steady-state with plasma lead) is an appropriate indicator of milk lead concentration in the mouse. The determination of plasma (or blood) lead concentrations in exposed lactating females could provide a measure of potential lead transfer to suckling infants. It is calculated (Barltrop and Hillier, 1974; O'Kell and Elliot, 1970) that human milk:serum calcium concentration ratios are only about 3:1, compared to the 30:1 ratio observed in mice (Section II-4). Therefore, it is probable that the human milk: plasma lead ratio is much less than the 23:1 ratio we reported for the mouse. Because of the high rate of lead transfer noted in continuously exposed lactating mice the investigation of milk lead concentrations in women exposed to lead is indicated to evaluate potential hazard of lead exposure of their infants through breast feeding.

III. GENERAL DISCUSSION AND SUMMARY

III. GENERAL DISCUSSION AND SUMMARY

The data presented in Section II can be integrated to generate a general hypothesis for the physiological basis of increased sensitivity of young animals to the toxic effects of lead. In the INTRODUCTION it was stated that the apparent sensitivity of the young to lead intoxication could be the result of a combination of possible factors including: increased exposure, increased gastrointestinal lead absorption, increased organ lead retention, differential lead distribution to organs (most importantly the brain), and increased sensitivity of the developing brain to the toxic effects of lead.

Evidence was presented which indicated that suckling and adult mice differ in absorption, distribution and elimination of lead. With the exception of the differences in gastrointestinal absorption, these differences were not large. Following an oral dose of lead it was found that relative distribution of lead to the brain of suckling mice was approximately 50% greater than lead distribution to adult brain. In addition, distribution of lead to kidneys of suckling mice was greatly reduced compared to adults (Section II-2). Data presented in Section II-1 indicate that there are not large differences in the rate of elimination of lead from the brain in suckling versus adult mice. Figure 6 (Section II-1) and Figure 14 (Section II-3) indicate that rates of elimination of lead from blood are similar in suckling and adult mice. The estimated half-life of bone lead was also similar in suckling and adult mice, but it is apparent (Section II-2) that skull sections of suckling mice retain a larger

relative fraction of the lead body burden than do adult skulls. This could be the result of differences in rates of bone ossification and subsequent lead deposition in young mice. In both suckling and adult mice the fraction of an oral lead dose retained was uniform over a wide dose range. These data support the hypothesis that in both suckling and adult mice lead absorption is non-concentration dependent process when relatively large doses of lead are administered.

Differences between suckling and adult mice in organ distributions and elimination of lead are probably not large enough to account fully for differences in lead toxicity. However, the differences which were noted in lead distribution to the brain may be a contributing factor in increased lead sensitivity and should be considered in evaluation of potential hazard.

Data presented in Sections II-4 and II-5 demonstrate the quantitative aspects of lead transfer in the milk of lead exposed mothers to their suckled pups. Following a single lead dose, a large fraction (25%) of the maternally absorbed dose was transferred to the litters. When mothers ingested lead (in the drinking water) during lactation the net lead transfer to the litters was greater than the net increase in maternal lead retention. Clearly much of the newly absorbed lead was eliminated via secretion in milk. In contrast, it was determined that when the adult female mice ingested lead prior to mating but not during gestation and lactation, only 3%

of the initial maternal body burden of lead was retained in suckled litters as a result of lead transfer in milk. This lead transfer was presumably the result of mobilization of lead sequestered in bone concurrent with the mobilization of bone mineral during lactation. In terms of toxicological hazard assessment it is apparent that continuous maternal exposure to lead during lactation represents a more serious hazard than exposure prior to lactation. Until additional human data are available, maternal lead exposure during lactation should be minimized.

A proposed mechanism (pinocytosis) to account for the observed differences in lead absorption between suckling and adult mice was presented in Section II-3. The results were obtained following oral dosing with solutions of lead acetate. However, childhood exposure to lead by the oral route consists of multiple sources of lead. Lead may be present in mother's milk or in formula preparations, may be ingested in paint chips, dust or other foreign material, or may enter the gastrointestinal tract by mucociliary clearance of inhaled lead particulates. Barltrop and Meek (1975) presented data which indicated that various inorganic lead salts are absorbed to different extents from the G.I. tract of adult rats. In a later report (Barltrop and Meek, 1979) they demonstrated that particle size is an important variable in determining absorption of metallic lead particulates from the adult rat gastrointestinal tract. The possible effects of chemical form of inorganic lead on absorption from the gastrointestinal tract have not been investigated. Because of the lack of knowledge

about the chemical and physical form of lead in the gut it is difficult to interpret the results of some dietary effect studies. In reports showing the effects of gross increases or decreases in dietary components the possible effect of these manipulations on chemical or physical form of lead have not been investigated. It may be possible that the observed effects on absorption may be the result of increasing or decreasing the amount of "available" lead in the gut. Lead binding to dietary components or formation of insoluble inorganic lead precipitates could be altered by dietary manipulations. Barton et al. (1978) demonstrated that the calcium chloride solutions in which they administered ^{203}Pb did not affect the formation of precipitates of lead *in vitro*. However, they did not investigate possible effects in the gut where lead interactions with many anions could occur. They did demonstrate that lead solubility is markedly reduced above pH 6. Since the pH of the small intestine is generally above pH 6 much of the luminal lead would exist as insoluble precipitates.

The concern about the interpretation of dietary effects on lead absorption may be most justified in the reports claiming to show an effect of dietary phosphate on lead absorption (Quarterman et al., 1978; Quarterman and Morrison, 1975; Barltrop and Khoo, 1975). Lead is known to form lead phosphate complexes *in vivo* (Clarkson and Kench, 1958) and like calcium phosphate complexes they are characterized by extremely low solubility at neutral or basic pH (Forth and Rummel, 1975). The inhibitory effect of high dietary

phosphate intake on lead absorption could be the result of increased formation of insoluble lead phosphate complexes which reduce the "availability" of absorbable lead. Thus increased lead absorption could occur with low dietary phosphate levels. This possibility warrants investigation.

Differences in the lead absorption mechanism between young and adult animals may be important in assessing the effects on absorption of different physical or chemical forms of lead. If the young or suckling animal is capable of gastrointestinal uptake of large molecular weight compounds and of particulates, then the effect of formation of lead precipitates (e.g. lead phosphate) would be an increase in the magnitude of G.I. lead absorption. If carrier-mediated (e.g. calcium binding protein) or diffusional processes were involved (as thought to be the case in adults) precipitate formation would probably reduce lead absorption. Le Fevre and Joel (1977) review the evidence that particulates of at least 1 μm can be taken up by pinocytosis in the intestinal mucosa in young animals. This evidence indicates that uptake of lead precipitates is possible in the neonatal gut.

The chemical form of lead in milk may be an important factor in lead absorption. Data presented in Section II-4 indicate a possible relationship between lead and calcium transmission into milk. The mechanism responsible for the transport of calcium into milk (where there is a concentration much greater than the plasma calcium concentration) has not been fully investigated. Baumrucker

and Keenan (1975) presented evidence that an ATP-dependent calcium transport mechanism may enable the accumulation of calcium in the Golgi apparatus. The high calcium concentration in the Golgi apparatus relative to the cytosol may be possible because of the formation of calcium phosphate complexes. The formation of these complexes, which are highly insoluble, could results in high total calcium concentrations, but low ionic or free calcium concentrations in milk. Since lead may exhibit affinity for some transport mechanisms it is possible, although there is no direct evidence, that this mechanism is also responsible for the large milk:plasma lead concentration ratio.

If lead in milk is transferred from lead exposed mothers in the form of lead phosphate complexes then it would be expected that a greater fraction of this lead would be absorbed than of an oral dose of lead acetate in suckling pups. This is based upon the prediction that lead phosphate complexes would be more efficiently absorbed by pinocytosis than a dose of ionic lead (which would be only partially complexed). This hypothesis is highly speculative, and is supported by only a small amount of evidence. The data presented in Figure 18 (Section II-4) indicate that 24% of the maternal lead dose is retained in the litter 4 days after maternal dosing. Figure 2 (Section II-1) and Figure 8 (Section II-2) show that following an oral lead dose (lead acetate) the entire dose is retained for at least 24 hours, after which time there is a rapid elimination of the "non-absorbed" lead in the feces. Following

exposure via mother's milk (Figure 18) there is a relatively smooth increase in litter lead retention, with no sudden drop attributable to defecation of "non-absorbed" lead. The approximate potential retention of milk lead can be calculated (Appendix I). Using the data presented in Section II-4 lead retention in pups of approximately 90% was determined following oral intake of lead containing-milk suckled from a lead injected mother. This greatly exceeds the extent of lead retention observed following *per os* dosing with lead acetate solutions (Sections II-1, 2, 3).

However, this finding is complicated by two additional variables. The actual lead dose delivered by the maternal milk was only 50 µg/kg, or a factor of 100 less than that used in the lead acetate administrations. In view of the noted dose-effect on lead retention (Section II-2) this could be important. However, a similar magnitude of lead transfer in milk to the litters was noted when lead doses 20-fold greater were administered i.v. to lactating mice. It is probable that the dose-effect on lead retention would be minimal in that case, given only a five-fold difference in the milk lead and lead acetate doses. The rate of lead passage through the intestine could also complicate analysis. Lead acetate was given as a single oral dose, while lead in maternal milk would have been ingested more slowly with nearly continual suckling. These differences would be narrowed by the intermittent sequence of gastric emptying, but it is not possible to directly ascertain its effect quantitatively.

The hypothetical calculation of lead retention following lead exposure via mother's milk is supported by experimental evidence (Appendix II). A much larger dose of lead (50 mg/kg) was administered i.v. to lactating mothers. This dose resulted in some maternal toxicity as evidenced by a 13% reduction in maternal weight during the first day after administration. This reduced the fractional transfer of lead to the litters perhaps as a result of decreased water intake resulting in decreased milk production. Actual dose to the pups was found to be 2.4 mg/kg transferred to the suckling pups in the mother's milk during a 24 hour period. This is similar to the oral doses of lead acetate (Sections II-1, 2, 3), although the possible confounding variable of "infusion rate" of lead into the intestine has not been eliminated. However, a large fraction (0.69) of the lead transferred from the lead exposed mother in maternal milk to the pups was retained two days after cross-fostering to non-exposed mothers. This value is much larger than the fractional retention of orally administered lead acetate and supports the hypothetical calculations in Appendix I.

These results indicate that chemical form of lead in the intestine is an important determinant in lead absorption in young animals. As mentioned above it is possible that the dietary manipulations which modified lead absorption in adults may have the opposite effect on lead absorption in young animals due to effects on the chemical form of lead in the gut. In any case considerable caution should be exercised in extrapolating the results of adult dietary-

effects studies to the assessment of lead absorption in young animals.

The augmentation of lead absorption by the chemical or physical form of lead in the suckling gut remains a distinct, but only weakly supported, hypothesis. Additional experimentation would be necessary to relate pinocytosis and uptake of insoluble lead complexes.

As discussed in Section II-3, diffusion and carrier-mediated transport may be involved in lead absorption in both suckling and adult mice. It has not been possible to define completely which differences in absorptive mechanisms between suckling and adult animals account for the increased absorption observed in suckling animals. However, it is apparent that pinocytosis in suckling mice accounts for a large fraction of the increased absorption. It has not been determined whether diffusional and carrier-mediated lead absorption are also increased in suckling mice relative to the adult. It is very likely that the mechanism(s) of lead absorption may be affected not only by the chemical form of lead, but also by the magnitude of the lead dose which is administered.

It was shown (Section II-2) that fractional lead retention (reflecting absorption) was greatly increased in both suckling and adult mice when carrier-free ^{203}Pb was administered compared to administration of doses larger than 5 mg/kg. It is possible that a larger portion of the carrier-free ^{203}Pb dose (or any low dose) could be absorbed through diffusional or carrier-mediated mechanisms than when larger lead doses are administered. Larger lead doses could "saturate" carrier mediated absorptive pathways, so that a

smaller fraction of the administered lead would be absorbed. The decreased fractional absorption of lead noted when lead doses of 5 mg/kg and greater are administered could be the result of "saturation" of an absorptive pathway (non-first-order). However, the difference in fractional absorption between suckling and adult mice when a carrier-free dose was administered indicates that age differences in absorptive mechanism occur even at very low doses. The constancy of absorption (non-concentration dependent) for doses larger than 5 mg/kg does not, however, implicate a "saturable" absorptive mechanism.

It is proposed that multiple absorptive pathways are involved in lead absorption in both suckling and adult mice. For very low doses of lead, carrier-mediated processes could be responsible for the greatest fraction of the absorbed lead. When larger doses are administered lead could be absorbed principally by diffusion in adults, and by pinocytosis in suckling mice. Flanagan et al. (1979) presented evidence which supports the hypothesis that lead is absorbed by carrier-mediated and diffusional processes in adults. The authors proposed that "non-saturable" diffusional lead absorption accounted for a large fraction of the absorbed lead, but that feeding of iron deficient diets resulted in some carrier-mediated lead absorption. This proposed model fits the published data, but remains largely speculative in view of the absence of specific proof.

It is apparent that dietary-effects experiments conducted with adult animals must be conservatively interpreted. It is not clear whether dietary effects on lead absorption are the result of effects

on carrier-mediated transport processes or simply the result of altered physico-chemical form of lead. Insufficient evidence exists to allow the extrapolation of proposed absorptive mechanisms in adults to suckling or young animals. The published reports concerning lead absorption in adults, and the data presented in this thesis concerning absorption in suckling mice indicate the involvement of multiple mechanisms of absorption. While both young and adult animals probably exhibit diffusional, carrier-mediated, and pinocytotic absorption of lead, the contribution of each absorptive component apparently varies with age. In suckling mice pinocytotic lead absorption accounts for a large fraction of the absorbed dose. In adults, the contribution of pinocytosis is apparently very small. It is appropriate that age differences in lead absorption be considered terms of qualitative as well as quantitative differences in the mechanism(s) of absorption.

It has been demonstrated that a large number of factors must be considered in the assessment of lead exposure in young and suckling animals. Large differences between suckling and adult mice were demonstrated in lead pharmacokinetic parameters, particularly in terms of lead absorption which was shown to be the result of increased pinocytotic activity in suckling mice. Suckling animals may be exposed to lead by a route (milk) to which adults are not exposed if there is significant maternal lead exposure. The developmental differences which contribute to the increased risk of lead intoxication in young animals accentuate the need for toxicological evaluation in

young animals. The extrapolation of results from adults to young animals is clearly not valid when large age differences occur which are relevant to the tested chemical. If there is possible exposure of young animals to the tested chemical then experimental studies should be conducted to ascertain differences in physiology, biochemistry and pharmacokinetics which might increase the sensitivity of young animals. Furthermore, if women of child-bearing age are potentially exposed the transfer of the chemical into milk should be evaluated, as well as the potential teratogenic and fetotoxic effects. Toxicological studies with adult animals may provide a useful starting point for the evaluation of toxicity in the young, but additional experimental studies would be required to formulate valid toxicity assessments. The age differences in lead pharmacokinetics outlined in this thesis may be applicable to a limited number of other chemicals. However, further research will almost certainly define other parameters relevant to increased sensitivity in young animals to the toxic effects of chemicals.

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APPENDIX I

Calculation of Absorption of Lead Transferred in Milk from the Lactating Mother Using Data taken from Section II-3

Introduction:

The retention (absorption) of lead delivered to suckling pups via maternal milk can be calculated using the results of experiments which were reported in EXPERIMENTAL RESULTS. The calculations indicate that there is greater retention of lead administered via maternal milk as compared to single oral doses of lead acetate. This is consistent with the hypothesis that the physical and chemical form of lead is an important factor in lead absorption.

Data

1) lead lost by excretion from suckling pups during the first day of exposure to lead in suckled milk is less than 1% of the administered dose (data from Figures 2 and 8).
2) growth of pups from day 0 to 1 and from day 1 to 2 was constant (approximately 1.5 g/day/litter), therefore volume of milk consumed per day (days 0-2) was relatively constant.
3) mean maternal milk lead concentration during 0 to 24 hours after maternal dosing was 380 ng/ml; during 24 to 48 hours, 100 ng/ml (calculated from Figure 19).
4) total lead content of each litter (5 pups each) was 1279 ng after 24 hours and 1524 ng after 48 hours (calculated from Figure 18).

Calculation:

Day 1: $\frac{\text{ng Pb in litter}}{\text{mean ng/ml in milk}} = \frac{1279 \text{ ng}}{380 \text{ ng/ml}}$

= 3.4 ml of milk produced by mother.

Day 2: ng Pb transferred to litter = 3.4 ml of milk × 100 ng/ml mean milk Pb concentration

= 340 ng (theoretical)

Actual Net Δ ng day 1 to day 2 in litter Pb content (day 2 - day 1) = 1524-1279

= 245 ng Pb

Net Δ (theoretical increase - actual net increase) in litter Pb content during day 2 = 340-245

= 95 ng

95 ng would be the "predicted" fecal lead elimination during day 2.

$\frac{1279\text{ng} - 95\text{ng}}{1279\text{ng}}$ = 93% theoretical retention of lead transferred in milk from lead-exposed lactating mothers.

APPENDIX II

Retention of Lead in Suckling Pups Following Lead Transfer From Exposed Lactating Mothers.

Experimental Design:

1) Two lactating BALB/c mothers injected i.v. with 50 mg/kg of ^{210}Pb-acetate (Day 0).
2) Each mother suckled five 12 day old (on Day 0) C129F, pups.
3) After one day of suckling by lead treated mothers, pups were cross-fostered to untreated mothers.
4) Lead content of pups determined by whole body gamma counting prior to cross-fostering and two days after cross-fostering (Day 3).

Results

1) Lead Dose to Pups (ingested via milk suckled from lead exposed mother):

   2.4 mg Pb/kg of pup's body weight

2) Lead Retention in Pups (n = 10 pups):

$$\text{Net Lead Retention} = \frac{\text{(mg Pb) 2 days after cross-fostering}}{\text{(mg Pb) in pups at cross-fostering}}$$

$$= 0.69 \pm 0.08 \text{ (SD)}$$

Conclusion: Lead delivered via milk from lead exposed mother is retained to a greater extent than lead acetate administered orally in an aqueous solution.