THE INFLUENCE OF NUTRITIONAL FACTORS ON THE ABSORPTION OF LEAD

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ABSTRACT

The nutritional factors influencing the absorption of lead from the gut have been studied in the rat using both intact animals and ligated gut loop preparations. Studies of 48 hour duration have been made in groups of animals fed synthetic diets of known composition containing 0.075% lead as $PbCl_2$ labelled with ^{203}Pb. Lead in the carcass and individual organs was determined by means of a whole-body counter for small animals and an auto-gamma counter. Lead absorption was enhanced by high fat, low mineral, low protein and high protein diets, but was decreased by high mineral diets. Low fat, low fibre, low vitamin and high vitamin diets had no effect on lead absorption. Calcium and phosphate were identified as the two principal minerals modifying lead absorption and their effects were found to be additive.

The interaction of lead with individual dietary components has been studied under controlled conditions using ligated gut loop preparations. With this technique the relative roles of luminal interaction between dietary components and lead have been investigated and the intestinal transport mechanisms and kinetics of lead absorption have been explored. Calcium and phosphate were found to modify lead transport across the gut wall and in addition, the role of vitamin D in the absorption of lead has been studied.

The significance of these findings is discussed in relation to the different responses to lead exposure which have been observed in various population groups. These results suggest that the nutritional determinants of lead absorption should be considered in relation to permissible limits of lead intake.

To my parents, brothers and Keat
for their love and encouragement.

TABLE OF CONTENTS

	Page No.
ACKNOWLEDGEMENTS	1
ABSTRACT	2
INTRODUCTION	12
Intake and Absorption	13
- Oral Intake	13
- Respiratory Intake	17
- Dermal Contact and Absorption	20
Normal Metabolism and Body Distribution	22
Toxic Effects of Lead	29
- Haemopoietic System	29
- Renal System	34
- Central Nervous System	36
- Other Organs	37
Sources of Excessive Lead for Humans	39
Metabolic Factors Influencing Uptake and Toxicity of Lead	41
- Chemical and Physical Form of Lead	41
- Age	43
- Season of the Year	45
- Dietary Protein	46
- Dietary Fat	47
- Dietary Minerals	47
- Dietary Vitamins	53
- Alcohol	54
- Milk	55
- Lactose	56

Page No.

- Citrate 57
- Chelating Agents 57
- Other Dietary Components 58

Biochemical Effects of Lead 63

- Subcellular Distribution of Lead 63
- Membranes 64
- Proteins 66
- Delta-Aminolaevulinic Acid Dehydrase 67
- Globin Synthesis 71
- Other Enzymes 72
- Nucleotides and Nucleic Acids 73

MATERIALS AND METHODS 74

Selection of Animals 74

Diet Preparation 74

Trial Procedure 80

Evaluation of Synthetic Diet 80

Selection of Cages 82

Preliminary Studies with ^{203}Pb 84

Establishment of Standard Conditions 87

- Dose 87
- Age and Weight 87

Analysis of Stable Lead 94

Ligated Gut Loops 98

Statistics 100

Page No.

RESULTS 101

Calorie Intake 101

Diets of Varying Nutritional Content 101

Protein 105

Fat 117

Fats of Varying Composition 117

Minerals 137

Minerals and Fat 165

Other Dietary Components 165

Diet Consistency 177

Ligated Gut Loops 181

- Varying Concentrations 181
- Varying Times 186
- Varying Calcium 186
- Varying Phosphate 191
- Inhibitors 198
- Intestinal Segments 198
- Vitamin D 198

DISCUSSION 203

REFERENCES 219

APPENDICES

A - Tissue Lead Values of Control Rats 257

B - Radioactivity Measurement 260

C - Composition of Oxoid Diet 264

D - Composition of Test Diets 265

E - Subsidiary Matter 272

LIST OF TABLES

		Page No.
1	Changes in the Concentration of Porphyrins and their Precursors in Lead Poisoning	32
2	Metabolic Factors Influencing Uptake and Toxicity of Lead	40
3	Composition of Control Diet	77
4	Effect of Synthetic Diet on Weight Gain	81
5	Distribution of ^{203}Pb after Intraperitoneal Injection	85
6	Distribution of ^{203}Pb after Feeding of ^{203}Pb in the Diet	85
7	Varying Age	88
8a	Varying Lead Concentration	95
8b	Influence of Age and Body Weight	97
9	Uptake of Lead from Control Diets	102
10	Decreased Calorie Intake	103
11	Different Diets	104
12a	Low Fibre	110
12b	High Fibre	110
13	Low Vitamin	111
14	High Vitamin	111
15	10% Lactose	112
16	0% Protein	113
17-23	Varying Protein	113-116
24-29	Varying Fat	122-127
30	Composition of Dietary Fat	134
31	Rapeseed Oil	135
32	Sunflower Oil	136

		Page No.
33	Coconut Oil	138
34	Butterfat	139
35	0% Mineral	140
36	0% Macromineral	142
37	0% Micromineral	143
38	0% K, Na, Cl	144
39	0% Calcium	145
40	0% Phosphate	146
41	0% Calcium and Phosphate	147
42	0% Magnesium	149
43	0.35% Calcium and 0.5% Phosphate	150
44	0.7% Calcium and 0.25% Phosphate	151
45	0.35% Calcium and 0.25% Phosphate	152
46	Decreased Minerals	153
47	High Mineral	154
48	High Macromineral	155
49	High Micromineral	157
50	1.4% Calcium and 0.5% Phosphate	158
51	0.7% Calcium and 1.0% Phosphate	159
52	1.4% Calcium and 1.0% Phosphate	160
53	2.8% Calcium and 0.5% Phosphate	161
54	0.7% Calcium and 2.0% Phosphate	162
55	2.8% Calcium and 2.0% Phosphate	163
56	Increased Minerals	164
57	0% Mineral and 15% Fat	169
58	Composition of Alginates	172
59	10% Alginate	173
60	1% Alginate	175

		Page No.
61	1% Phytate and 1% Pectin	178
62	Varying Water Content	179
63	Diet Consistency	183
64	Ligated Gut Loops and Varying Lead Concentrations	184
65	Varying Incubation Time	187
66	Varying Calcium and 1 μg Pb/ml	189
67	Varying Calcium and 10 μg Pb/ml	193
68	Varying Phosphate and 1 μg Pb/ml	196
69	0.025 mM Phosphate and 1, 5, 10 μg Pb/ml	197
70	Lead Phosphate and Lead Chloride	199
71	Inhibitors	200
72	Intestinal Segments	201
73	Vitamin D	202

LIST OF FIGURES

		Page No.
1	Ecological Pathways of Lead	14
2	The Synthesis of Haemoglobin	31
3a	All-Glass Small Animal Metabolism Chamber with Stick Feeder	75
3b	All-Glass Small Animal Metabolism Chamber with Powdered Diet Feeder	76
4	Food Mould	79
5	Polythene Cage	83
6-10	Tissue Lead and Diets of Varying Lead Concentration	89-93
11	Influence of Age on Kidney Lead Concentration	96
12-15	Tissue Lead and Diets of Varying Composition	106-109
16-19	Tissue Lead and Diets of Varying Protein Composition	118-121
20-24	Tissue Lead and Diets of Varying Fat Composition	128-132
25-26	Tissue Lead and Diets of Varying Mineral Content	166-167
27	Kidney Lead and Diets of Varying Calcium and Phosphate Content	168
28	Lead Uptake from Diets of Varying Fat and Mineral Content	170
29	Lead Uptake from 10% Alginate Diet	174
30	Lead Uptake from 1% Alginate Diet	176
31	Lead Uptake and Additional Water	180
32	Blood Lead and Varying Dietary Water Content	182

		Page No.
33	Rate of Lead Transport from Intestinal Loops	185
34	Distribution of 1 µg Lead in Intestinal Loops	188
35	Varying Calcium Concentration and Lead Transport (1 µg)	190
36	Varying Calcium Concentration and Lead on Gut Wall (1 µg)	192
37	Varying Calcium Concentration and Lead Transport (10 µg)	194
38	Varying Calcium Concentration and Lead on Gut Wall (10 µg)	195

INTRODUCTION

As civilisation has progressed, man has increasingly extracted and utilised the metallic elements. This has resulted in their redistribution over the surface of the earth. The potential biological consequences of this environmental pollution with the metals and their compounds has caused great concern because their redistribution is a progressive and permanent process. Interest in the problem has been intensified by the development of more sensitive methods of detection and analysis. This is particularly true of lead which is one of the most useful and abundant metals known to man.

Lead has been used for about 6000 years. Evidence for this is a lead figure that was made before 3800 BC which is now in the British Museum (Hunter, 1969). The early Egyptians, Hebrews and Phoenicans are known to have used lead before 2000 BC and the Babylonians had lead pans to hold plants in their Hanging Gardens. When the Romans discovered lead in Britain, they began to exploit the lead mines in the Mendips, Shropshire, Derbyshire and Flint. This lead was mainly used for making lead pipes for sanitation, water supplies and domestic utensils. In modern times the United States of America, Mexico, Australia and Canada are the main lead-producing countries.

Since lead is a soft, heavy, malleable and ductile metal which is protected from corrosion by the formation of a thin coating of oxide it has found many applications.

Roofing, tank linings and water pipes have contained lead. It is used in casting type as an alloy of antimony, and alloyed with tin, it is used in solder.

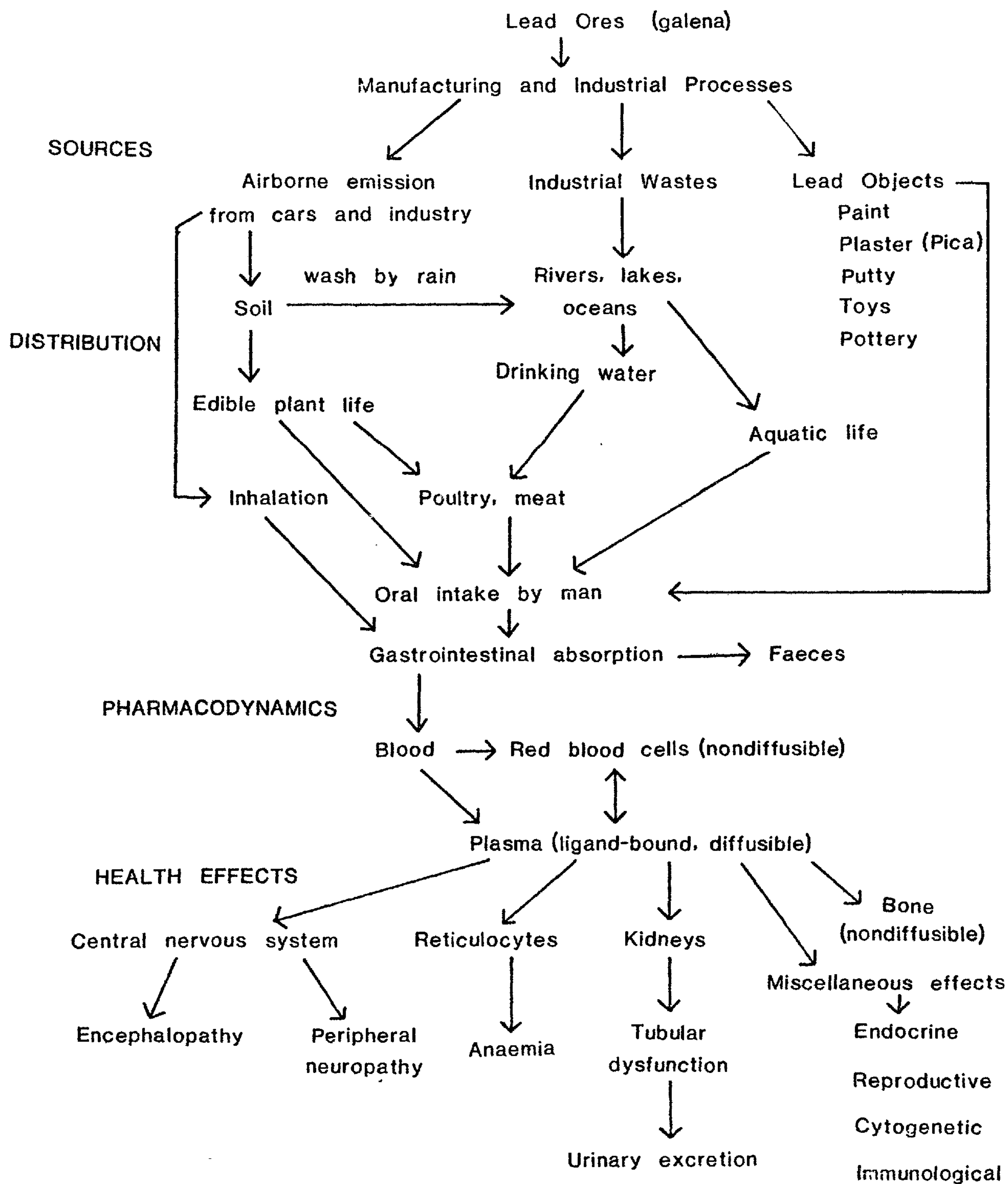
The modern usage of lead in the United States has been reported in detail by Ziegfeld (1964). About 1,100,000 short tons is utilised per annum. Of this total consumption, 35% is used in the manufacture of storage batteries, 16% as alkyl additives to petrol, 11% in building, 15% in brass, ceramics, type and paint and 23% in other unnamed processes. Thus, 175,000 tons is being used in the production of petrol much of which is ultimately discharged into the atmosphere in automobile exhaust. 2,500 tons of lead arsenate are also produced annually to be applied as pesticides to food-crops. A total of 177,500 tons of lead is therefore being distributed into the ecosystem which will contribute to the human body burden of lead via the atmosphere and food. All other sources of emission of lead in the United States equal less than 4000 tons, or about 2% of the lead emitted from petrol exhausts (Goyer and Rhyne, 1973). A scheme of the ecological pathways by which lead enters the metabolism of man is shown in Fig. 1.

Oral Intake

Lead has been considered toxic to man for more than 2,000 years. Although its acute toxicity is relatively low, it may accumulate slowly in certain tissues when intake exceeds elimination. Under normal conditions, food, water and other beverages are the major sources of lead in man and

Figure 1

Ecological Pathways of Lead*



* Adapted from Goyer and Chisolm (1972)

probably in most animals. There have been numerous studies of the concentration of lead in various foods and beverages.

Foodstuffs

Kehoe et al (1933) found lead in all food items obtained from the field and from dwellings of a primitive region far from industrial and mining activities. Patterson (1965) estimated that the natural lead content of food should be 0.01 μg/g wet weight and concluded that most of the lead present today in food is from industrial sources. Thus Schroeder et al (1961) found present day food contained lead in concentrations from 0 to 2.5 μg/g wet weight, fish and other seafoods exhibiting the greatest values within the range. The 1975 survey of lead in food in the United Kingdom (Ministry of Agriculture, Fisheries and Food, 1975) reported similar values in all the foods analysed. However, certain canned foodstuffs and vegetables grown in areas with soils of high lead content, in areas of atmospheric contamination or where sewage sludge is spread on the land, were found to have increased lead content.

Water

The lead content of public water supplies ranged from traces to 63 μg/l for 100 United States cities (Durfor and Becker, 1964). Kehoe (1961) found values of 3-40 μg/l but reported lead concentrations as high as 920 μg/l in water where lead piping or lead joint luting was used.

In the United Kingdom only 0.2% of 10,899 samples of drinking water contained lead greater than 100 µg/l and 1% contained 50 µg/l. However, samples of water from old homes in Glasgow contained an average of 350 µg/l and 930 µg/l from homes with lead-lined storage tanks (Ministry of Agriculture, Fisheries and Food, 1975).

From these findings, it is evident that drinking water contributes, on average, only a small amount of lead to the body burden. Assuming an average daily intake of 1 litre of water containing 20 µg/l, then the lead consumed from water would only be 20 µg/day compared with a total daily intake of 200 ug from foodstuffs.

Daily Intake

The daily combined food and water lead intake for an average United States adult has been estimated to range from 100 to 500 µg/day (Schroeder and Tipton, 1968). Other studies have estimated 300 µg/day (Cholak and Bambach, 1943; Kehoe, 1961; Lewis, 1966; Harley, 1970). These estimates are based on surveys of lead in foodstuffs but do not take into account the contribution from utensils and any liquids used in cooking. However, the mean daily lead intake of 200 µg for the general public in the United Kingdom has been based on food provided as typical meals and including cooking (Ministry of Agriculture, Fisheries and Food, 1975).

The daily dietary intake of children without unusual non-dietary exposure has been estimated at 130 µg (Chisolm and Harrison, 1956; Barltrop and Killala, 1969). King (1971) has found a daily intake for children varying from about 75 to 170 µg/day.

Gastrointestinal Absorption

Gastrointestinal absorption of lead by an adult male has been measured by a number of workers. Kehoe (1961; Patterson (1965) and Schroeder and Tipton (1968) found that 10% of the lead ingested by humans is absorbed. Other estimates vary from 1% (Fairhall, 1938; Hursch and Suomola, 1968) to 40% (Imamura, 1967). However, the most widely accepted estimate is Kehoe's (1961) measurement of 10% for an adult.

Little information is available for the uptake of dietary lead by infants and children. Alexander et al (1972) gave a value of 50% absorption but a more recent study has suggested that an absorption of 25% is more likely (Strehlow, personal communication).

Net absorption also varies with species. Sheep and rabbits absorb 1.3% (Blaxter, 1950) while adult rats absorb 1% (Kostial et al, 1971a). A number of factors have been shown to affect the rate of lead absorption from the gastrointestinal tract.

Respiratory Intake

The petroleum industry is the world's second largest consumer of lead, using 20% of the total for petrol anti-knock additives. The combustion of petrol is responsible for 98% of the airborne lead that can be traced to its source (National Academy of Sciences, 1972).

As the concentration of lead in ambient air is closely correlated with the density of vehicular traffic, it is reasonable to assume that it would decrease as one progressed from large cities to rural areas. This has been confirmed by McMullen et al (1970) whose data showed an air lead concentration of 0.21 $\mu g/m^3$ near to a city, 0.096 $\mu g/m^3$ at an intermediate distance from the city and 0.022 $\mu g/m^3$ in remote areas. Ambient air lead in most United States cities has been found to range from 1 to 5 $\mu g/m^3$ (Kehoe, 1964).

In spite of the rapid increase in the consumption of lead used in petrol, the concentration of lead in urban air has increased relatively slowly, presumably because of dispersion. Lead is removed from the air by aggregation and by precipitation. Thus, the concentration of lead in rainfall in a particular area can also be correlated with the volume of petrol used in that area (Lazrus et al, 1970).

Cigarettes also constitute a source of lead for the smoker. Goghill and Hobbs (1957) found 1-2 µg of airborne lead was released by the burning of one cigarette. Cigarette smokers also had consistently higher blood lead concentrations (Lehnert et al, 1967).

Rate of Absorption from the Respiratory Tract

The rate of absorption of lead from the respiratory tract varies with the particle size of the lead inhaled. Kehoe (1961) reported that lead deposition in the respiratory tract associated with exposure to smaller particles

(average diameter 0.05 μm with 90% from 0.02 to 0.09 μm) was 36% while deposition of larger particles (average diameter 0.9 μm with 90% less than 2 μm) was 46%. Nozaki (1966) confirmed Kehoe's results with particles varying in size from 0.05 to 1 μm. When the excretion of inhaled lead was measured, there was an increase in faecal lead with the larger particles (Kehoe, 1961) which was attributed to the passage of lead from the respiratory tract into the gastrointestinal tract. The larger lead particles deposited in the nasopharyngeal region may be swallowed. Particles deposited in the trachea and bronchi may also migrate up to the pharynx by ciliary-mucous transport and be swallowed.

Until recently, very little was known about the clearance of lead from the respiratory tract. Chamberlain et al (1975) have measured percentage deposition in the lung, uptake to blood and excretion of lead in humans exposed to the exhaust lead aerosols from engines burning tetraethyl lead labelled with ^{203}Pb. The particle size of the lead aerosol produced in this way was approximately 0.5 μm. It was found that the percentage deposition in the lung depended on the length of the respiratory cycle thus confirming Nozaki's (1968) observation that shallow respiration resulted in lower rates of deposition of lead particles than with deep respiration. Chamberlain et al (1975) found that for a 4 second cycle (15 breaths/min), the deposition was 35%. Kehoe (1961), Mehani (1966), and Nozaki (1966) all reported a similar figure of 37%.

Chamberlain et al (1975) showed that most of the lead passed from the lung to the blood plasma from which half was removed to the bone and other tissues and half becomes attached to the red blood cells. The biological half-life of lead in the blood was found to be 16 days. Faecal clearance averaged 6% after the first week and renal clearance averaged 4.3 ml/hour (^{203}Pb excreted in the urine per hour divided by ^{203}Pb concentration in the blood). Unlike Kehoe's (1961) study where an estimated 40% of the lead particles deposited in the lungs was transferred to the gastrointestinal tract, in this study only a small percentage of the inhaled lead was brought up by ciliary action and swallowed. This was not unexpected as the particles involved in Kehoe's (1961) study were larger and so deposition in the tracheo-bronchial region would have been greater.

The data from the study by Chamberlain et al (1975) indicate that continuous (24 hours/day) exposure to 1 $\mu g/m^3$ of exhaust lead over a few months would given an average contribution of 1 µg/100ml to whole blood. This figure appears consistent with data from other work (Williams et al, 1969; Cole and Lynam, 1973; Knelson, 1974).

Dermal Contact and Absorption

Although a finite amount of lead is continuously coming into contact with the skin from air, water and clothing, these contacts are not considered significant (Cantarow and Trumper, 1944; Tepper, 1966). However, fat-soluble lead

alkyl compounds, tetraethyl and tetramethyl lead are readily absorbed from the skin and constitute an occupational hazard (National Academy of Sciences, 1972; Gething, 1975).

LEAD METABOLISM

Normal

Although the transfer of lead from the external environment to the body is not clearly understood, there is extensive information concerning the manner in which lead circulates in the blood. In a review of this topic, Goyer (1971) stated that lead in blood is in a nondiffusible form bound to the red cells and a diffusible form in the plasma. Most of the lead, 99% of the circulating total, is bound to the red blood cells and 1% is in the plasma. Clarkson and Kench (1958) suggested that the lead bound to the red cells is in the form of a peptized lead phosphate sol and that it is only slowly exchangeable with lead in plasma. In cases of lead poisoning, Lloyd Davis and Rainsford (1967) reported that the diffusible lead content of blood increases to only 3 or 4% of the total lead in the blood.

With dietary lead intake variation, the changes in concentration of lead in blood and urine occurred more or less proportionally, with blood exhibiting a lesser fluctuation than urine (Kehoe, 1961). The concentration of lead in blood was considered more useful than that of urine as an indication of the body lead burden because blood is not subject to large fluctuations in water content or to the influence of changes in renal excretory capacity.

Blood lead concentration is thus still the most widely used index of recent and current absorption of lead. However, estimates of the range of blood lead values found

in the general population have varied, as might be expected when samples are taken from groups with presumed differences in exposure. The most comprehensive report has been that of Goldwater and Hoover (1967) which determined blood lead concentrations from persons in sixteen different countries. From their data and those of others (Kehoe et al, 1940; Kubota et al, 1968), it may be concluded that blood lead concentrations greater than 50 µg/100g are unlikely to occur in persons with no more than the general environmental exposure to lead and that the range of typical values lies between 10 and 40 µg/100g of whole blood. In general, urban mean values tend to be greater than rural mean values. The subject of increased blood lead concentrations in exposed individuals has been reviewed by the National Academy of Sciences (1972). Blood lead concentrations of 80 µg/100g were described as moderately increased and might produce symptoms of lead poisoning. Sustained concentrations of 100 µg/100g of blood would usually be associated with deranged haem synthesis, as well as other clinical manifestations of lead poisoning.

Distribution of Lead in the Body

Under normal steady state conditions, over 90% of the total amount of lead in the body is in the skeleton (Schroeder and Tipton, 1968). The concentration found in tissues is variable, being greatest in bone, intermediate in liver, kidney and aorta, and low in muscle and brain. The concentration in most other tissues is intermediate

between those of kidney and muscle.

The exposure of the general population to lead results in some accumulation in the bone up to and perhaps beyond the age of 50, as determined by analysis of tissues (Barry and Mossman, 1970). However, lead in soft tissues showed values that did not increase after the second decade. The total lead content in the soft tissues appeared to be relatively stable and did not correlate with levels in bone. A later study (Gross et al, 1975) demonstrated a similar increase in dense bone lead with advancing age while spongy bone lead remained constant or decreased in later years. Although the overall content of lead increased with age due to the skeletal lead burden, many soft tissues did not change and several tissues actually decreased in lead concentration with age. The liver, kidney, pancreas, jejunum, stomach and adrenal decreased significantly while decreases in blood, skin, caecum and bladder almost achieved statistical significance. Correlation between blood lead concentrations and that in the other tissues of the same individual have not been sought.

Bone Lead

Aub and his colleagues (1926) drew attention to the considerable amount of lead that can be accommodated in the skeleton without apparent ill-effect to the individual. However, it is not known in what manner lead is deposited in bone. It has been suggested that a major part of the lead

is fixed by ion exchange with calcium within the bone crystal or incorporated into the lattice interstices (MacDonald et al, 1951).

The biological half-life of lead varies with metabolic activity. Using ^{210}Pb as a tracer, Strehlow (1971) has shown that maximal uptake occurs at the ends of long bones, especially in the region of calcification and then in descending order to the vertebrae, scapulae, ribs, maxilla, long-bone shafts to the calvarium. Kehoe (1964) also reported lead concentrations in the long bones to be greater than in the flat bones in cases of chronic lead poisoning, but in acute cases the content was greatest in the flat bone. Willoughby et al (1972) reported higher concentrations of bone-lead in the more active epiphyseal sections of long bones than in the mid-shaft regions.

Various studies (Horiuchi et al, 1959; Schroeder and Tipton, 1968; Barry and Mossman, 1970; Gross et al, 1975) have indicated that concentrations of lead in bone increased with age. The significance of lead in bone has been reviewed by Barltrop (1973) who concluded that the affinity of lead for bone resulted in the removal of soft tissue lead in excessive exposure and bone lead could serve as an index of long-term exposure. However, lead in bone could become a potential source of endogenous lead in conditions of bone resorption and contribute to the body burden of internal radiation from the natural isotope, ^{210}Pb.

Soft Tissue Lead

The most extensive studies of soft tissue lead distribution have been made by Schroeder and Tipton (1968) and recent surveys have confirmed their data (Barry and Mossman, 1970; Gross et al, 1975).

The concentration of lead in soft tissues varies from less than 0.1 μg/g in organs such as muscle and heart to over 2 μg/g in the aorta (Barry, 1975). However, the form in which lead is present at normal levels in soft tissues is not known.

Normal values for lead in bone, blood, kidney, liver, spleen and muscle of rats are given in Appendix A.

Hair Lead

Lead concentrations in hair and nails were found to be higher than soft tissue concentrations but varied widely (Barry, 1975). Thus hair lead measurements were not considered to provide a reliable assessment of lead absorption. Recent studies however, have suggested that the concentration of lead in hair may be used as an indicator of previous lead exposure (Barltrop et al, 1974; Kopito and Shwachman, 1975).

Tooth Lead

Deciduous teeth have been shown to accumulate lead (Altshuler et al, 1962; Strehlow, 1971; Carroll et al, 1972; Needleman et al, 1972; Needleman and Shapiro, 1972; Albert et al, 1974). Children known to have had lead poisoning have

significantly greater concentrations of this element in their circumpulpal dentine than normal children. Thus this tissue can be used to identify past exposure even in asymptomatic children.

Normal Excretion of Lead

Although much is known about the principal routes of entry of lead into the body, there is less information available concerning the excretion of absorbed lead. The body seems to have adapted to the presence of lead by being able to remove or sequester lead in an inert form. The presence of over 90% of body lead in an inert form in the skeleton represents a mechanism by which the body can prevent the accumulation of toxic amounts in soft tissues. The formation of intranuclear inclusion bodies of lead-protein complexes binding lead in a nondiffusible form in renal cells (Goyer et al, 1970) may also represent an adaptive mechanism.

Excretion of absorbed lead can take place via two routes. The gastrointestinal route has been suggested to contribute as much as the urinary route to total lead excretion in humans (Booker et al, 1969). From experimental studies in dogs and rats, it has been shown that lead is excreted from the liver mainly in bile (Blaxter and Cowie, 1946; Castellino et al, 1960; Cikrt, 1972). The role of the entero-hepatic circulation in lead metabolism has not been quantitatively studied in man.

Goyer and Mahaffey (1972) have reported that lead is excreted by the kidney in two ways: by glomerular filtration

and transtubular flow. Vostal and Heller (1968) showed transtubular transport of lead from the renal tubular capillary through the tubular lining cell to the tubular lumen of the avian kidney. Only diffusible or ligand-bound lead can be transported across membranes and through renal tubular lining cells. It has been demonstrated that in lead poisoning, the increase in urinary lead is largely ligand- or organic bound lead (Dinischiotu et al, 1960).

Lead may also be excreted in sweat, hair and desquamated skin. Shiels (1954) and Kehoe (1961) found that the concentration of lead in sweat approximated that of urine. The daily volume of sweat produced is approximately one-third that of urine. Thus the excretion of lead in the sweat does contribute substantially to the total excretion of lead from the body.

TOXIC EFFECTS OF LEAD

The pathological effects of lead are manifested in three major body systems:- haemopoietic, renal and central nervous systems.

Haemopoietic System

Lead poisoning has been reported to give rise to a mild hypochromic and sometimes microcytic anaemia. The anaemia is also associated with shortened red-cell life-span, reticulocytosis and the presence of basophilic stippled cells in the peripheral blood (Leikin and Eng, 1963; Griggs, 1964; Bessis and Jensen, 1965; Waldron, 1966; Berk et al, 1970). Anaemia due to lead poisoning has many morphological features in common with the anaemia of iron deficiency and thalassaemia; hence these three conditions are not always easily distinguished. Six and Goyer (1970, 1972) have produced a significant hypochromic anaemia and reticulocytosis in rats by giving the animals 200 µg lead/ml in their drinking water.

Teisinger et al (1958) suggested that lead was fixed by the red cells, possibly by physical adsorption on the surface of the erythrocytes or possibly by simultaneous chemical fixation, and considered that there was probably a chemical bond with the lipoprotein component of erythrocytes. Since membrane lipoprotein is essential to the integrity of the red cell membrane, it may be that chemical combination with lead interferes with its stabilizing properties.

This could account for the phenomena of reduced red cell survival time, increased red cell fragility and accelerated red cell destruction, which are characteristic of lead poisoning (Hardy, 1966; Hunter, 1969; Haeger-Aronsen, 1971).

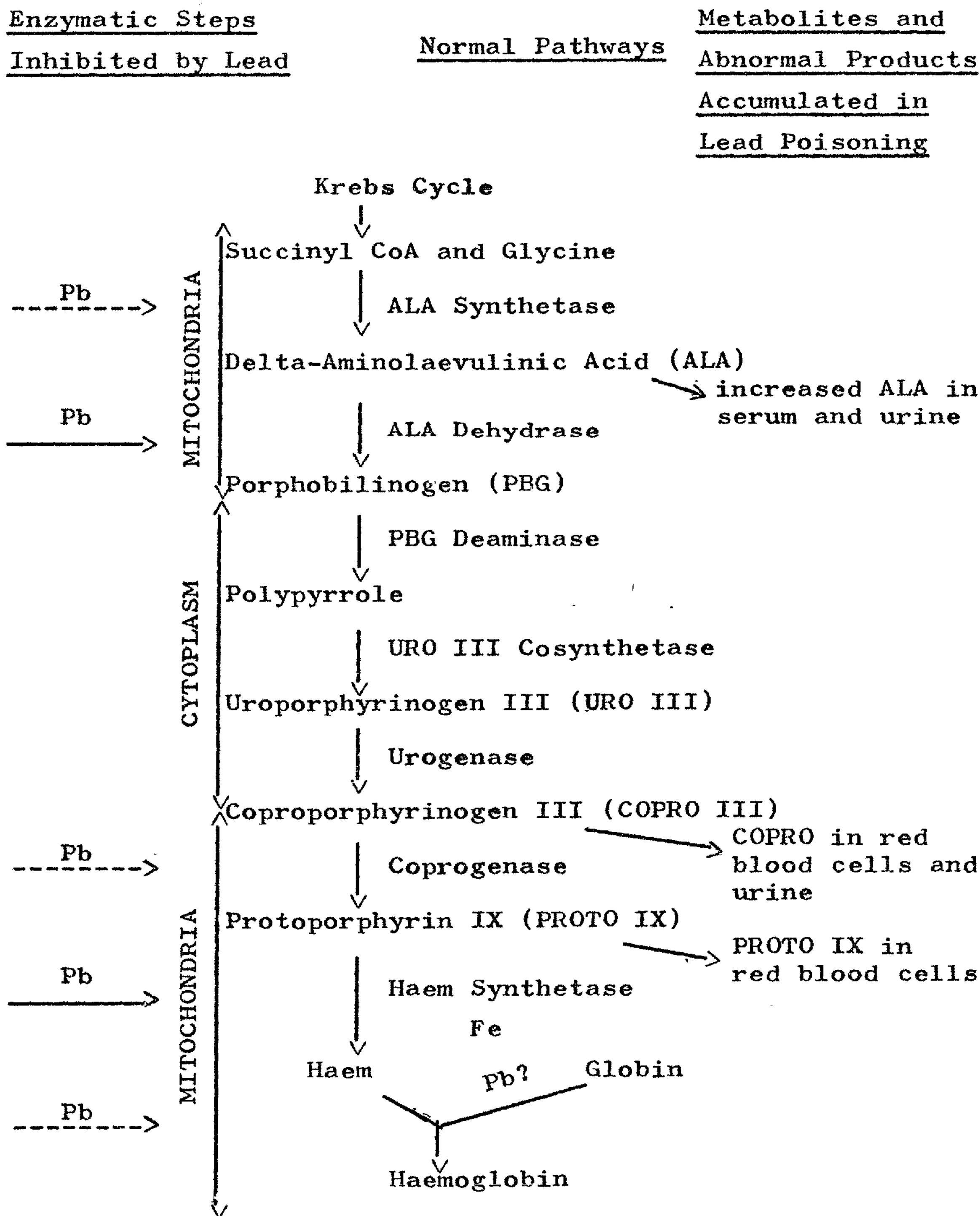
Current knowledge of the biosynthesis of haem is based largely on experimental studies. It has been reviewed by Lascelles (1964) and Goodwin (1968). The metabolic pathway shown in Figure 2 is the common pathway in mammals for the formation of haem. Lead inhibits the formation of haem at several points indicated in Figure 2. Evidence for these inhibitions comes from work reported by Lichtman and Feldman (1963), Chisolm (1964), Griggs (1964), Bessis and Jensen (1965), Waldron (1966) and Ulmer and Vallee (1969).

The results of disturbances of haem synthesis caused by lead are summarized in Table 1. The urinary excretion of delta-aminolaevulinic acid (ALA) and coproporphyrinogen III (COPRO III) is markedly enhanced in lead poisoning. There is also accumulation of non haem iron and protoporphyrin IX (PROTO IX) in erythrocytes and accumulation of ALA in the serum (Haeger-Aronsen, 1960; Feldman et al, 1969). Haeger-Aronsen (1960); Gibson et al (1968); Berk et al (1970) reported increased urinary porphobilinogen (PBG) and uroporphyrinogen III (URO III) in human lead poisoning only in cases of severe plumbism.

The transfer of iron from transferrin into human reticulocytes is partially inhibited (Jandl et al, 1966). However, electron microscope studies (Bessis and Jensen, 1965; Jensen et al, 1965) revealed the accumulation of non-haem

Figure 2

The Synthesis of Haemoglobin*



*Adapted from the National Academy of Sciences (1972).

Table 1

Changes in the Concentration of Porphyrins and their Precursors in Lead Poisoning*

	Blood			
	Erythrocytes	Plasma	Urine	Faeces
ALA		+	+	
PBG		N	N	
URO	±	N	±	N
COPRO	+	±	+	N
PROTO	+	±	N/±	N

N = Normal

ALA delta-aminolaevulinic acid

PBG porphobilinogen

URO uroporphyrinogen

COPRO coproporphyrinogen

PROTO protoporphyrin

*Adapted from Chisolm (1967); Waldron and Stofen (1974).

iron in the perinuclear mitochondria of developing erythrocytes in the form of "ferruginous micelles" which are responsible for the ringed sideroblasts characteristic of lead poisoning and dense aggregations of ferritin in damaged mitochondria.

The enzyme-dependent steps involved in the synthesis of haem which are most susceptible to high concentrations of lead are therefore the conversion of ALA to PBG, resulting in increased blood and serum concentrations of ALA and the conversion of PROTO IX to haem by the coupling of iron, resulting in high erythrocyte concentrations of PROTO IX. Some in vitro studies have suggested that there is inhibition at some stage before the formation of ALA but there is no evidence of inhibition in vivo (Morrow et al, 1969). Chisolm (1964, 1968) and Lascelles (1964) reported that both ALA and PROTO IX are substrates of SH-dependent enzymes, but COPRO III was apparently not. Thus the factors responsible for the increased urinary excretion of COPRO III in human lead poisoning have not yet been defined.

Although lead in high concentrations may inhibit a number of enzymes in the haem synthesis pathway that most specifically associated with lead posioning is the inhibition of ALA dehydrase (ALAD). The associated increase in ALA levels in both urine and serum is therefore a prominent feature of lead poisoning (Haeger-Aronsen, 1960). Nakao et al, (1968) and Hernberg et al, (1970) have shown that the logarithm of the activity of ALAD in haemolysates has an inverse linear relation with the concentration of lead in

whole blood in the range 5 and 95 µg/100g. They suggested that impaired ALAD activity in red blood cells was the earliest evidence of an adverse effect of environmental exposure to increasing concentrations of lead.

Goyer (1971) has proposed that the effect of lead on haem synthesis is a reflection of the action of lead on the mitochondria in haemopoietic cells. Many of the enzymes involved are located within the mitochondria and are dependent on normal mitochondrial function (Rimington, 1966). The studies of Bessis and Jensen (1965) suggest a correlation between damaged mitochondria and abnormal deposition of iron with defective haem synthesis in lead poisoning.

McRoberts (1973) reported that a shift in fractionated blood lead concentrations was associated with evidence of pharmacological or clinical intoxication. This step may represent a biochemical or biophysical trigger mechanism which induces a state of intoxication.

Renal System

The characteristic renal response to the lesions of acute lead poisoning resembles the Fanconi syndrome of aminoaciduria, glycosuria and phosphataemia (Clarkson and Kench, 1958; Chisolm, 1962). Goyer (1971) considered that the impaired functions of the kidneys would be largely accounted for by mitochondrial dysfunction. In the kidney, transport functions, particularly in the proximal tubular lining cells, depend on energy derived from oxidative phosphorylation in the mitochondria. Impairment of these functions could lead to many of the clinical signs observed.

At the histological level, the characteristic finding is the presence of intranuclear inclusion bodies in the cells of the proximal tubule. The nature of the inclusion bodies has been investigated by Castellino and Aloj (1969), Goyer and Krall (1969) and Goyer et al (1970). They indicated that the inclusion bodies represent an inert lead-protein complex. Goyer et al (1970) have proposed that the inclusion bodies represent a storage mechanism to protect renal cells from high concentrations of cytoplasmic lead. Moore et al (1973) have suggested that the protein involved in the lead-protein complex comes from the insoluble acidic fraction of nuclear proteins.

Goyer (1968) observed that intranuclear inclusion bodies were evident in rat renal tubular lining cells after 5 - 8 weeks on a 1% lead diet. They became progressively larger and more numerous and at 20 weeks aminoaciduria was observed. Choie and Richter (1972) however, found that a single dose of lead (0.05 mg/g body weight) given to rats induced characteristic inclusion bodies in the cells of the proximal tubule within 1 - 6 days. Reviews on the role and significance of inclusion bodies have been presented by Richter et al (1968) and Goyer et al (1970).

Aminoaciduria is considered to be a result of impaired reabsorption of amino acids by the proximal tubular cells (Goyer, 1968). Goyer et al (1970) and Six and Goyer (1970) have demonstrated that aminoaciduria does not occur until lead in excess of 1200 ppm is given in drinking water.

At 400 ppm however, intranuclear inclusion bodies had already appeared and these are considered to be the most sensitive index of lead intoxication. Urinary ALA and renal oedema were not significant until 1000 ppm was given (Goyer et al, 1970).

An additional consequence of the chronic renal injury due to lead is the syndrome of saturnine gout caused by lead interfering with urate excretion by the renal tubules (Emmerson, 1968; Ball and Sorensen, 1969).

Central Nervous System

Neurological symptoms attributable to lead poisoning have been recognized since antiquity. However, experimental information on the adverse effects of lead on the function of the nervous system is limited. Most of the information has been limited to clinical and post-mortem findings. While lead encephalopathy is not uncommon in lead poisoning in children it is now rare in adults. Little has been done, however, to gain an understanding of the pathogenesis of lead encephalopathy.

There are a number of experimental studies on animals but these may not be relevant to human poisoning because greater doses are required to produce encephalopathy and the clinical features and the lesions induced may differ. Pentschew and Garro (1966) reported that the lesions produced by feeding 4% lead to pregnant rats and their offspring include vascular damage, serous exudation and cellular proliferation in the central nervous system of the young rat. The pathology

of lead encephalopathy in the baboon was reported by Hopkins and Dayan (1974). The main findings were of widespread cerebral oedema and focal cortical necroses. Zook (1971) reported demyelination in the brain and spinal cord of lead intoxicated primates in zoos. Peripheral neuropathy involving axon degeneration and demyelination has been reported and can be produced in experimental animals (Fullerton, 1966). Acute amaurotic epilepsy was observed in non-human primates with lead poisoning (Zook et al, 1972).

Toxic Effects on Other Organs

A large number of diverse toxic effects have been reported in association with lead absorption and lead poisoning in both animal and human subjects. These include a teratogenic effect (Ferm, 1969); impaired thyroid acitivity in man and rats (Sandstead et al, 1969); impaired function of the pituitary-adrenal axis (Sandstead et al, 1970); increased abortion and infertility in humans (Hamilton and Hardy, 1949); ultrastructural changes in cardiac tissue (Asokan, 1974; Moore et al, 1975); decreased antibody formation (Koller and Kovacic, 1974); increased susceptibility to bacterial infections (Schroeder et al, 1965; Hemphill et al 1971); carcinogenic effects in the kidneys and other organs in rats and mice (van Esch et al, 1962; Roe et al, 1965; Mao and Molner, 1967; van Esch and Kroes, 1969; Oyasu et al, 1970; Goyer and Rhyne, 1973), and diminished body weight gain (Six and Goyer, 1970; Goyer, 1971).

Secchi et al (1971) reported marked increases in the serum levels of liver-specific enzymes such as sorbitol dehydrase consistent with liver damage. Both cytoplasmic and mitochondrial enzymes were increased while normal levels of lysosomal enzymes were observed. A recent study by Trejo et al (1972) indicated hepatic derangement in rats after the administration of lead acetate. Ultrastructural studies (Hoffman et al, 1972) revealed fine granular electron dense material in the mitochondria, endoplasmic reticulum and smooth membrane vesicles in Kupffer cells, liver parenchymal cells and splenic macrophages in lead poisoned rats.

Sources of Excessive Lead for Humans

Uncontrolled industrial exposure to airborne lead, the eating of lead-based paints by children with pica, and the drinking of illicitly distilled lead-contaminated whisky are responsible for most cases of overt clinical illness due to lead poisoning today. The burning of battery casings for fuel in the home and contamination of food and drink by improperly lead-glazed vessels have also resulted in severe illness. Sporadic cases of human lead poisoning have also been traced to lead-painted children's toys and furniture, lead toys eaten by children, lead nipple shields, white lead dusting powders, artists' paint pigments, lead dust in shooting galleries, soluble lead compounds in lead pipes, some patented "medications", jewellers wastes and lead type in schools for the blind (Barltrop, 1968; National Academy of Sciences, 1972).

Table 2

Metabolic Factors Influencing Uptake and Toxicity of Lead

1. Chemical and physical form of lead involved
2. Age
3. Season of the year - body temperature
   - dehydration
   - ultraviolet light and vitamin D
4. Dietary protein
5. Dietary fat
6. Dietary minerals
   - a) Calcium, Phosphorus
   - b) Zinc
   - c) Cadmium
   - d) Iron
   - e) Thallium
   - f) Sulphur
   - g) Copper
7. Dietary vitamins
   - a) Nicotinic acid
   - b) Vitamin B
   - c) Vitamin C
   - d) Vitamin D
   - e) Vitamin E
8. Alcohol
9. Milk
10. Lactose
11. Citrate
12. Chelating agents
13. Other dietary components
    - a) Alginates
    - b) Phytates
    - c) Pectins

Although it has been shown that various sources such as air, water and foodstuffs contribute to the total body burden of lead, the actual uptake of lead may be modified by several factors. The various factors which have been reported to influence susceptibility to lead uptake and toxicity are given in Table 2.

Chemical and Physical Form of Lead Involved

Lead exists in the environment in many different forms, lead sulphide or lead chloride in the mining industry, lead oxide in soil adjacent to smelters, lead chromate, lead carbonate and other lead salts in older paint. Today the paint industry uses lead octoate, naphthenate and tallate as driers. Although it has been suggested that different compounds of lead are not absorbed to the same degree from the gut or respiratory tract, earlier studies were conflicting and poorly documented (Fairhall and Sayers, 1940; Allcroft, 1950). Some evidence from the mining industry suggested that lead sulphide is less toxic than lead chloride. The absorption of lead from soils by plants has been related to the solubility of the lead compounds concerned in water (MacLean et al, 1969). Karhausen (1972) has also suggested that lead compounds which are soluble or able to be converted to soluble compounds in the gut are more readily absorbed.

The influence of particle size on the absorption of inhaled lead has not been extensively studied. Although Chamberlain et al (1975) have reported that most of the

deposited lead passed from the lungs to the blood plasma, they used only particles of one size (0.5 μm). Kehoe's (1961) study of the inhalation of lead sesquioxide used particles of two sizes (small particles of average diameter 0.5 μm and larger particles of average diameter 0.9 μm). He found that only 36% of the small particles was deposited and all the deposited particles were subsequently absorbed. By contrast, 46% of the large particles were deposited in the airways. However, 40% of these particles were subsequently removed to the gastrointestinal tract by ciliary action thus leaving 60% to be absorbed from the lungs.

By contrast, a recent study (Rendall et al, 1975) reported that the rate of absorption of lead into blood from coarse (mean diameter 1.6 μm) airborne particles is faster and reaches a higher level than from fine (mean diameter 0.8 μm) particles. This effect is probably due to the slow, continuous absorption of the larger particles from the alimentary tract added to the quick, intermittent absorption of the inhaled particles.

There was no early information on the influence of particle size on the absorption of ingested lead although it was believed that there was none. However, a recent study by Barltrop and Meek (1976) has revealed a striking relationship between particle size of metallic lead and absorption from the gut in rats. Lead absorption increased with a decrease in particle size. This increase was marked for particles below 75 μm but was less marked for larger particles.

Age

Since the introduction of improvements in industrial hygiene and occupational health standards in the lead industry, severe occupational lead poisoning has been reduced. In contrast, acute lead poisoning is now mostly seen in children between the ages of 2 and 5 years (Ingalls et al, 1961; Christian et al, 1964; Rennert et al, 1970). Two factors in childhood have a particular influence on the intake of lead and its significance for the child, namely growth and behaviour. Growth results in changes in the relative weights of organs and tissues in a non-uniform manner and this may influence the site of action and storage of absorbed lead. Growth and activity in children also result in increased metabolic needs for nutrients and oxygen from the diet and atmosphere. A behavioural characteristic of children is the chewing, sucking and ingestion of substances not normally regarded as food (pica). This may make them more prone to over-exposure to lead than adults (Barltrop, 1972).

Hardy (1966) has reviewed some of the possible explanations for the greater vulnerability of the young to lead. These include the vulnerability of young growing tissue and the greater variation in gastrointestinal acidity and alkalinity which might be more likely to dissolve and hence increase the amount of lead absorbed.

Kostial et al (1971a) reported that 5 to 7 day old rats retained 55% of a radioactive dose of lead 40 and 80 hours after feeding whereas adult rats retained 1%. Using carrier-free ^{212}Pb, Forbes and Reina (1972) also demonstrated that

age and weight affect lead absorption with immature rats absorbing more lead than do mature animals. Alexander et al (1972) have given a value of 50% absorption of lead from the gastrointestinal tract in children. This is 5 times greater than Kehoe's (1961) estimate of 10% for an adult man not occupationally exposed to lead. Thus the greater susceptibility of children appears to be due to a greater gastrointestinal absorption of lead. Other evidence, however, has suggested an alternative explanation.

Marked differences in the kinetics of lead retention and distribution were observed in suckling as compared to adult rats (Momcilovic and Kostial, 1974). There was increased retention in the whole body as well as an 8-fold increased concentration of lead in the brain of the suckling as compared to adults. The latter observation could partly explain the lead-induced encephalomyelopathy of suckling rats (Pentschew and Garro, 1966) and mice (Rosenblum and Johnson, 1968) with doses tolerated by the nursing mothers. Conversely, experimental lead toxicity studies (Kostial et al, 1974) showed that lead had a lower toxicity in young animals than in adult animals. Thus the greater risk of lead poisoning and more severe picture of lead poisoning seen in the young is more likely to result from a greater exposure to lead than an increased sensitivity of the immature organism.

Another possible explanation not considered extensively in the literature is the effect of nutritional factors in the apparent difference in response of children and adults

to lead. Barltrop (1969) has suggested that varying dietary patterns may play a role in the lead metabolism of children. Thus a relative deficiency of minerals or excessive fat and vitamin D intake may occur in childhood resulting in the modification of lead absorption.

Seasonal Variation

The incidence of lead poisoning varies with the season of the year with an increased incidence of lead poisoning in the summer (Baetjer, 1959). The reasons for this have been postulated to be increased vitamin D formation from the sun's ultraviolet irradiation and increased environmental temperature. Increased dietary vitamin D is known to increase lead uptake (Sobel et al, 1938). Blackman (1937) found that rabbits, chronically poisoned with lead, when placed at 37°C, died much sooner than those kept at room temperature. Germuth (1948) noted that lead-poisoned rabbits survived much longer in winter than in summer. Baetjer et al (1960) confirmed that mice injected with lead and exposed to a higher temperature (35°C), had a greater mortality and shorter average survival time than mice exposed to a lower temperature (22°C). The higher temperature resulted in an added burden of dehydration to the animals which then excreted less lead in their urine and faeces. However, Rapoport and Rubin (1941) have reported no effect of exposure to 40°C for 4 hours per day in rats fed a high lead diet.

The increased incidence of lead poisoning in the summer has been attributed to the influence of seasonal variation on nutritional and metabolic abnormalities which in turn influence the organism's response to lead toxicity (Goyer and Mahaffey, 1972).

Contrary to the clinical and experimental data, Kehoe et al (1943) found that during long term constant dietary intake of lead, accumulation in the body decreased in the summer months. They attributed this to increased losses of lead in urine, sweat and perhaps faeces.

Proteins

Proteins have been shown to increase the resistance of rats to lead toxicity (Baernstein and Grand, 1942; MacDonald et al, 1953). Gontzea et al (1964) also showed that a protein-deficient diet resulted in a 40 to 73% increase in lead uptake by liver, spleen and tibia of rats injected subcutaneously with lead acetate. The protein used in these 3 studies was casein. Other work has been reported using serum albumin to prevent lead poisoning (De Renzi and Ricciardi-Pollini, 1952; Odeschalchi, 1956; Merli, 1957; Biondi, 1959). By adding methionine and cysteine to the diet of rabbits receiving lead, there was increased resistance to intoxication (Buckup et al, 1956). Conversely, high protein diets will also result in increased intestinal absorption of lead in rats (Milev et al, 1970).

Fat

There have been conflicting reports on the effect of fat on lead absorption from the gut. Weyrauch and Necke (1933) and Sand (1965) thought that fat would form a complex with ingested lead and bile which would be more readily absorbed from the gut. However, Tompsett (1939) could not find any influence of dietary fat on lead absorption. Thus the use of whole milk or skimmed milk in lead prophylaxis has also been questioned. Tanquerel des Planches (1848) believed fat food would decrease lead uptake. Later workers, however, advocated the use of skimmed milk or diets low in fat (Buckup et al, 1956; Boyadzhiev, 1960; Zielhuis, 1960; Sand, 1965).

More quantitative work was provided by Weyrauch and Necke (1933) who showed that oil and margarine added to ordinary diets containing lead increased lead absorption 10-fold in rabbits. Recent work by Kello and Kostial (1973), however, reported no correlation between lead absorption and dietary fat content of 6 week old rats.

Minerals

Interaction of Lead with Calcium, Phosphorus and Vitamin D

Early studies (Sobel et al, 1938; Leaderer and Bing, 1940; Shields and Mitchell, 1941; Longley, 1967) suggested that calcium, phosphorus and vitamin D had marked influences on lead absorption and metabolism. Shields and Mitchell (1941) demonstrated that increased dietary calcium and phosphorus decreased lead absorption and decreased dietary

calcium increased lead uptake. An earlier study had associated increased vitamin D intake with increased gastrointestinal absorption of lead (Sobel et al, 1938). This effect of vitamin D was observed in diets containing low calcium (0.03%) and low phosphorus (0.246%), low calcium (0.03%) and high phosphorus (0.846%), high calcium (1.0%) and low phosphorus (0.246%). The lead content of bone ash and fat-free femora were all doubled in animals fed vitamin D.

Dietary levels of calcium and phosphorus influenced the deposition of lead in the bones of rats fed a diet containing 0.8% lead (Sobel et al, 1940). Diets containing low calcium (0.03%) and low phosphorus (0.26%) resulted in the greatest concentration of bone lead. The addition of either calcium or phosphorus to the low calcium-low phosphorus diet caused a decrease in lead deposition. These authors developed a theory that the deposition of calcium and phosphorus in bone is independent and that adequate dietary levels of calcium and phosphorus at a Ca/P ratio of between 1 and 2 were necessary to minimise lead retention. The addition of vitamin D to any of the diets studied resulted in enhanced lead uptake, thus confirming the earlier study (Sobel et al, 1938).

Recent work by Six and Goyer (1970) demonstrated that low calcium diets (0.1%) fed to rats ingesting lead acetate in drinking water resulted in enhanced lead concentrations in the blood, soft tissues and bone. Other symptoms of lead poisoning, including anaemia, increased urinary ALA

excretion, increased frequency and size of renal intranuclear inclusion bodies, increased kidney size and aminoaciduria, were also observed in the low calcium animals. Quarterman et al (1974) confirmed that rats on low calcium-low phosphorus diets had greatly increased amounts of lead incorporated into the body tissues but the uptake and release of lead were not related to the Ca/P ratio of the diet. Crawford and Crawford (1969) have shown that residents in hard water areas had diminished bone lead content compared with residents in soft water areas.

After lead exposure had ceased, addition of vitamin D to the diet produced a decrease in blood lead concentration and a decreased loss of bone lead (Sobel and Burger, 1955). Rapoport and Rubin (1941) have also reported a direct relationship between the incidence of lead poisoning in children and the level of sunlight and supplementary vitamin D. Recently, Thawley (1975) has shown a parallel increase in lead uptake as vitamin D concentrations in the diet changed from deficient to excessive.

Lead and Zinc

The interactions of dietary lead and zinc have not been extensively studied. There are conflicting reports of the effect of zinc on lead toxicity. The study by Hsu et al (1975) demonstrated that zinc potentiates lead toxicity in growing pigs. It was postulated that zinc prevents the normal assimilation of lead and calcium which, in turn,

enhances the absorption of lead. The observations of Willoughby et al (1972) are contrary to these findings. They reported that toxic amounts of zinc might prevent the development of clinical signs in the experimentally lead-poisoned young growing horse through the impairment of zinc metalloenzyme function. Thawley (1975) has also reported that addition of zinc to the diet of rats decreased lead levels of bone, kidneys, liver and blood.

Lead and Cadmium

The earliest study of the interaction of cadmium and lead was the work of Ferm (1969) who observed that cadmium potentiated the teratogenic effects of lead. Challop (1971) proposed that cadmium may play a role in childhood lead poisoning because of the presence of cadmium in old paint. He noted that many cases of childhood lead poisoning exhibited raised blood cadmium concentrations and increased renal implications possibly due to cadmium intoxication. In cases of renal disease associated with illicit alcohol consumption, elevated blood cadmium concentrations have also been reported. There appears to be a synergistic effect of lead and cadmium in these cases. A significant correlation between whole blood concentrations of lead and cadmium in children has been reported (Bogden et al, 1974).

Lead and Iron

The effects of experimental iron deficiency on the rat's susceptibility to lead toxicity have been studied by Six

and Goyer (1972). Urinary lead and delta-aminolaevulinic acid were elevated for the low iron diet. Retention of lead in bone was also tripled although soft tissue lead remained approximately the same as on the nutritionally adequate diet. There are differences, however, in the mechanisms by which calcium and iron deficiencies enhance susceptibility to lead toxicity. With the low calcium diet soft tissue lead was approximately 25 to 30 times that of the nutritionally adequate or iron-deficient diet (Six and Goyer, 1970). A low calcium diet therefore greatly modifies the distribution of lead, especially the partitioning of lead from bone to soft tissues.

A decreased intestinal absorption of lead in iron-supplemented rats has also been reported (Kochen and Greener, 1975). They demonstrated both in vivo and in vitro binding of lead by ferritin and postulated that binding of lead with ferritin leads to mucosal exfoliation and decreased lead absorption. However, the findings of Angle et al (1975) appeared to be inconsistent with the hypothesis that administration of iron supplements to correct iron deficiency in children will decrease blood lead concentration. They suggested that iron therapy at physiological levels may actually enhance lead absorption, possibly by facilitation of ferritin binding.

Lead and Thallium

A survey of blood lead and thallium concentration in children has shown no correlation between the content of the two metals in whole blood. It suggests that exposure to and/or absorption of these substances are different (Singh et al, 1975).

Lead and Sulphur

In ruminants the sulphur content of the diet influences the utilization of metals which form insoluble sulphides. Lead toxicity was markedly enhanced in animals receiving low sulphur diets (Morrison et al, 1975). In rats, the addition of sulphur amino acids to the diet did not produce any clear increase or decrease in gastrointestinal lead absorption (Quarterman, 1975).

Lead and Copper

The relationship between lead toxicity and the metabolism of copper was indicated in a report by Rubino et al (1958) which showed that there was an elevated copper content of red blood cells of patients suffering from lead poisoning. The elevated copper content was also correlated with an increase in red blood cell protoporphyrin content. A correlation between an increased lead level in grass with normal copper and molybdenum content and copper insufficiency in lambs was reported by Alloway (1969). Following this, Klauder et al (1973) found that dietary copper deficiency resulted in increased lead toxicity in male rats. There was also evidence that increased dietary copper markedly reduced the absorption of lead.

LEAD AND VITAMINS

Lead and Nicotinic Acid

In experimental lead poisoning, nicotinic acid synthesis may be impaired (Benko, 1942; Sales Vazquez, 1943; Acocella, 1966; Pecora et al, 1966). However, Tenconi and Acocella (1966) could not find any changes in tryptophan metabolism in rats fed a high lead diet. Kao and Forbes (1973) found that a ten-fold dietary excess of niacin provided no significant protection against toxic reactions from lead in terms of in vivo blood haem levels, in vitro haem synthesis or urinary aminolaevulinic acid.

Lead and Vitamin B

Beneficial effect of vitamin B_6 therapy during lead poisoning have been reported (Pokotilenko, 1964). Vitamin B_{12} and ascorbic acid treatment have also been applied to alleviate and reduce clinical and experimental lead poisoning symptoms (Holmes et al, 1939; Pillemer et al, 1940; Harada et al, 1955; Saita et al, 1955; Garminati, 1959; Dhar et al, 1960; Aldenazarov and Sabdenova, 1961; Lukidi, 1969).

Lead and Vitamin C

Holmes et al (1939) and Gontzea (1963) suggested the addition of large amounts of ascorbic acid to the diet of industrial workers as a means of alleviating symptoms of lead poisoning. In experimental lead poisoning, guinea pigs fed vitamin C-deficient diets were more susceptible to lead toxicity than animals fed diets adequate in vitamin C (Pillemer et al, 1940). However, other studies (Evans et al, 1943; Dannenberg et al, 1940) have reported no effects of vitamin C on lead toxicity.

Lead and Vitamin E

De Rosa (1954) claimed that vitamin E had a protective action against subacute lead poisoning in rabbits. This finding was confirmed by Levander et al (1975) who demonstrated that vitamin E deficiency enhanced the susceptibility of rats to the in vivo haemolytic effects of lead poisoning.

Lead and Alcohol

Cramer (1966) reported an association between the incidence of lead poisoning and alcohol consumption in lead workers. Lead poisoning has also been related with consumption of illicit alcohol (Morgan et al, 1966; Sandstead et al, 1970). Despite these clinical associations between lead poisoning and alcohol ingestion, little is known about the basis for the apparent synergism between alcohol and lead. There are similarities in the cellular pathology of lead and alcohol which means that alcohol could be enhancing the toxic effects of lead. Another possible mechanism is through the introduction of nutritional deficiencies by alcoholism which result in enhanced lead absorption. It is known, for example, that a high intake of alcohol is accompanied by lowered intake of nutrients in general (Mahaffey et al, 1974). Decreased intake of certain nutrients, including calcium, phosphorus (Sobel et al, 1938; Six and Goyer, 1970), iron (Six and Goyer, 1972) and proteins (Gontzea et al, 1964) have all been reported to increase

the absorption of lead. Experimental studies of rats fed isocaloric diets of controlled nutritional content (Mahaffey et al, 1974) showed that the synergistic effect of alcohol on lead toxicity is slight. It suggests, therefore that the apparent synergism between alcohol and lead is more likely to be due to the lowered intake of nutrients than a mutual enhancement of the closely related effects of these two toxins.

Lead and Milk

Weyrauch and Necke (1932) could not find any effect of milk supplements on lead absorption, confirming the similar studies of Aub et al (1926). However, other experiments on animals have demonstrated that milk can reduce considerably the absorption of lead from the gut of mice (Miyasaki, 1930; Tompsett, 1939), dogs (Horwitt and Cowgill, 1939) and rats (Ardelean et al, 1955). Buckup et al (1956) demonstrated that less lead was deposited in the skeleton of rabbits fed milk than in those not fed milk. The administration of milk to lead workers was recommended by Tanquerel des Planches (1848), Feissinger (1900), McKenna (1913), Hunter (1969) and Krook (1974). However, conflicting reports have been made on the use of milk as a prophylactic agent in human subjects.

Dizon et al (1950); Troisi (1950); Collier (1952); Travers et al (1956) and Schweigart (1957) considered that milk did have some value as a prophylactic agent but Vigliani (1954); Boyadzhiev (1960); Schiemann (1960) and

Lockhart (1963) considered that it did not. Experimental evidence that milk had no prophylactic effect against lead poisoning and that it may actually enhance lead toxicity has been provided by Wittgens and Niederstadt (1955). Their observations have been confirmed by Kello and Kostial (1973) who demonstrated that rats fed a diet with added milk absorbed more ^{203}Pb than those fed a milk-free diet. The milk was shown to be directly affecting lead absorption from the gut because it did not influence the absorption of lead injected into the peritoneal cavity.

These conflicting reports concerning the use of milk in lead prophylaxis have arisen because the experimental studies have not been uniform in the diets of tested animals, the modes of administration of lead and the total amount of lead given (Stephens and Waldron, 1975). They concluded that whole milk could not be regarded as a useful agent in lead prophylaxis because its overall effect was to promote the absorption of lead from the intestinal tract although it did contain components which might hinder lead absorption.

Lactose

Although it is well known that dietary lactose enhances calcium absorption (reviewed by Lengemann, Comar and Wasserman, 1957), no work has been done to assess the effect lactose may have directly or indirectly on lead absorption. It may be important since it is the most abundant substance in milk (4.8 g/100ml; Macy and Kelly, 1961) which makes up a substantial part of a child's diet.

Citrate

A number of authors (Kety and Letonoff, 1941; Shiels et al, 1950; Hardy et al, 1951; Moeschin and Schechterman, 1952; Suntych, 1953; Rossi et al, 1954; Shibata, 1957; Hsu and Yao, 1958; Mokranjac et al, 1958) have suggested the use of sodium citrate in lead prophylaxis because it increased urinary lead excretion. However, Masuda (1959) has found no therapeutic effects of potassium sodium citrate on lead-poisoned rabbits. The inhalation of aerosols of calcium sodium citrate and zirconium citrate had no protective effect against lead either (Schubert and White, 1952; Niemoller, 1957). Sano (1953) found that administering sodium citrate orally had no effect on urinary excretion of ingested or inhaled lead. This could be explained by the fact that ingested citrate would be rapidly metabolized in the body via the Krebs cycle. Schubert and Lindenbaum (1960) demonstrated this by inhibiting the Krebs cycle enzymes with monofluoroacetic acid so that there was an accumulation of citric acid. Then injection of small doses of sodium citrate did protect the treated rats against lead.

Chelating Agents

It has been reported that chelating agents, sodium citrate, D-penicillamine, calcium disodium ethylenediaminetetra-acetate and 2, 3-dimercaptopropanol, given orally caused an increase in the gastrointestinal absorption of lead (Jugo et al, 1975). The amount of lead retained in the body was also

increased by all the chelating agents studied except CaNa EDTA. The mechanism has been suggested to be the formation of a Pb chelate that is readily absorbed (Selander, 1967) from the intestine.

In a recent study, McClain and Siekierka (1975a) demonstrated that some lead-chelate complexes exhibited less foetotoxic and teratogenic effects as compared to the effects of the administration of an equimolar amount of lead alone. They went on to show that the membrane permeability and the placental transfer of lead could be increased with lead-chelate complexes (McClain and Siekierka, 1975b) but this factor was balanced by more rapid maternal elimination and faster termination of the foetal exposure to lead when present in its chelated form.

Lead and Other Dietary Components

Alginates

The addition of alginates to the diet of animals and children has resulted in a decreased uptake of strontium (Kostial et al, 1969; Sutton et al, 1971). A similar effect was observed in newborn rats (Kostial et al, 1971b) where alginate supplements to the diets resulted in a selective reduction of the absorption of lead but not of calcium. However, this effect of alginates was not observed in 7 to 8 week old rats fed solid food (Carr et al, 1969) or man (Harrison et al, 1969) although there was a slight reduction in lead absorption in rats fed a milk diet (Carr et al, 1969).

Phytates

It has been suggested that a high phytate intake may promote the uptake of lead by a secondary effect because it minimises calcium absorption (Stephens and Waldron, 1975). However, no studies have been conducted on the direct effect of a high phytate diet on gastrointestinal absorption of lead.

Pectins

Pectin injected into animals intoxicated with lead served as an effective chelating agent (Arkhipova, 1964). It was thus recommended that pectin-containing food should be given to lead workers. However, studies on the effect of orally ingested pectin on lead absorption have not been reported.

Clinical Study on Relationship of Diet to Lead Absorption

The only clinical study to determine the relationship of diet to lead absorption has been conducted in children (Mooty et al, 1975). They found that the average daily dietary intake of calories, protein and iron in both groups of children (controls with blood lead 10 to 25 μg/100ml) and subjects with blood lead greater than 50 μg/100ml) were not significantly different. They thus concluded that other non-dietary factors were contributing to the increased blood lead concentration of the subjects. Unfortunately, the daily dietary intake of calcium, phosphorus, vitamins and milk which might be expected to modify lead

absorption to a greater extent than calories, protein or iron were not assessed.

Although the studies reviewed above have drawn attention to the effects of various individual factors on lead absorption, their non-uniform experimental design have rendered it difficult to compare the relative effects of each factor. The studies have been conducted with animals of varying age and weight, varying modes of administering lead, varying compounds of lead, varying doses of lead and carrier-free isotopes of lead which may not necessarily behave like stable lead under all experimental conditions. These have therefore resulted in conflicting observations. Comparison would thus require careful control of the age and weight of the experimental animals, the mode of administration of lead, the chemical and physical form of lead supplied, the dose of lead and specific activity of any isotope used.

This work was started with these conditions in mind. Preliminary studies were conducted to select animals of suitable age and weight and fed lead chloride labelled with ^{203}Pb to a known activity which was then incorporated into solid food. The diets were compounded in the laboratory so that the exact composition of the diet was known and different nutritional factors could be varied.

A related aspect of lead absorption which has not been studied extensively is the mechanism of lead transfer through the intestinal wall. Although it is known that different factors affect lead absorption and toxicity, the mechanisms of their action are not understood. There have been some

studies to determine the mechanism of lead transfer through the intestinal wall using in vitro techniques.

Cikrt (1970) used everted intestinal sacs of rats to study the uptake of lead by the duodenal and ileal wall. He found increasing concentrations of lead bound on both the duodenal and ileal walls after 10 to 60 minutes of incubation. The amount of lead associated with and transported across the ileal wall was higher than the corresponding amount for the duodenum.

In contrast, Gruden and Stantic (1975) found that lead transport through and lead uptake by the gut wall was the same throughout the whole length of the gut. They also reported that there was no active transport of lead and no age dependence effect in 6 and 26 week old rats. An earlier study (Gruden et al, 1974) found that oral doses of lead acetate decreased the transfer of both calcium and strontium across the duodenal wall using everted gut sacs. However, the retention of both calcium and strontium in the intestinal wall was not affected by lead treatment.

Another study (Gerber and Derov, 1974) has reported that absorption of lead was higher in the jejunum than in the duodenum or colon using an in vivo preparation. Absorption was greater in 4 than in 10 week old rats and maintaining the animals on a low calcium diet decreased rather than increased lead absorption.

These conflicting reports are confusing because both in vivo and in vitro methods have been used. So more studies have to be conducted before the kinetics and mechanism of

lead transport can be elucidated. In vitro studies may not necessarily provide a valid model for an intact animal. It has been shown that zinc absorption is increased by using an in vivo method instead of everted gut sacs (Davies, 1973, 1975). Using a method in which the gut loop is left inside the body with its blood supply intact may result in more physiological responses (Davies and Nightingale, 1975). Thus a series of ligated gut loop experiments were conducted in animals of the same age and weight as in the feeding studies to determine the kinetics and mechanism of lead transport across the intestinal wall and the effect of various factors on this.

BIOCHEMICAL EFFECTS OF LEAD

Lead shows a strong affinity for ligands such as phosphates, cysteinyl and histidyl side chains of proteins, purines, and porphyrins. Consequently, lead may inhibit enzymes having functional sulphydryl groups, may induce catalytic activity in certain enzymes, for example, by substituting for zinc atoms at the active centre of bovine pancreatic carboxypeptidase A (Vallee and Ulmer, 1972), may modify the conformation of nucleic acids and may disrupt pathways of oxidative phosphorylation by inhibiting cytochrome oxidase (Mashakev and Verbolovich, 1967).

Subcellular Distribution of Lead

An understanding of the distribution of lead at the cellular level is essential to determine the basis of clinical lead poisoning. Some organelles within the cell seem particularly susceptible to lead, including mitochondria (Castellino and Aloj, 1969; Goyer et al, 1970; Barltrop et al, 1971), nuclei (Castellino and Aloj, 1969; Goyer et al, 1970) and microsomes (Castellino and Aloj, 1969; Barltrop et al, 1971). The binding of lead to the mitochondrial membrane may cause the inhibition of intramitochondrial enzymes of haem synthesis such as ALA synthetase, coproporphyrinogen oxidase, protoporphyrinogen oxidase and ferrochelatase (Moore, 1975).

Lead alters mitochondrial structure and cellular oxidative phosphorylation in the human placenta (Dawson et al, 1969), rat liver (Ulmer and Vallee, 1969), rat kidney (Goyer and Krall, 1969) and in corn mitochondria (Koeppe and Miller, 1970). In vitro, mitochondria bind and accumulate lead in

the absence of inorganic phosphate. However, the presence of ATP markedly increases the damage that lead causes to heart mitochondria (loss of cristae and impaired oxidative phosphorylation) in vitro in the presence of inorganic phosphate because of the formation of a Pb-ATP complex which renders more of the metal soluble (Parr and Harris, 1975a). Addition of dextran and sucrose result in a diminution of oxidative phosphorylation in mitochondria exposed to lead in vitro presumably by a similar mechanism of solubilizing lead phosphate (Parr and Harris, 1975b).

Lead binds to ferritin in vitro (Kochen and Greener, 1975) and in vivo, in the liver, spleen and kidney of lead-poisoned cattle (Russell, 1970) suggesting that iron storage may be altered in this condition. Lead can generate ferritin (Allen and Jandl, 1960) and abnormal deposits of iron, termed ferruginous micelles, may be detected in mitochondria (Bessis and Jensen, 1965; Jensen, 1965).

The intranuclear inclusion bodies containing lead which have been isolated from kidney tubular cells of chronically poisoned rats are also present in rabbits and man (Macadam, 1969; Goyer et al, 1970). They are known to contain protein because trypsin partially destroys their central core while RNAse or DNAse do not (Goyer et al, 1970). Calcium, iron, zinc, copper and cadmium are also found in the lead-protein complexes (Moore and Goyer, 1974).

Membranes

Decreased osmotic fragility and increased mechanical fragility of erythrocytes were among the earliest observations

in the field (Aub et al, 1926). However, the biochemical basis for these phenomena is still uncertain. Vallee and Ulmer (1972) have reviewed the various theories postulated for the interaction of lead with the red cell membrane. Damage to the red cell membrane may be caused by liberation of free acids, subsequent to the formation of a lead-inorganic phosphate complex with red cells or plasma. The formation of lead diglyceral sulphate and deposition of a peptide lead phosphate sol on the cell membrane may alter its structural integrity. In support of the hypothesis that lead binds to the red cell membrane, Hoogevoon (1970) has demonstrated that lead binds firmly to phosphatidylcholine membranes in vitro.

Contrasting evidence, however, has been presented by Barltrop and Smith (1971). Lead was found to bind to the cell contents rather than to the stromal material of human erythrocytes in vitro. Haemoglobin had a greater affinity for lead than erythrocyte stroma or membrane material. In addition, gel filtration showed the presence of lead binding to some low molecular weight material. Similar results were obtained by Bruenger and Stevens (1973) for beagle erythrocytes in vivo and in vitro. These authors reported that about 90% of the lead found in the red cells was associated with the cytoplasm and less than 10% was bound to the stroma. Further studies (Barltrop and Smith, 1975) suggested the existence of two binding sites in erythrocytes differing in their affinity for lead.

Incubation of human erythrocytes with lead in vitro causes leakage of potassium (Joyce et al, 1954). Hasan et al

(1967a) also found increased potassium loss in blood cells from men exposed to lead. This may be due to decreased activity of erythrocyte membrane Na^+/K^+ ATPase (Hasan et al, 1967b; Secchi and Alessio, 1969). Selhi and White (1975) have presented evidence that the spatial rearrangement of the red cell membrane proteins induced by lead poisoning is responsible for the inhibition of the erythrocyte membrane Na^+/K^+ ATPase. The incorporation of radiophosphate into phosphatidic acid in the red cells of lead poisoned humans and rabbits is decreased (Westerman and Jensen, 1975). It is significant that incorporation of radiophosphate into phosphatidic acid of normal red cells is sulphydryl-dependent and occurs at the cell surface. Lead also inhibits Na^+/K^+ ATPase from renal homogenates of lead-poisoned animals and thereby interferes with tubular sodium reabsorption (Secchi et al, 1969).

Proteins

Lead forms mercaptides with the -SH group of cysteine. Thus proteins with a large number of free -SH groups, such as thionein, will bind to lead firmly both in vitro and in vivo (Ulmer and Vallee, 1969). Lead has also been used to fractionate serum proteins by the formation of Pb-albumin and Pb-α-globulin complexes (Aoki and Mori, 1964). Although enzymes bearing a single functional -SH group are inhibited by lead, the concentrations of the metal required usually exceed those observed in tissues and fluids of lead intoxicated individuals. Certain enzymes, however, are inhibited by lower

concentrations of lead. Lipoamide dehydrogenase is inhibited by 6.5×10^{-6} M lead (Ulmer and Vallee, 1969) possibly due to the interaction with active-site dithiols (Massey and Veeger, 1961). Erythrocyte Na^{+}/K^{+} ATPase is also inhibited by lead (Hasan et al, 1967b) but cysteine reverses the inhibition.

Delta-aminolaevulinic acid dehydrase

The inhibition of delta-aminolaevulinic acid dehydrase (ALAD) in human erythrocytes by lead has been extensively studied and this has been proposed as a biochemical index for the identification of asymptomatic exposure to lead. An inverse correlation has been reported between the enzymatic activity of ALAD and blood lead content of lead workers (Tola et al, 1973) and of seemingly normal individuals who live in urban environments (Hernberg and Nikkanen, 1970). Others have extrapolated from these observations to predict the existence of unknown disease or clinically undiscernible lead toxicity at blood lead concentrations generally accepted to be within the normal range (Millar et al, 1970; Weissberg et al, 1971). Criticism of such extrapolations have been made for both biochemical and medical reasons by Vallee and Ulmer (1972).

Kinetic standards for the assay system of ALAD have not been established. Thus the K_m, V_{max}, pH optimum and other kinetic parameters for the human erythrocyte enzyme have not been recorded and the product or products generating colour

development with Erlich's reagent have not been identified. Temperature and pH affect the activity of the red cell enzyme both in vivo and in vitro in 'normal' and lead-exposed persons (Tomokuni, 1974; Chiba, 1976). This raises the possibility that a labile protein inhibitor modulates activity perhaps by allosterism.

The correlation of diminished enzyme activity with blood lead concentration would reflect any variability in the measurement of lead in blood. It is known that at the low concentrations of blood lead reported in these studies, measurement of lead in blood may yield inconsistent results. Conflicting information on the effect of lead on haem synthesis has been reported so that lead (10^{-7} M) stimulated rather than impaired haem synthesis in rabbit bone marrow (Pecora et al, 1965).

It has been assumed that inhibition of ALAD activity in human red cells is due to an excess of lead in blood. However, there has been no systematic examination of the enzyme's activity in a 'normal' population or in general medical disorders. For example, alcohol is known to inhibit ALAD activity (Moore et al, 1971). The possible role of other metals in the activity of ALAD has also been neglected although studies by Lauwerys et al (1973) and Roels et al (1975) showed no correlation between cadmium in human blood and ALAD activity. Zinc has been found to activate rat erythrocyte ALAD and to reactivate ALAD activity in lead-expsoed erythrocytes (Finelli et al, 1975). No data are available on the effect of zinc on the human erythrocyte enzyme.

The evidence available indicates that ALAD activity in erythrocytes from blood containing lead 10 μg/100 ml is reduced to one-third its activity at a blood lead concentration of 40 μg/100 ml. However, there is no evidence to suggest that this decrease as such has any significance for the health and well-being of the individual. Haem synthesis is not affected if ALAD activity is greater than one-third normal level (Zielhuis, 1972).

Contrasting evidence on the relation of blood lead concentrations to ALAD activity has been presented. Sakurai et al (1974) plotted means of log ALAD activity in workers against each 5 μg/100 ml increment in blood lead from 30 up to 65 μg/100 ml and found that the regression was not linear. In workers with blood lead values of less than 30 μg/100 ml, no correlation was found. The data suggested an inflection point in the range 25 to 30 μg Pb/100 ml. Moreover, in the range of blood lead 0 to 30 μg/100 ml, the ALAD in non-exposed controls was greater than in exposed workers.

Maxfield et al (1972) demonstrated that dogs fed with lead acetate (100, 500 and 1000 ppm in their diet) were similar to controls in their haematological functions, although blood ALAD values were depressed to 7% of pre-exposure values by the lead diet. After the dogs had been returned to normal diets (without added lead), ALAD activity was restored to normal pre-exposure values more quickly than the diminution of blood lead concentrations (Maxfield et al, 1975). Thus normal ALAD activity and elevated blood lead

concentration may co-existe at least soon after exposure. It was also found that some control animals which had not been exposed to lead had as little ALAD activity as animals whose ALAD had been depressed by lead. Sassa et al (1975) have also reported that there would be no effect on human red cell ALAD activity at blood lead concentrations lower than 15 μg/100 ml. These results were therefore in disagreement with the demonstration by Herberg and Nikkanen (1970), Millar et al (1970) and Weissberg et al (1971) of the absence of a no-effect level for the relationship between blood lead concentration and ALAD activity.

Other enzymes in the haem synthesis pathway (Fig. 2) may also be inhibited by lead. It inhibits the step prior to condensation of glycine with succinyl coenzyme A to form delta-aminolaevulinic acid (ALA). It is possible that lead inhibits the lipoic acid enzyme essential for conversion of citrate to succinyl coenzyme A via the Kreb's cycle (Brown, 1959). Lead may also interact with pyridoxal phosphate which is required for glycine activation in haem synthesis (Feldman and Lichtman, 1967).

ALA synthetase is inhibited by lead in chicken red cells but not in rabbit or guinea pig liver mitochondria (Vallee and Ulmer, 1972). In human erythrocytes and avian red cells, the conversion of uroporphyrinogen to coproporphyrinogen by uroporphyrinogen decarboxylase is inhibited (Dresel and Falk, 1956; Lichtman and Feldman, 1963). The conversion of coproporphyrinogen to protoporphyrinogen IX is inhibited by lead competing with iron (Ulmer and Vallee, 1969).

The incorporation of iron into protoporphyrinogen IX to form haem is also inhibited in human erythrocytes and rabbit ferritin (Dresel and Falk, 1956; Grinstein et al, 1959; Morgan and Baker, 1969).

From the available evidence, it is clear that lead may affect virtually all of the steps in the haem synthesis pathway. However, the degree to which each step is inhibited and the concentration of lead required varies considerably. Inhibition of ALAD and ferrochelatase (the enzyme involved in the incorporation of iron into PROTO IX) are consistent with observed accumulation and excretion of porphyrins and precursors in lead poisoned man and experimental animals. However, the rate-limiting enzyme in haem synthesis is ALA synthetase (Lehmann and Huntsman, 1974). Therefore, the inhibition of ALA synthetase would appear to be the more important cause of lead-induced anaemia than the inhibition of ALAD or ferrochelatase.

Globin Synthesis

There is some evidence that globin synthesis may also be modified by lead. Glycine incorporation into globin in duck erythrocytes (Kassenaar et al, 1957) and globin synthesis in rabbit reticulocytes (Waxman and Rabinowitz, 1966) are inhibited by lead. However, the inhibitory effect of lead can be largely overcome by haem (White, 1975). It is therefore possible that the major effect of lead is on haem synthesis which, via the formation of an inhibitor, modifies globin synthesis. There is some evidence that lead also acts

directly on globin synthesis. In vitro studies with reticulocytes indicated that lead is bound to the ribosome fraction of the cell and thus may have a direct effect at the level of translation of globin mRNA (White, 1975). If this is substantiated, then inhibition of globin synthesis by lead probably acts at two levels - the first, and major effect, is secondary to haem deficiency, and the second is probably a primary effect of lead per se on globin synthesis.

Other Enzymes

Data on the effect of lead on other enzymes is conflicting and reports of both inhibition and enhancement of the same enzyme have been presented. Enzymatic activities enhanced by lead in man include serum aldolase and catalase; those in animals include alkaline phosphatase, cytochrome oxidase, glucose-6-P-dehydrogenase, glutamic pyruvate transaminase, glutamic dehydrogenase, glutamic oxaloacetate transaminase, lactic dehydrogenase, sorbitol dehydrogenase and sterol 3-beta-ol-dehydrogenase. Enzymatic activities inhibited by lead in man include alkaline phosphatase, cholinesterase and carbon anhydrase; those in animals include acetyl cholinesterase, acid phosphatase, alkaline phosphatase, aminopeptidase, ATPases, cytochrome oxidase, diaphorase, fructose 1,6-diphosphatase, glucose 6-P-dehydrogenase, glutamic dehydrogenase and succinic dehydrogenase. These have been reviewed by De Bruin (1971), Vallee and Ulmer (1972) and Waldron and Stofen (1974).

Nucleotides and Nucleic Acids

It is not clear whether lead binds directly to nucleic acids or not, but protein synthesis is adversely affected by lead. Ulmer and Vallee (1969) reported that lead inhibits ^{14}C-leucine incorporation into E. coli tRNA, either by inhibiting the synthesizing enzyme or by binding to, and hydrolyzing tRNA. Lead will also cause disaggregation of polyribosomes in rabbit reticulocytes (Waxman and Rabinowitz, 1966) and cause diminished amino acid acceptance by tRNA (Farkas et al, 1972). Brief exposure of phenylalanyl and lysyl tRNA to lead inhibits their binding to ribosomes (Farkas et al, 1971). RNA from lead-poisoned horses contains substantial quantities of lead (>100ug/g) (Ulmer and Vallee, 1969). Chromosomal aberrations were increased in leukocyte cultures from mice fed on diets containing 1% lead acetate. Such changes however, may also occur in response to a variety of drugs, ionising radiation, deficiencies and infections (Muro and Goyer, 1969). Choie and Richter (1974) found that administration of 5 mg lead/kg body weight to mice stimulated the DNA synthesis of renal tubular cells.

Lead has been reported to increase the malformation rate in hamster (Ferm and Carpenter, 1967), rat and mice fetuses (McClain and Becker, 1970; McClain and Siekierka, 1975a). However, despite the evidence in laboratory animals on very high dosage of lead, teratogenic effects of lead have not been seen in cattle or sheep (James et al, 1966; Shupe et al, 1967).

MATERIALS AND METHODS

Selection of Animals

The rat was selected as the experimental animal as the absorption of lead in this species has been studied extensively by many workers. For each dietary variation 8 male Wistar rats of 100-110g body weight and age 30-32 days were used. Two animals were killed on the first day of each trial as controls to confirm that they had not been previously exposed to lead (Table A-2). The remainder were used as test animals and housed in individual cages. All-glass metabolic chambers (Fig. 3a and 3b) were used initally but were found to have no advantages over polythene and stainless steel cages.

Diets

Commercially available diets could not be used as the nutritional factors could not be varied individually. A synthetic diet was therefore used (Six and Goyer, 1970) in which each of the constituents of the diet could be varied independently and the caloric content of the varying diets estimated.

The synthetic diet was prepared to contain 20% casein, 28% sucrose, 33% corn starch, 3% cellulose, 5% corn oil, 5% molasses, mineral mix and vitamin mix (Table 3). The mineral mix was prepared from two separate mixes - a micromineral mix (comprising KIO_3, $(NH_4)_6 Mo_7O_{24}.4H_2O$, $CuSO_4$, $ZnCO_3$, $FeSO_4$, $MnSO_4$) and a macromineral mix (comprising NaCl, KCl, $MgCl_2$, $CaCO_3$, $NaH_2PO_4.2H_2O$). Cellulose or corn starch was added to the micromineral mix to facilitate dispersion of the

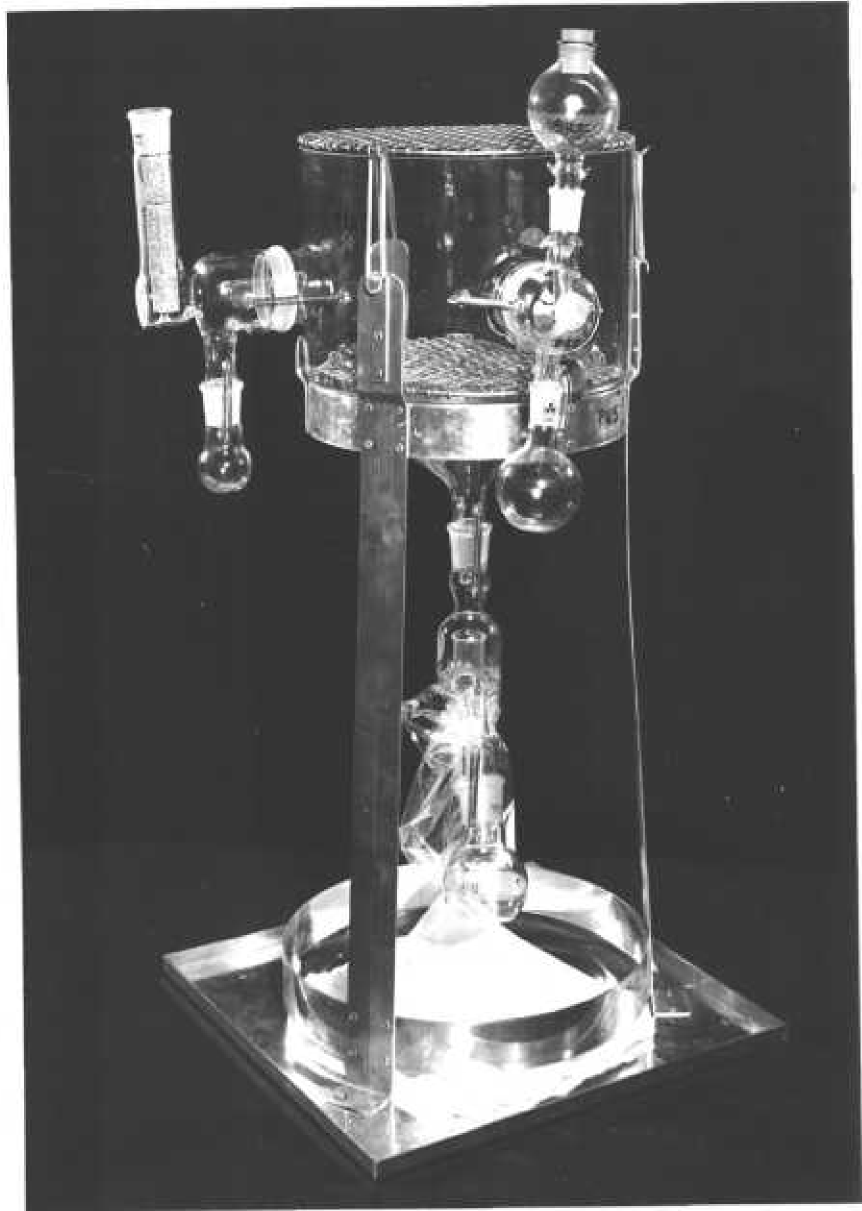


Figure 3a

All-Glass Small Animal Metabolism Chamber With Stick Feeder.

Figure 3b

All-Glass Small Animal Metabolism Chamber With Powdered Diet Feeder.

Table 3

COMPOSITION OF CONTROL DIET

			g
Protein	-	Casein	20.0
Fat	-	Corn Oil	5.0
Fibre	-	Cellulose	3.0
	-	Molasses	5.0
Carbohydrate	-	Sucrose	28.0
	-	Corn Starch	33.0
	*	Minerals	5.38
	±	Vitamins	0.62
			100.00

* The mineral mix provided the following quantities of nutrient in grams per kilogram of diet:-

$NaH_2PO_4.2H_2O$, 25.13; $CaCO_3$, 17.5; $MgCl_2.6H_2O$, 5.43; KCl, 3.43; NaCl, 1.27; $MnSO_4.H_2O$, 0.168; $FeSO_4.7H_2O$, 0.105; $ZnCO_3$, 0.050; $CuSO_4$, 0.020; $(NH_4)_6\ Mo_7O_{24}.4H_2O$, 0.005 and KIO_3, 0.0003.

± The vitamin mix provided the following per kilogram of diet:-

Vitamin A, 19,800 international units (I.U.) Vitamin D, 2,300 I.U.; Vitamin E, 7 I.U. choline chloride, 4.65g; Ascorbic acid, 991 mg; p-aminobenzoic acid, 110 mg; inositol, 110 mg; niacin, 99.1 mg; calcium pantothenate, 68 mg; menadione, 49 mg; thiamine hydrochloride, 22 mg; pyridoxine hydrochloride, 22 mg; riboflavin, 22 mg; folic acid, 1.98 mg; D-biotin, 0.44 mg and Vitamin B_{12}, 0.029 mg.

small amount of micromineral mix in the diet. The vitamin mix was prepared with added cellulose or corn starch.

When other nutrients were varied, the proportion of sucrose and corn starch were adjusted accordingly to give an approximately isogravic and isocaloric diet. The protein and fat content were varied by adjusting the content of casein and corn oil (Appendix C). Fibre was varied by the addition of different proportions of cellulose (Appendix D). Molasses were added to bind the other dietary components which were mainly in powder form.

Each diet was prepared in the following sequence. The calculated amounts of stable $PbCl_2$, macromineral mix, cellulose-micromineral mix and cellulose-vitamin mix were combined and mixed well with a mortar and pestle. Subsequently, casein, sucrose, corn starch and corn oil were added slowly in sequence to the mixture. Finally, the molasses were dissolved in 10ml warm deionised water and the appropriate volume of ^{203}Pb solution added to the solution which was then thoroughly mixed with the dry components. With the exception of diets containing fat in the range 15 to 40%, feeds were prepared by moulding into sticks (Fig. 4) and heating at 100°C for 20 minutes. These sticks were then fed to the experimental animals in 3 to 4 cm length portions. The high fat diets were fed in powder form to the rats kept in individual glass metabolic chambers. Each animal was given 30 to 35 g of feed for each 48 hour experimental period. Any residual diet after 48 hours was weighed and the lead ingested determined by difference.

Figure 4

Aluminium Food Mould

The diameter of each hole is 0.5 inches.

Trial Procedure

After each experimental period, the rats were killed by ether anaesthesia. Approximately 3 ml of blood was obtained by venepuncture and collected into heparinized vials. The radioactivity of the carcass was measured in a Packard Armac small animal whole-body counter. After counting, each carcass was dissected and the gastrointestinal tract, kidneys, liver and femur were removed. The lead content of residual food, carcass without the gastrointestinal tract, eviscerated carcass, whole liver, kidneys, and femur were determined by measurement of the gamma-emission of ^{203}Pb. Residual food, carcass and liver were counted in the Packard Armac whole-body counter. Specimens of blood, kidneys and femur were counted in a Hewlett-Packard Auto-Gamma counter. The ingested dose was determined by the difference between the food given and the residual food. Lead content of the carcass and the individual organs were expressed as percentages of ingested dose.

Evaluation of Synthetic Diet

In order to evaluate the effects on growth and palatibility of the synthetic diet, one group of rats provided with the synthetic diet was compared with another group fed with standard laboratory diet Oxoid 41B (See Appendix C for composition) ad libitum for one week. The weight of food and volume of water ingested as well as weight gain were measured (Table 4). No significant differences were observed in the amount of food or water consumed.

Table 4

Effect of Synthetic Diet on Weight Gain

	Synthetic Diet	41B	"t" Test (p)
No. of animals	5	6	
Food ingested daily	7.9 ± 0.1 g	7.2 1.1 g	N.S.
Water ingested daily	8.8 ± 0.4 ml	8.4 ± 1.5 ml	N.S.
Total weight gain	30.2 ± 3.3 g	16.5 ± 7.1 g	$<.005$

However, a significantly greater weight gain was observed in the animals receiving the synthetic diet. The reason for this difference may be due to the greater caloric content of the synthetic diet. The synthetic diet was fully accepted by the animals and no ill-effects were observed.

Selection of Cages

Initially, balance studies were planned using individual metabolism chambers. The chambers, mounted with either a stick feeder (Fig. 3a) or a powdered diet feeder (Fig. 3b) and a water bottle were designed to separate faeces and urine into different collecting vessels. In practice, it was found that instead of gnawing at the food in the feeder, the rat pulled the pelleted food into the chamber piece by piece. Powdered diets were similarly found to be unsuitable because the animal tended to remove food from the feeder into the metabolism chamber. In both cases the faeces and urine collected were contaminated with food particles. The all-glass chambers proved to be difficult to clean and more easily damaged. It was therefore decided to employ ordinary small-animal polythene cages with stainless steel covers in which the food was available to the rat only through the wires of the cage cover (Fig. 5). These cages had the advantage of being easily cleaned and dried. The amount of food ingested was estimated by measuring the amount given and the residual food at the end of the experimental period.

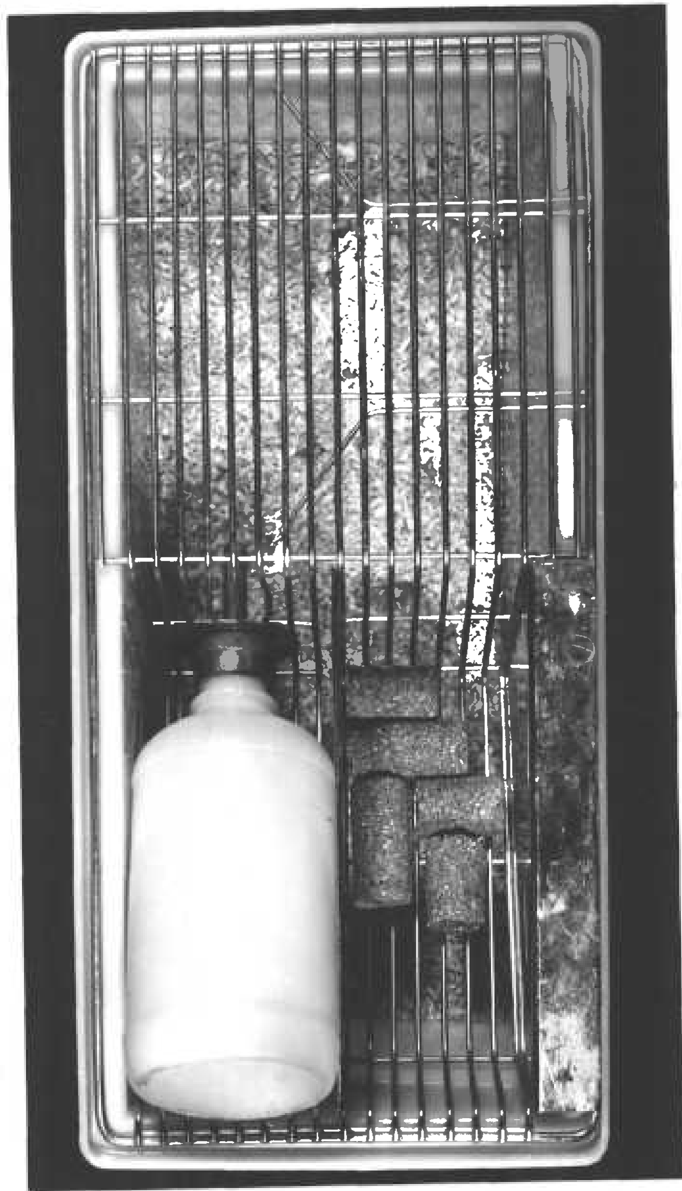


Figure 5

Polythene Cage Showing Arrangement of Food and Water Bottle

Preliminary Studies with ^{203}Pb

Preliminary studies were undertaken in individual animals, firstly by intraperitoneal injection and subsequently, by feeding. The purpose of these studies was to determine the distribution of the tracer and evaluate the counting techniques, and secondly to determine an appropriate specific activity for use in the studies.

The first study involved the use of a carrier-free dose of ^{203}Pb solution which was administered by intraperitoneal injection. The animal was sacrificed after 24 hours and the distribution determined in individual organs, i.e. kidney, liver, gastrointestinal tract and blood, as well as in the carcass before and after dissection. It was found that no suppression of counts occurred when the rat was counted intact. This finding was not unexpected because of the high energy of the gamma-emission from ^{203}Pb (279 KeV). However, it had to be verified because of the potential effects of absorption by the body tissues contributing differences into counting efficiency.

The percentage distribution of the ^{203}Pb (Table 5) showed that 51.6% of the initial dose was found in the eviscerated carcass. The relatively high percentage found associated with the gut (12.3%) may have been due to surface adsorption after intraperitoneal injection. The blood concentration (0.5%/g) was less than the other tissues studied but the fraction in the total blood volume (8ml/100g) of the animal would be greater than 4%.

Table 5

Distribution of ^{203}Pb after Intraperitoneal Injection

Organ	Percentage of Initial Dose after 24hr
Whole body without gut, kidneys and liver	51.6
Kidneys	18.4
Liver	7.5
Gut	12.3
Blood/g	0.5

Table 6

Distribution of ^{203}Pb after Feeding of ^{203}Pb (90 nCi/g of food) in the Diet

Organ	Percentage of Initial Dose after 48hr
Kidneys	0.16
Liver	0.15
Femur	0.10
Muscle	none
Heart	none
Spleen	none

The kidney fraction was 18.4% suggesting that the smaller mass of the kidneys had taken up more lead per unit mass. The percentage of the intraperitoneal dose in the whole carcass may partly be due to peritoneal adsorption.

The second study was concerned with the incorporation of labelled Pb into the diet. A dosage of 10nCi/g food was incorporated into the synthetic diet and fed to an animal for 48 hours. After sacrifice, the animal was dissected as before and the activity in individual organs determined in the Armac whole-body counter. It was found that with this dose, the counter was too insensitive to detect activities in any of the tissues studied. Therefore, a further study using 90nCi/g food was undertaken. The animal was prepared as before but this time the smaller organs (kidneys, femur, spleen, heart and muscle) were counted in a Hewlett-Packard Auto-Gamma counter. This proved to be more sensitive but the activities in the spleen, heart and muscle were still too low to be detected (Table 6). This dosage resulted in very high counts for the whole carcass in the Armac whole-body counter. Thus it was decided to select a dose of 100nCi/g food in subsequent experiments because it was sufficient to be counted accurately in the Armac and low enough to conserve the limited supplies of ^{203}Pb.

Appendix B gives the data for the efficiencies and counting windows of the Armac whole-body counter and Hewlett-Packard Auto-Gamma counter. A computer programme was written to carry out the necessary calculations.

Establishment of Standard Conditions

Dose

To establish a standard dose of stable lead to be used as a carrier in the diets, an experiment was designed to obtain a dose-response curve. 42 animals of age 30-32 days and body weight 100-110g were randomly allocated into groups of 6 and fed diets with lead chloride ranging in concentration from 0.03% to 1% for 48 hours. Tissue lead concentrations increased with increasing lead dose (Table 7, Figs. 6-10). The value of 0.075% was chosen since it gave an increase in tissue lead concentrations after 48 hours. It also lay on the most sensitive portion of the dose response curves. Although greater tissue lead concentrations could have been attained with greater doses, the value was kept as low as possible because greater concentrations of lead may not be of significance in practical nutrition.

Age and Weight

Forbes and Reina (1972) demonstrated that in rats aged 20 to 32 days, the absorption of ingested lead decreased from 90% to 16% with increasing age. However, the study was conducted with carrier-free lead so that their findings might not be applicable to larger doses. 42 rats aged 20 days were randomly allocated into groups of 6 and fed with control Oxoid diet 41B. At intervals in the age range 20 to 40 days, the control diet was withdrawn from successive groups and a diet containing 0.075% Pb was substituted 48 hours before sacrifice. Kidney lead concentration as a function of the

Table 7

Uptake of Lead from Diets of Varying Lead Concentration

Dietary Pb (%)	Whole body without gut (mg)	Liver (μg)	Kidneys (μg)	Blood/g (μg)	Femur (μg)
0.030	3.3 ± 0.8	374 ± 137	182 ± 58	14.4 ± 6.8	96 ± 27
0.075	7.3 ± 3.9	851 ± 241	371 ± 102	24.9 ± 7.0	205 ± 69
0.100	10.2 ± 4.8	951 ± 274	444 ± 153	38.1 ± 18.4	258 ± 217
0.200	16.8 ± 4.7	1630 ± 436	869 ± 196	52.8 ± 28.3	547 ± 171
0.500	25.9 ± 7.2	1760 ± 406	1142 ± 330	91.6 ± 27.7	660 ± 150
1.000	30.9 ± 8.9	3180 ± 463	1160 ± 461	96.5 ± 43.3	739 ± 248

Each value represents a mean ± S.D. (n = 6)

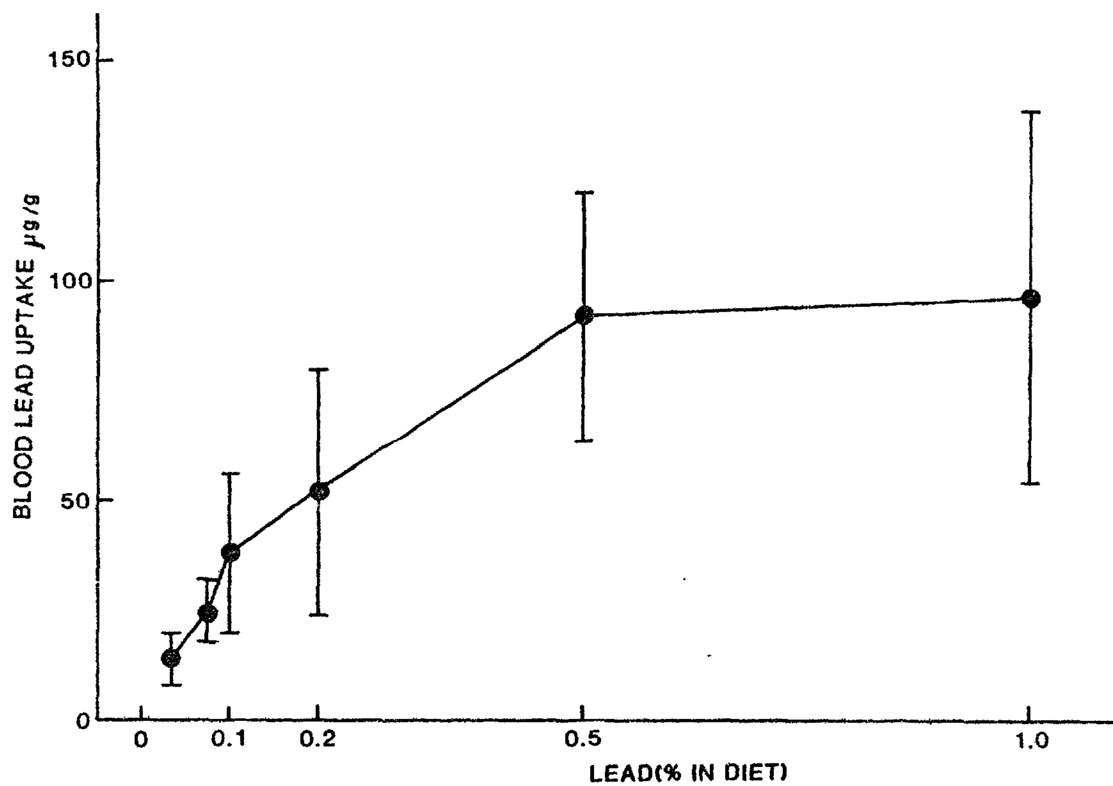
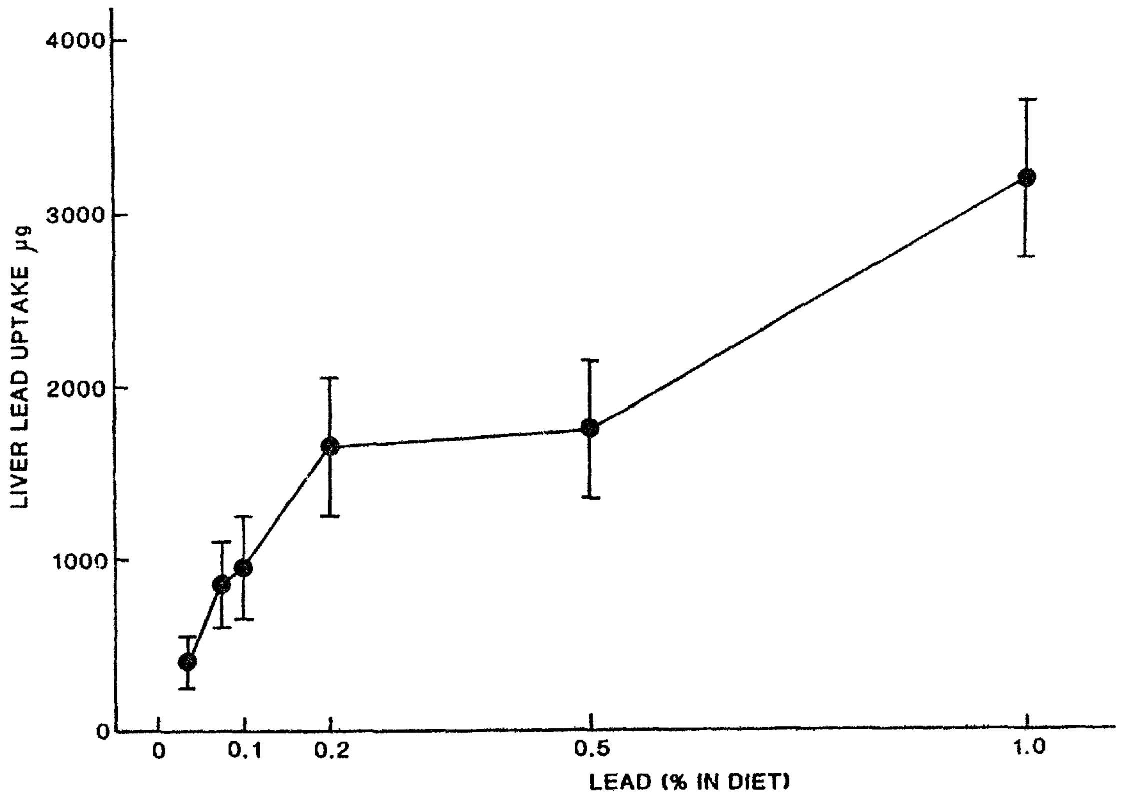


Figure 6

Mean Blood Lead Uptake ± S.D. for Diets of Varying Lead Concentration (n = 6).



<u>Figure 7</u>

Mean Liver Lead Uptake ± S.D. for Diets of Varying Lead Concentration (n = 6).

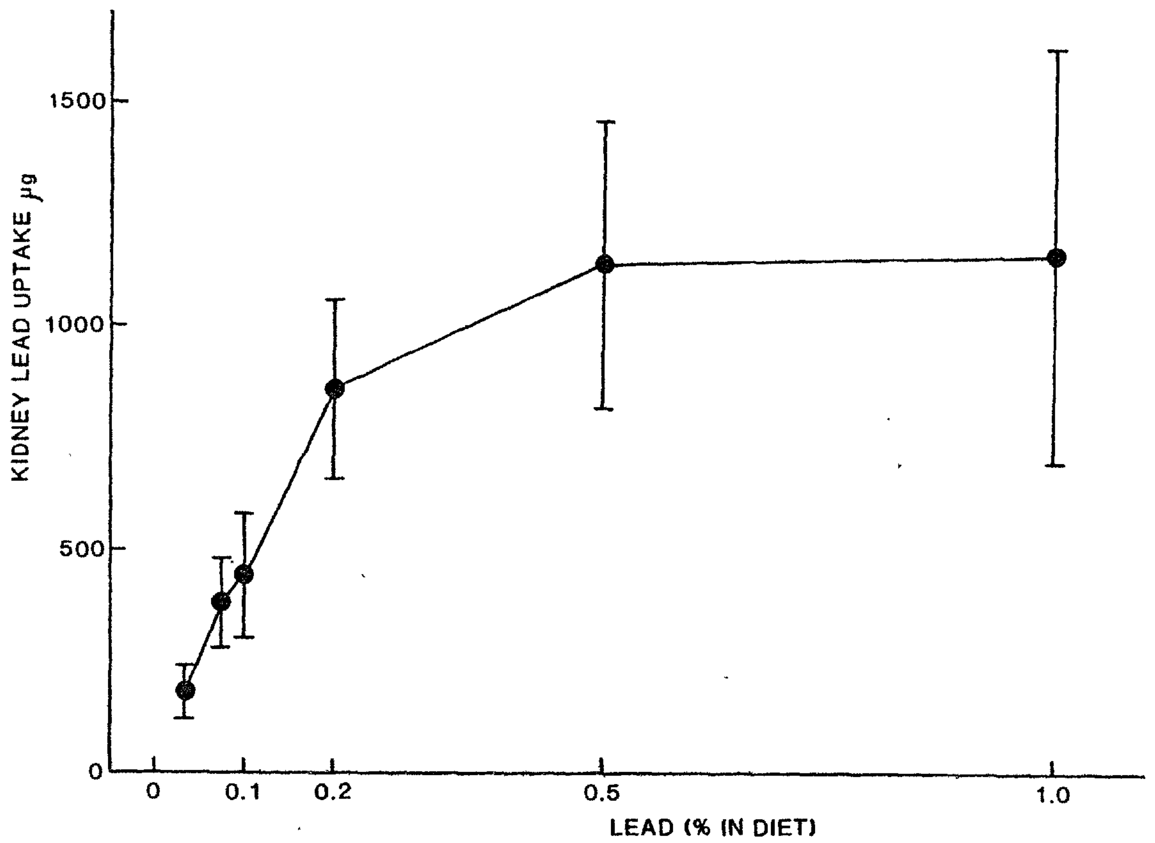


Figure 8

Mean Kidney Lead Uptake ± S.D. for Diets of Varying Lead Concentration (n = 6).

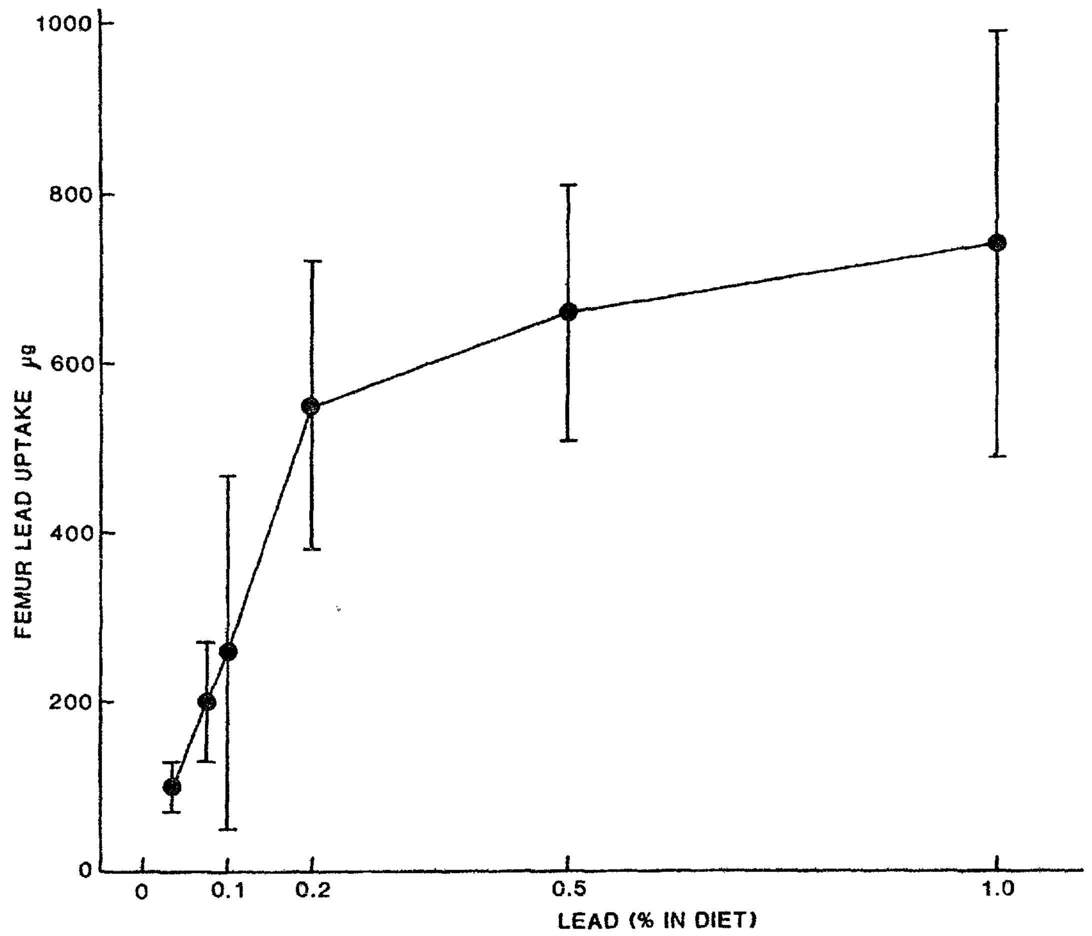


Figure 9

Mean Femur Lead Uptake ± S.D. for Diets of Varying Lead Concentration (n = 6).

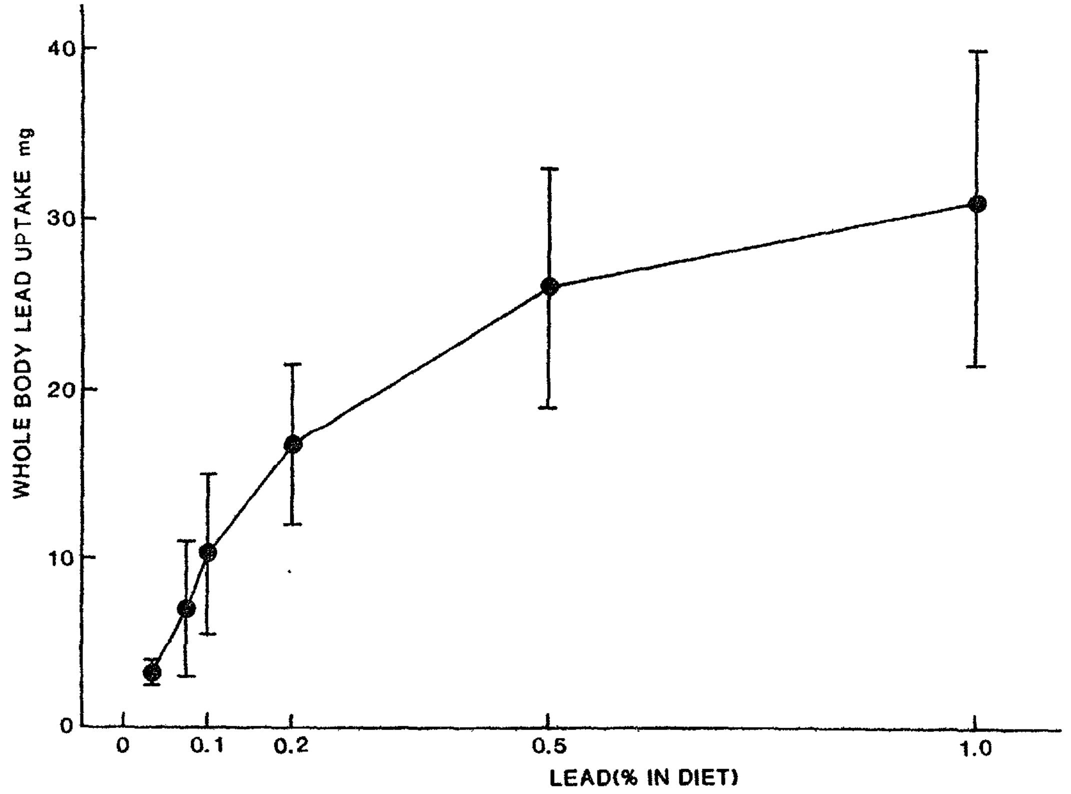


Figure 10

Mean Whole Body Lead Uptake ± S.D. for Diets of Varying Lead Concentration (n = 6).

ingested dose decreased from 10.82 x 10^{-2}% to 2.70 x 10^{-2}% between the ages of 20 to 40 days. Although this effect was small, rats aged 30 to 32 days were selected to minimize variation from this source (Table 8a, Fig. 11).

At a given age, the body weight may also influence lead absorption (Forbes and Reina, 1972). To verify this, 3 groups of 6 animals were selected so that 2 groups were of the same body weight but of differing ages and 2 groups were of the same age but differing in body weight (Table 8b). There was a significant difference between the blood lead concentration of 35 day old rats compared with 30 day old rats of the same body weight. The older and heavier (35 days, 89g) animals also had greater blood lead concentration than the younger and lighter (30 days, 74g) animals. No other differences were observed in blood or kidney lead content. The results suggest that only age affects lead uptake because the 2 groups of rats of the same age but differing body weight did not show any differences in blood or kidney lead concentration. Thus, to minimize any variation due to age and body weight, only animals aged 30 to 32 days and weighing 100-110g were used.

<u>Analysis of Stable Lead</u>

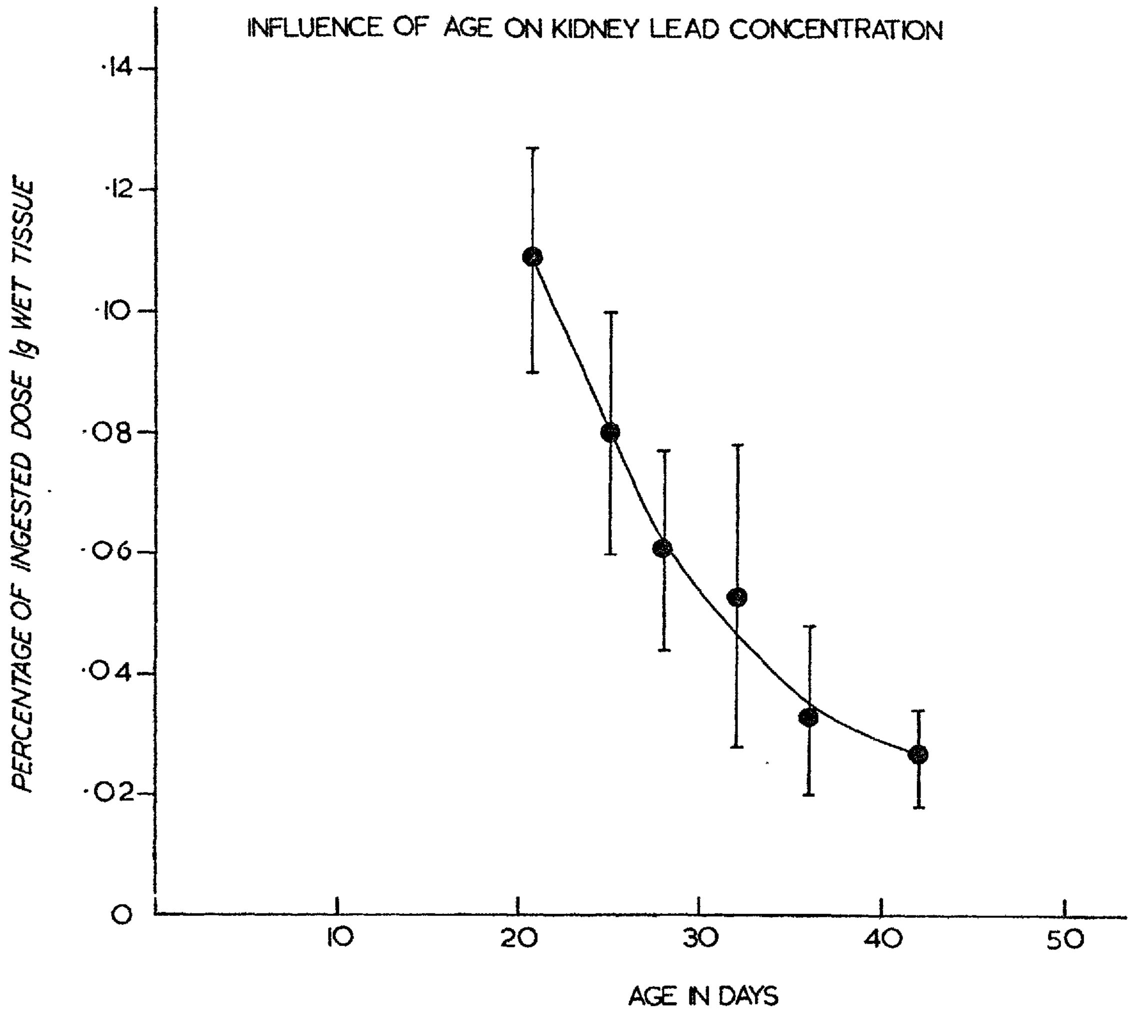
In trials where ^{203}Pb was not used, lead analysis was carried out using a semi-automated method (Browett and Moss, 1965). Blood and kidney specimens were wet-ashed and taken to dryness using 5% sulphuric acid, 50% nitric acid, and 35% perchloric acid digestion mixture on a sandbath at 350°C.

Table 8a

Effect of Varying Age on Lead Absorption

Age (days)	Body Weight (g)	Pb Ingested* (mg)	Total Kidney Pb* (μg)	Blood Pb* (μg/100g)	Total Femur Pb* (μg)
21	37 ± 4	13 ± 2	7.5 ± 2.2	57 ± 14	3.5 ± 1.9
23	49 ± 9	18 ± 4	8.7 ± 2.9	49 ± 7	5.4 ± 2.0
25	46 ± 3	16 ± 2	8.5 ± 1.2	69 ± 9	5.7 ± 0.9
28	54 ± 5	16 ± 2	7.7 ± 1.6	47 ± 10	5.8 ± 1.6
32	70 ± 11	18 ± 4	8.1 ± 1.6	44 ± 9	3.9 ± 1.0
36	110 ± 6	24 ± 1	10.3 ± 3.9	48 ± 10	5.1 ± 1.7
42	120 ± 4	24 ± 1	9.9 + 2.8	70 ± 20	4.7 ± 0.9

* Mean ± S.D. (n = 6).



<u>Figure 11</u>

Percentage Ingested Dose of Lead/g Wet Weight Kidney as a Function of Age for Groups of 6 Animals.

Table 8b

Influence of Age and Body Weight

On Lead Absorption

Weight (g)	Age (days)	Blood Pb μg/100g*	Kidney Pb μg/100g*
74.0 ± 3.1	30	28.2 ± 4.2 [a c]	7.1 ± 1.5 [d f]
90.0 ± 1.5	30	30.7 ± 7.6 [a b]	6.2 ± 1.2 [d e]
89.0 ± 2.7	35	45.6 ± 11.6 [b c]	6.0 ± 1.4 [e f]

*Mean ± S.D. (n = 6)

a, d, e, f, - no significant difference ("t" test)

b, c - $p < .001$ ("t" test)

Bones were dry ashed in a muffle furnace for successive periods of 20 hours at 450°C and 600°C. The ash was weighed and dissolved in 5ml concentrated hydrochloric acid and diluted to 10ml with deionised water. An aliquot was evaporated to dryness at 85-95°C. In each case the residues were taken up in the acid ammonium citrate buffer and analysed by means of the semi-automated dithizone method.

Ligated Gut Loops

Ligated gut loop experiments were conducted on groups of 6 male Wistar rats weighing 100-110g aged 30-32 days. All the rats were allowed food and water ad libitum because starvation might modify the transport of materials from the gut.

Each rat was anaesthetised with an intraperitoneal injection of pentobarbitone 6 mg/100g body weight (Nembutal, Abbott Laboratories, Ltd, Queensborough, Kent). The duodenum and jejunum were located through a mid-line incision and the bile duct ligated. A loose ligature was made around the gut 2 cm from the distal end of the stomach. 2.5ml of isotonic saline at 37°C was injected into the gut and the ligature tightened. The saline was moved along the gut gently for about 15 cm from the first ligature and a second ligature applied at this point. The ligatures were placed quickly and as close as possible to the duodenal wall without restricting blood flow to the intestinal loop. 1 ml of the perfusate (labelled $PbCl_2$ in saline or labelled $PbCl_2$ with other nutrients in saline) was then injected into the gut

loop at the proximal end through a no. 20 needle. After injection, the intestinal loop was returned to the abdomen with its blood supply intact and the skin incision closed. Absorption was allowed to proceed for a measured interval after which the rat was killed with pentobarbitone. The gut loop was removed, weighed and the length measured. The residual solution in the gut was collected and the gut flushed with 5ml warm, isotonic saline. The amount of lead transported was assessed from the loss of activity from the gut loop to carcass. The activity in the washed length of gut was also determined to measure the amount of residual lead associated with the gut wall. All measurement of gamma-emission were made in a Packard-Armac whole-body counter.

To establish a dose-response relationship, varying concentrations of lead, 1µg to 200µg/ml were introduced into the gut loop for 60 minutes.

A dose of lead (1µg/ml) was selected because it was at the lower end of the linear range in the relationship between the lead transferred across the gut wall and the lead concentration of the initial perfusate (Table 64, Fig. 33). A concentration of 1µg/ml is also a possible physiological dose assuming a 100g rat ingests an amount of lead relative to the 300µg ingested daily by a 70kg man. Incubation time was varied from 10 to 120 minutes. An incubation time of 30 minutes was selected as the standard duration of further experiments because it was convenient and was on the linear range in the relationship between the lead transferred across

the gut wall and incubation time (Table 65, Fig. 34).

Subsequently, calcium was added to 1 and 10µg Pb/ml and incubated for 30 minutes in order to assess the effect of calcium on lead absorption. The calcium concentration was varied from 100 to 1000 µg/ml.

The effect of phosphate on lead absorption was also studied using varying concentrations of phosphate (2.5mM to 10 mM) and lead (1, 5 and 10 µg/ml).

Inhibitors of oxidative phosphorylation (2, 4 dinitrophenol) and the sodium pump (ouabain) were added to 1µg Pb/ml to investigate the dependence of lead transport on the presence of ATP and the sodium pump.

A 15cm segment of the ileum was also studied for comparison with the 15cm duodenal-jejunal segment.

Preliminary studies to investigate the effect of vitamin D_3 on lead absorption were also initiated by introducing 25ng of 1,25 dihydroxycholecalciferol in ethanol intravenously 4 hours before measuring the transport of 1µg Pb/ml from the gut loop by the standard method.

<u>Statistics</u>

Standard statistical techniques were used in interpreting the data, namely Student's 't' test and the non-parametric Mann Whitney 'U' test for the significance of differences between lead uptake of given tissues.

RESULTS

The values for lead retention by animals fed on the control diet are given in Table 9. Whole-body retention was only 0.65% of the ingested dose compared to the value of 10% that has been reported for human adults (Kehoe, 1961) but agrees with the value of 0.7% for adult rats (Kostial and Momcilovic, 1972). In the organs, liver had the greatest retention of lead with progressively lesser values in kidney, bone and blood respectively. However, the fraction in the total blood volume (8ml in a 100g rat) and the total skeletal tissues of the whole animal would be considerable. The smaller mass of the kidneys means that this soft tissue takes up more lead per unit mass from the lead in food as well as from parenterally adminstered lead.

Calorie Intake

There was 2 -fold difference in lead uptake (expressed as percent ingested dose) by all tissues studied when the calorie intake of the animals was halved (Table 10). This implies therefore that the absolute amount of lead absorbed is the same. All further studies were thus conducted with the total calories ingested kept as constant as possible.

Diets of Varying Nutritional Content

The effects of various experimental diets on lead absorption and distribution among the organs are given in Table 11. All results which were not significantly different from the controls are presented with the experimental to

Table 9

Uptake of Lead from Control Diets

Organs	Pb in Organs As % Ingested Dose
Blood	0.20 ± 0.05*
Femur	2.08 ± 0.51*
Kidneys	2.82 ± 0.50*
Liver	6.62 ± 1.20*
Whole body without gut	0.65 ± 0.12

*$(\bar{x} \pm S.D.) \times 10^2$

<u>Table 10</u>

<u>Effect of Decreased Calorie Intake on Lead Absorption</u>

Food Ingested:	10-20g	5-10g			
Organ	% Ingested Dose $\bar{x} \pm$ S.D	% Ingested Dose $\bar{x} \pm$ S.D	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	4.46 ± 1.19	11.25 ± 5.82	<.01	<.002	2.5
Femur*	2.54 ± 1.14	7.83 ± 3.74	<.005	<.002	3.1
Liver*	10.04 ± 4.08	25.10 ± 14.20	<.02	<.02	2.4
Blood/g*	0.406 ± 0.144	0.989 ± 0.377	<.005	<.002	2.4
Whole body without gut	0.884 ± 0.372	2.377 ± 1.379	<.02	<.002	2.7

*$(\bar{x} \pm$ S.D.$) \times 10^2$

Table 11

Effect of Different Diets on Lead Absorption

(Ratio of Mean Retention, Experimental : Control)

Diet	Blood	Kidneys	Femur	Liver
Low Protein	5.1	2.5	2.8	2.2
High Protein	1	3.7	2.6	1
Low Fat	1	1	1	1
High Fat	9.6	7.6	4.8	4.2
Low Minerals	17.7	11.9	13.7	8.8
High Minerals	0.2	0.2	0.1	0.1
Low Fibre	1	1	1	1
High Fibre	1	1	1	1
Low Vitamins	1	1	1	1
High Vitamins	1	1	1	1

control ratio as 1. In the low factor diets, the nutritional factor concerned was omitted while in the high factor diets, it was increased 3 to 4-fold.

Low protein, high fat and low mineral diets increased, and high mineral diet decreased blood lead concentration (Fig. 12). The lead concentration of liver showed a similar relationship (Fig. 13). In the kidneys and femurs, however, a high protein diet was also associated with an increased lead content (Fig. 14 and 15). Low fat (Table 24 and 25), low fibre, high fibre, low vitamin, high vitamin and 10% lactose diets had no effect on lead absorption (Tables 12-15).

Protein

Detailed studies of varying dietary protein confirmed the findings in the preliminary investigations. Diets containing 10% and 15% protein had no significant effects on lead absorption (Table 18 and 19) but diets containing 0% and 5% protein increased the lead retention in all the organs studied (Table 16 and 17). Conversely, doubling the recommended protein content to 40% by weight resulted in greater lead uptake in the kidneys and a lower lead retention in the liver (Table 20). The lowered liver lead uptake was significant only by means of the Mann-Whitney "U" test. Increasing the protein content further to 50, 60 and 80% increased lead uptake in both kidneys and femur but did not produce any significant changes in the blood and liver uptake compared to controls ingesting 20% protein (Tables 21-23). Figures 16 to 19 illustrate the effects of dietary

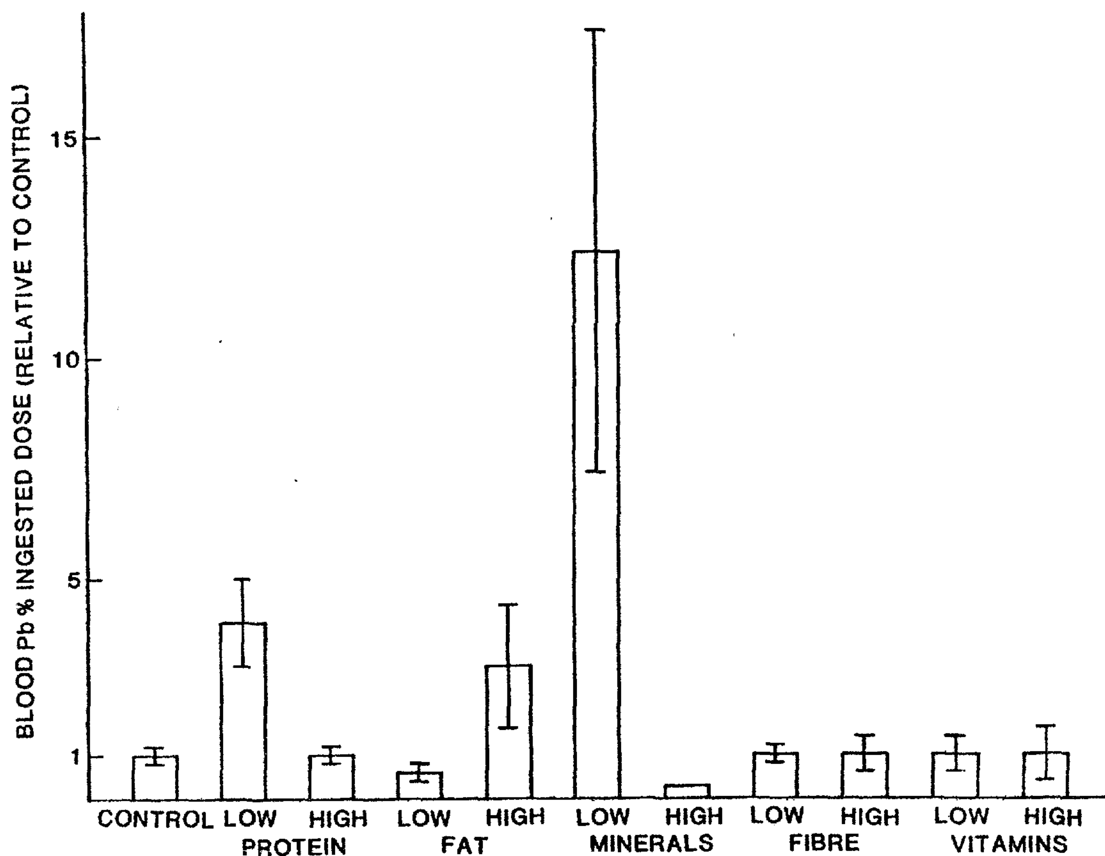


Figure 12

Mean Blood Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Composition Relative to Control Diet (n = 6).

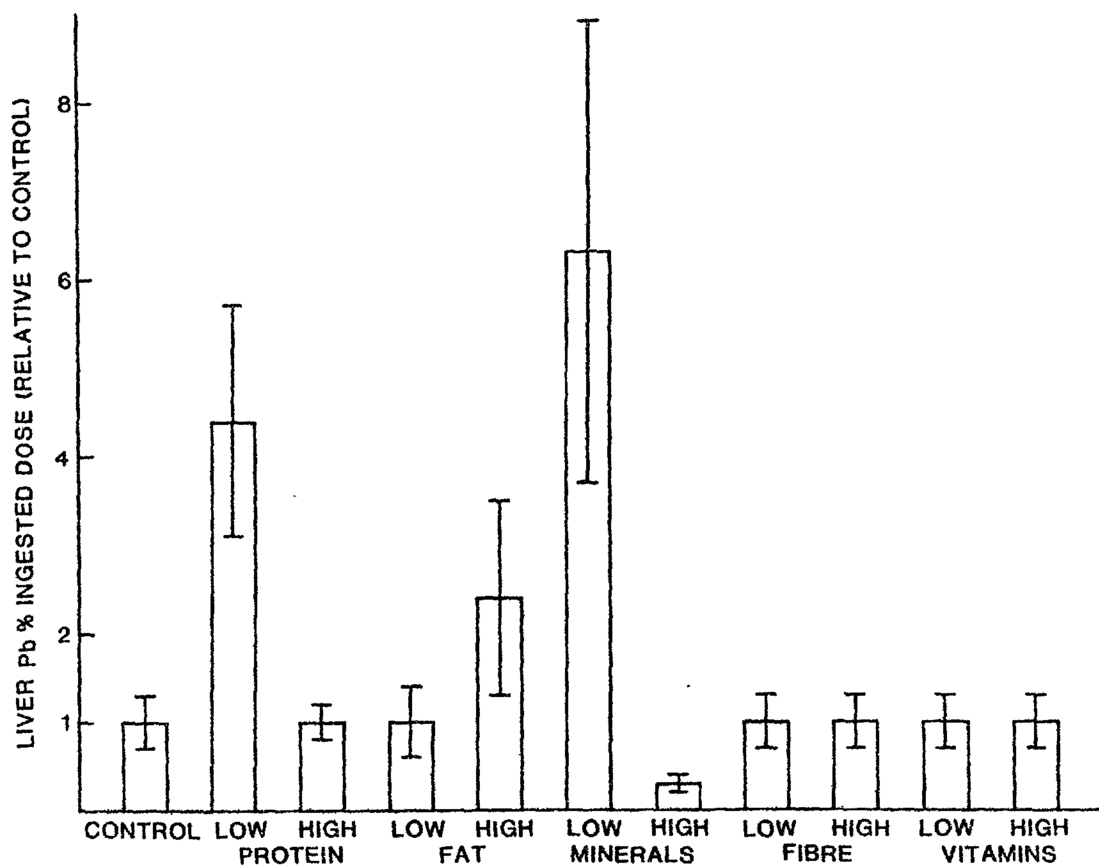


Figure 13

Mean Liver Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Composition Relative to Control Diet (n = 6).

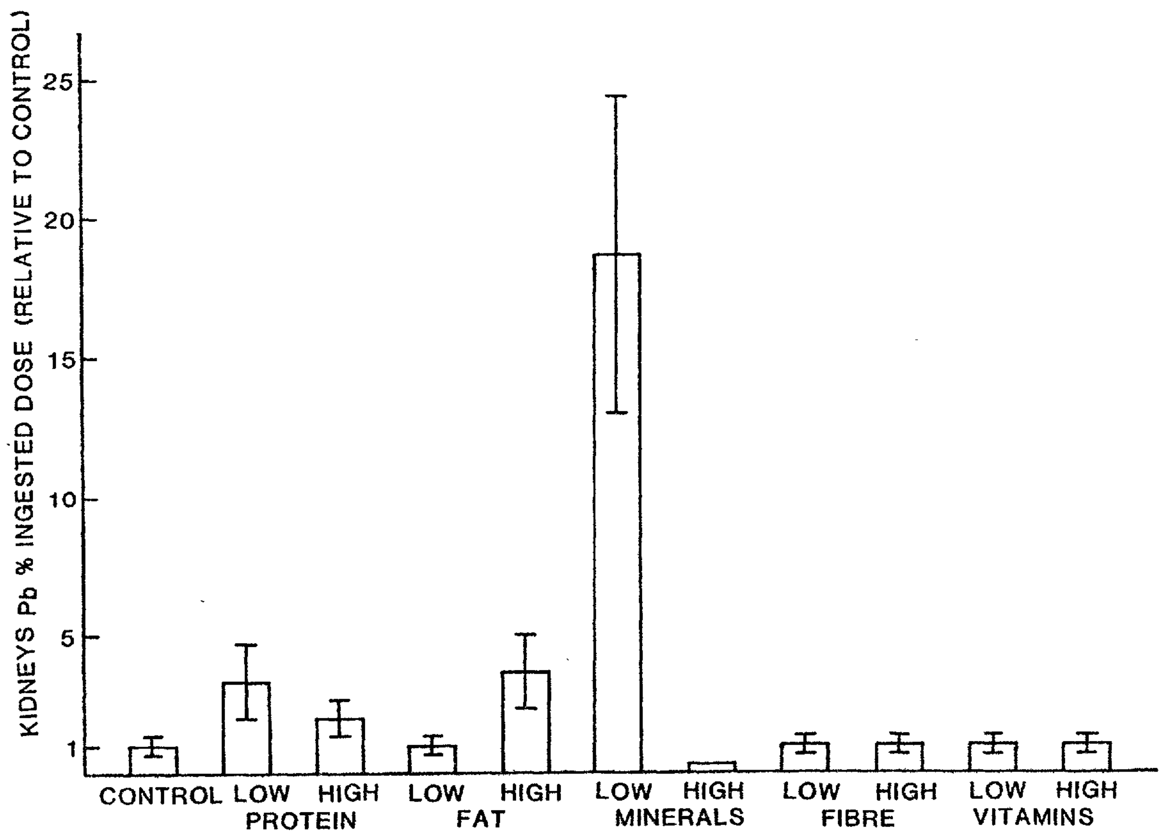


Figure 14

Mean Kidney Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Composition Relative to Control Diet (n = 6).

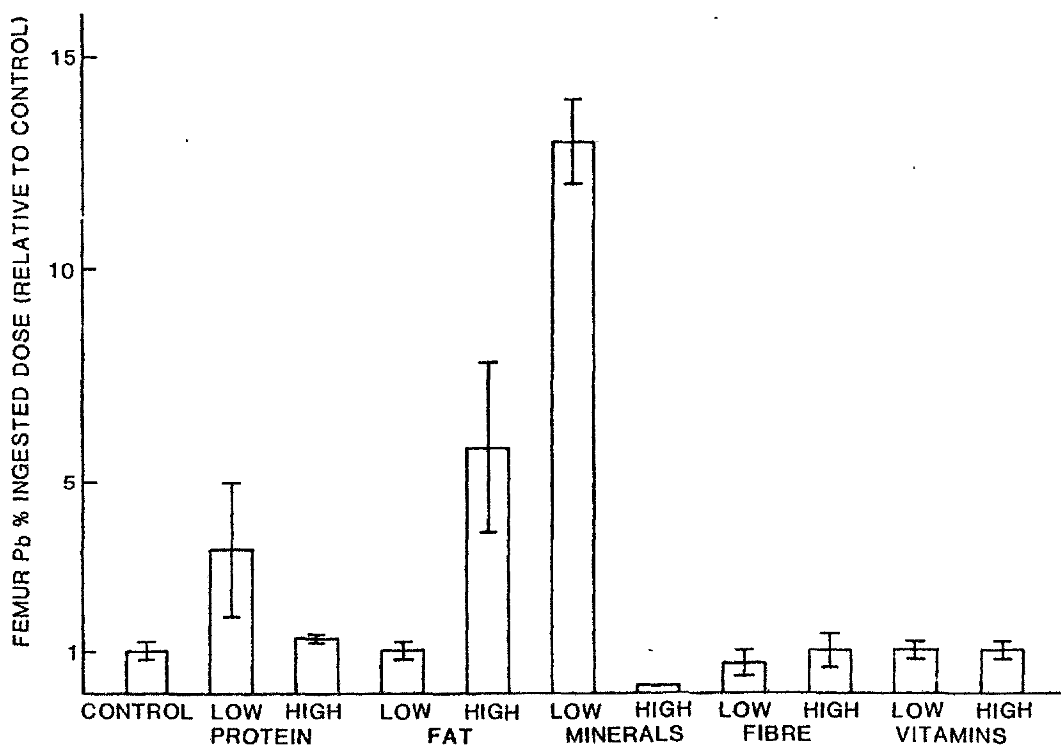


Figure 15

Mean Femur Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Composition Relative to Control Diet (n = 6).

Table 12a

Uptake of Lead from Low Fibre Diet

Organ	Control (3% Cellulose)	Low Fibre (0% Cellulose)	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	6.43 ± 3.48	5.70 ± 1.48	N.S.	N.S.	-
Femur *	2.25 ± 0.72	1.53 ± 0.56	N.S.	<.03	0.7
Liver *	7.66 ± 3.70	7.31 ± 2.04	N.S.	N.S.	-
Blood/g *	0.470 ± 0.350	0.340 ± 0.090	N.S.	N.S.	-

Table 12b

Uptake of Lead from High Fibre Diet

Organ	Control (3% Cellulose)	High Fibre (12% Cellulose)	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	2.78 ± 0.61	3.32 ± 1.40	N.S.	N.S.	-
Femur *	1.51 ± 0.66	1.48 ± 0.66	N.S.	N.S.	-
Liver *	5.14 ± 1.45	5.75 ± 1.91	N.S.	N.S.	-
Blood/g *	0.323 ± 0.098	0.225 ± 0.095	N.S.	<.02	0.6

* $(\bar{x} \pm S.D.) \times 10^2$

Table 13

Uptake of Lead from Low Vitamin Diet

Organ	Control (0.62% Vitamin)	Low Vitamin (0% Vitamin)	"t" Test (p)	"U" Test (p)	Test/Control
Kidneys*	2.23 ± 0.57	2.98 ± 0.79	N.S.	N.S.	-
Femur*	1.10 ± 0.27	1.24 ± 0.40	N.S.	N.S.	-
Liver*	4.84 ± 0.95	6.86 ± 0.31	N.S.	N.S.	-
Blood/g*	0.173 ± 0.052	0.184 ± 0.078	N.S.	N.S.	-

Table 14

Uptake of Lead from High Vitamin Diet

Organ	Control (0.62% Vitamin)	High Vitamin (2.48% Vitamin)	"t" Test (p)	"U" Test (p)	Test/Control
Kidneys*	1.78 ± 0.85	2.19 ± 0.62	N.S.	N.S.	-
Femur*	0.79 ± 0.50	0.80 ± 0.31	N.S.	N.S.	-
Liver*	4.02 ± 2.28	3.34 ± 1.08	N.S.	N.S.	-
Blood/g*	0.106 ± 0.078	0.119 ± 0.038	N.S.	N.S.	-

* $(\bar{x} \pm S.D.) \times 10^2$

Table 15

Uptake of Lead from 10% Lactose Diet

Organ	Control (28% Sucrose)	Lactose (10% Lactose 18% Sucrose)	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	4.62 $\pm$ 1.80	5.84 $\pm$ 2.92	N.S.	N.S.	-
Femur *	2.92 $\pm$ 1.28	3.11 $\pm$ 1.33	N.S.	N.S.	-
Liver *	15.52 $\pm$ 5.21	9.48 $\pm$ 4.44	N.S.	N.S.	-
Blood/g *	0.342 $\pm$ 0.167	0.397 $\pm$ 0.264	N.S.	N.S.	-
Whole body without gut	0.972 $\pm$ 0.320	0.871 $\pm$ 0.349	N.S.	N.S.	-

*$(\bar{x} \pm \text{S.D.}) \times 10^2$

Table 16

Uptake of Lead from 0% Protein Diet

Organ	20% Protein (Control)	0% Protein	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	2.06 ± 0.60	5.10 ± 2.62	<.01	<.002	3.4
Femur*	1.35 ± 0.57	3.76 ± 2.03	<.01	<.004	3.4
Liver*	5.20 ± 2.06	11.55 ± 4.07	<.001	<.002	4.4
Blood/g*	0.125 ± 0.042	0.633 ± 0.389	<.001	<.002	4.0

Table 17

Uptake of Lead from 5% Protein Diet

Organ	20% Protein (Control)	5% Protein	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	2.06 ± 0.60	5.72 ± 3.23	<.005	<.002	2.8
Femur*	1.35 ± 0.57	4.43 ± 3.10	<.005	<.02	3.3
Liver*	5.20 ± 2.06	15.38 ± 7.72	<.001	<.002	3.0
Blood/g*	0.125 ± 0.042	0.485 ± 0.393	<.01	<.002	3.9

*$(\bar{x} \pm S.D.) \times 10^2$

Table 18

Uptake of Lead from 10% Protein Diet

Organ	20% Protein (Control)	10% Protein	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	2.06 ± 0.60	3.10 ± 2.53	N.S.	N.S.	-
Femur*	1.35 ± 0.57	1.97 ± 1.62	N.S.	N.S.	-
Liver*	5.20 ± 2.06	7.16 ± 4.79	N.S.	N.S.	-
Blood/g*	0.125 ± 0.042	0.286 ± 0.265	N.S.	N.S.	-

Table 19

Uptake of Lead from 15% Protein Diet

Organ	20% Protein (Control)	15% Protein	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	2.06 ± 0.60	1.74 ± 0.75	N.S.	N.S.	-
Femur*	1.35 ± 0.57	1.11 ± 0.75	N.S.	N.S.	-
Liver*	5.20 ± 2.06	4.33 ± 2.88	N.S.	N.S.	-
Blood/g*	0.125 ± 0.042	0.137 ± 0.056	N.S.	N.S.	-

*$(\bar{x} \pm S.D.) \times 10^2$

Table 20

Uptake of Lead from 40% Protein Diet

Organ	20% Protein (Control)	40% Protein	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	5.99 ± 2.60	11.73 ± 4.70	<.05	<.004	2.0
Femur *	4.20 ± 1.57	5.41 ± 1.64	N.S.	N.S.	-
Liver *	14.32 ± 6.01	7.67 ± 4.52	N.S.	<.03	0.5
Blood/g *	0.622 ± 0.383	0.359 ± 0.246	N.S.	N.S.	-

*($\bar{x}$ ± S.D.) x 10^2

Table 21

Uptake of Lead from 50% Protein Diet

Organ	20% Protein (Control)	50% Protein	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	18.54 ± 3.20	36.11 ± 10.18	<.01	<.002	2.0
Femur *	9.87 ± 3.10	13.07 ± 1.20	<.05	<.009	1.3
Liver *	23.73 ± 2.22	21.71 ± 3.37	N.S.	N.S.	-
Blood/g *	1.220 ± 0.520	1.130 ± 0.190	N.S.	N.S.	-

Table 22

Uptake of Lead from 60% Protein Diet

Organ	20% Protein (Control)	60% Protein	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	5.99 ± 2.60	21.88 ± 3.02	<.001	<.002	3.7
Femur*	4.20 ± 1.57	10.92 ± 2.93	<.001	<.002	2.6
Liver*	14.32 ± 6.01	21.41 ± 7.67	N.S.	N.S.	-
Blood/g*	0.622 ± 0.383	0.710 ± 0.339	N.S.	N.S.	-

$*(\bar{x} \pm S.D.) \times 10^2$

Table 23

Uptake of Lead from 80% Protein Diet

Organ	20% Protein (Control)	80% Protein	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	5.99 ± 2.60	15.07 ± 4.33	<.005	<.002	2.5
Femur *	4.20 ± 1.57	6.25 ± 1.58	<.05	N.S.	1.5
Liver *	14.32 ± 6.01	11.16 ± 3.86	N.S.	N.S.	-
Blood/g *	0.622 ± 0.383	0.431 ± 0.119	N.S.	N.S.	-

protein content on lead retention in the blood, liver, kidneys and femur respectively.

Fat

Data showing the relationship between tissue lead and varying dietary fat are given in Tables 24 to 29. Decreasing the dietary fat content from 5% to 0 and 2.5% (Table 24 and 25) did not affect the lead retention of any of the tissues studied. By contrast, increasing the fat content to 10% (Table 26) resulted in a 2-fold increase in retention in all the tissues studied by means of the "t" test although the Mann-Whitney 'U' test resulted in a significant increase for the whole body only. Further increases in the dietary fat content enhanced lead uptake in all the tissues studied. Tables 27 to 29 present the results of 15, 20 and 40% fat diets on lead uptake. The greatest increases were observed with the 40% fat diet which increased lead uptake 14-fold in the kidneys, 11-fold in the femur, 7-fold in the liver, 14-fold in the blood and 9-fold in the carcass (Table 29). The enhancement of lead uptake was greatest in the kidneys and blood for all the increased fat diets. Fig. 20 to 24 illustrates the effect of varying dietary fat content in the blood, liver, kidneys, femur and carcass respectively.

Fats of Varying Composition

Since increased dietary fat was shown to enhance lead absorption, further studies were undertaken to identify any particular fat fractions which might be significant in this

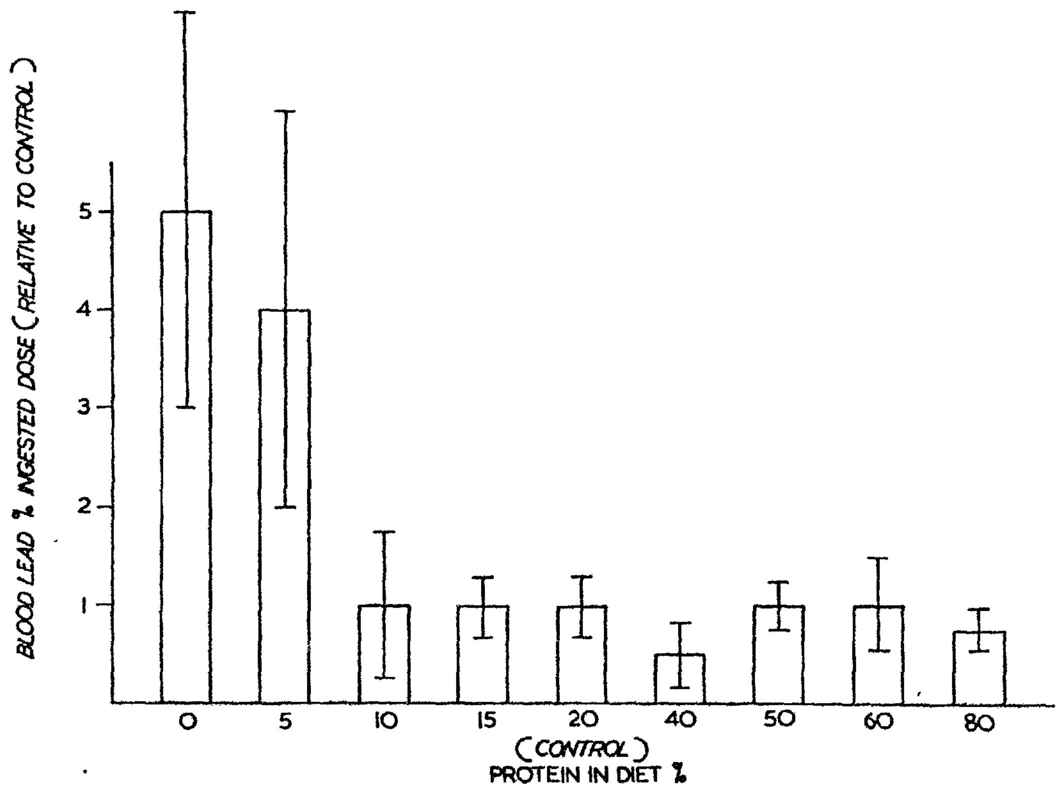


Figure 16

Mean Blood Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Protein Composition Relative to Control Diet (n = 6).

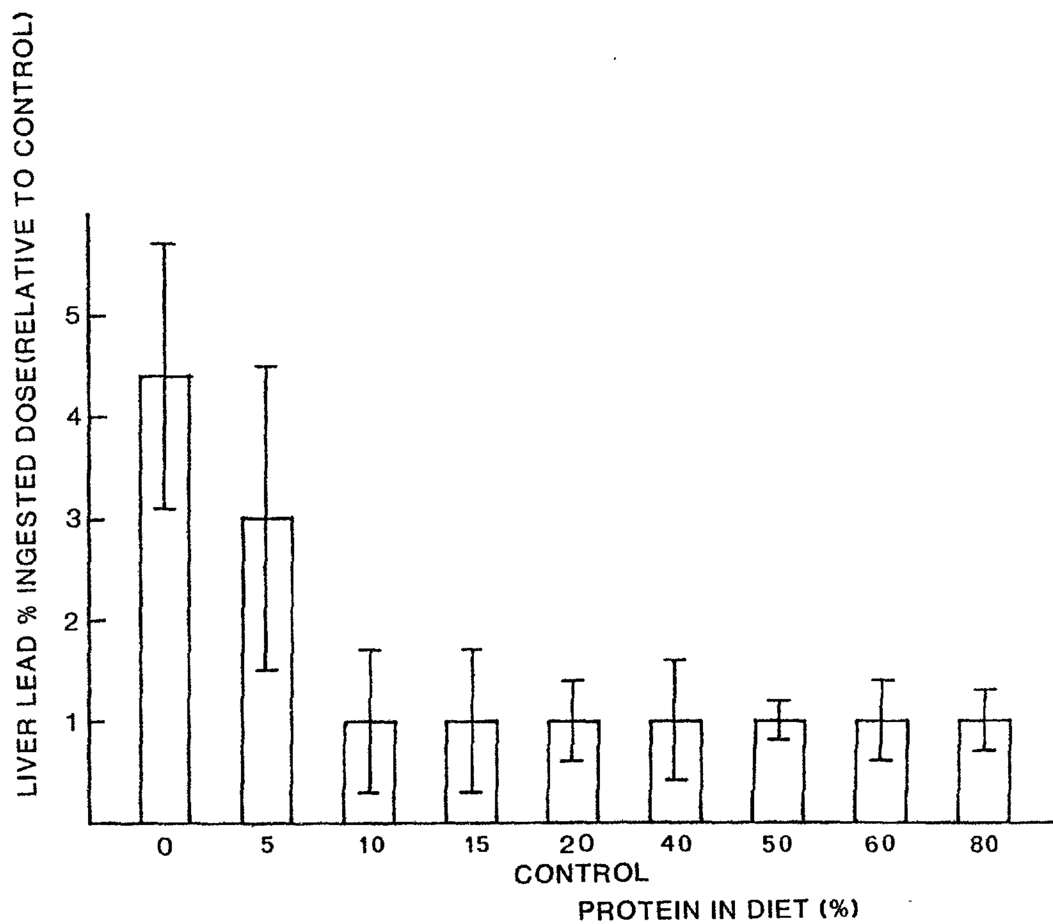


Figure 17

Mean Liver Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Protein Composition Relative to Control Diet (n = 6).

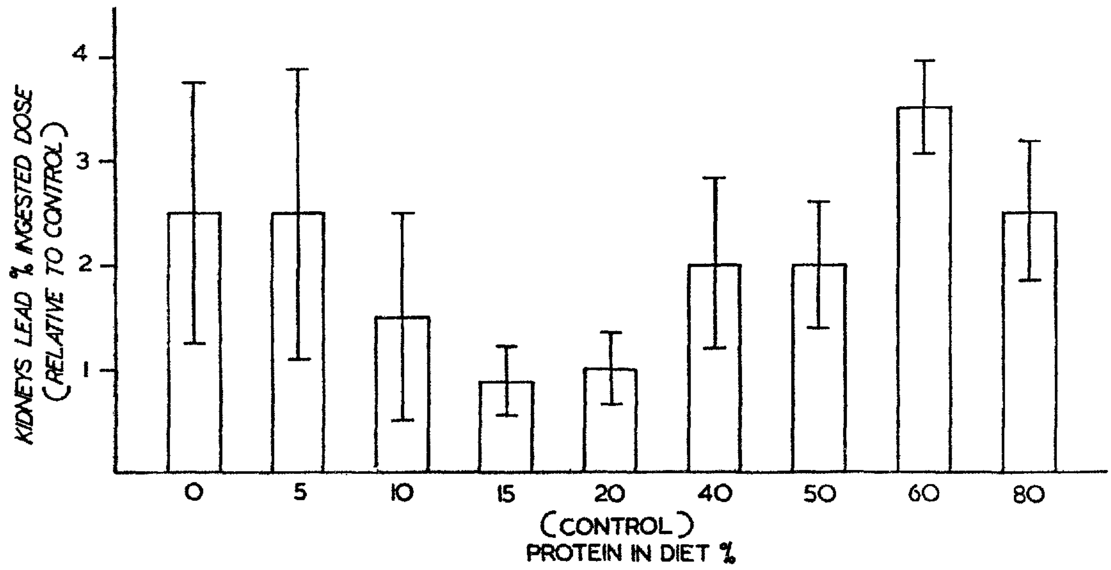


Figure 18

Mean Kidney Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Protein Composition Relative to Control Diet (n = 6).

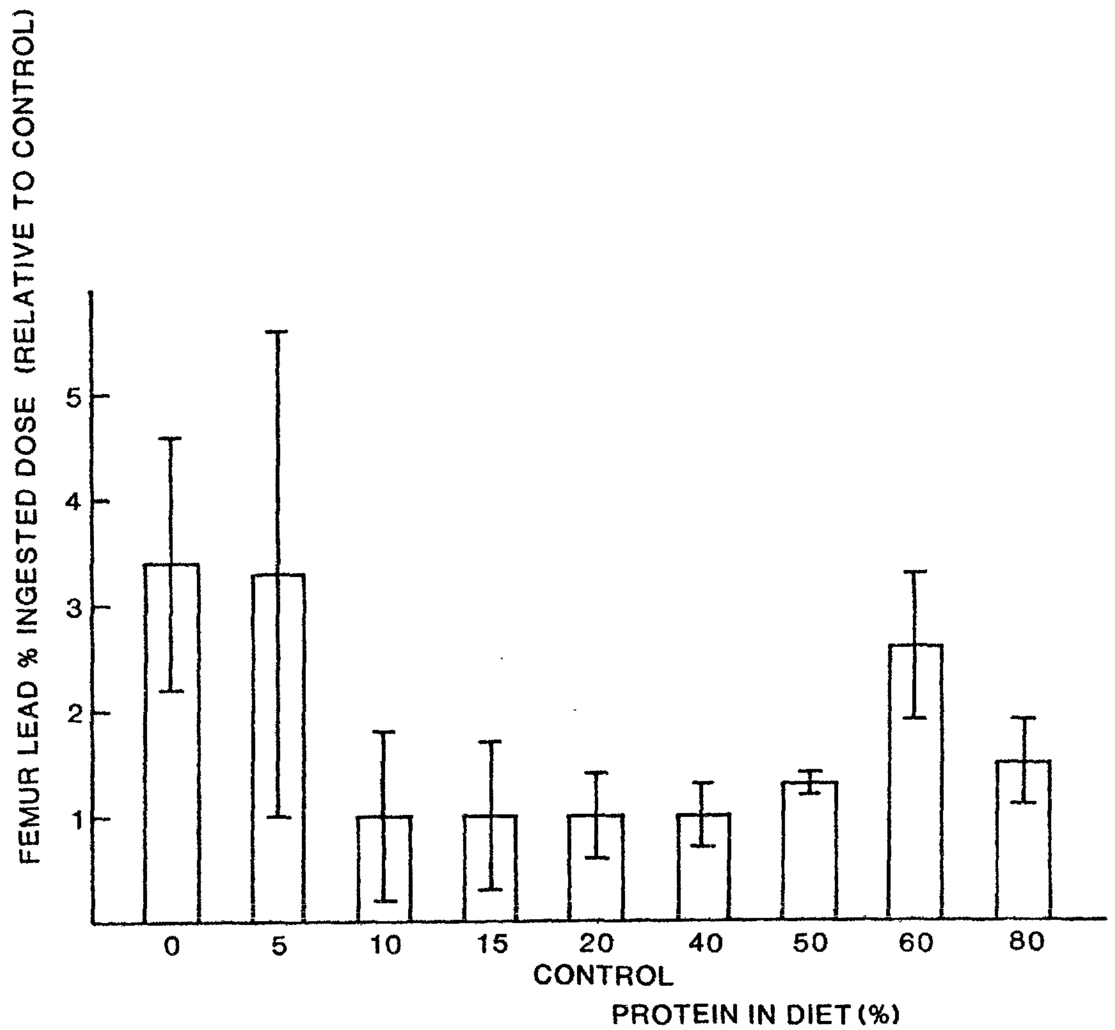


Figure 19

Mean Femur Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Protein Composition Relative to Control Diet (n = 6).

Table 24

Uptake of Lead from 0% Fat Diet

Organ	5% Fat (Control)	0% Fat	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	2.82 ± 0.49	2.29 ± 0.76	N.S.	N.S.	-
Femur*	2.07 ± 0.51	1.54 ± 0.73	N.S.	N.S.	-
Liver*	6.61 ± 1.20	4.48 ± 1.82	N.S.	N.S.	-
Blood/g*	0.195 ± 0.124	0.116 ± 0.051	N.S.	N.S.	-
Whole body without gut	0.646 ± 0.124	0.522 ± 0.213	N.S.	N.S.	-

*$(\bar{x} \pm S.D.) \times 10^2$

Table 25

Uptake of Lead from 2.5% Fat Diet

Organ	5% Fat (Control)	2.5% Fat	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	2.82 ± 0.49	3.15 ± 1.01	N.S.	N.S.	-
Femur*	2.07 ± 0.51	2.40 ± 0.97	N.S.	N.S.	-
Liver*	6.61 ± 1.20	6.88 ± 2.63	N.S.	N.S.	-
Blood/g*	0.195 ± 0.124	0.198 ± 0.116	N.S.	N.S.	-
Whole body without gut	0.646 ± 0.124	0.665 ± 0.244	N.S.	N.S.	-

*($\bar{x}$ ± S.D.) x 10^{2}

Table 26

Uptake of Lead from 10% Fat Diet

Organ	5% Fat (Control)	10% Fat	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	2.82 ± 0.49	5.81 ± 3.22	<.01	N.S.	2.1
Femur *	2.07 ± 0.51	3.21 ± 1.26	<.02	N.S.	1.5
Liver *	6.61 ± 1.20	10.18 ± 4.82	<.05	N.S.	1.5
Blood/g *	0.195 ± 0.124	0.364 ± 0.213	<.02	N.S.	1.9
Whole body without gut	0.646 ± 0.124	1.144 ± 0.485	<.005	<.002	1.8

*($\bar{x}$ ± S.D.) x 10^2

Table 27

Uptake of Lead from 15% Fat Diet

Organ	5% Fat (Control)	15% Fat	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	2.82 ± 0.49	21.49 ± 9.00	<.001	<.002	7.6
Femur *	2.07 ± 0.51	10.03 ± 3.42	<.001	<.002	4.8
Liver *	6.61 ± 1.20	27.77 ± 13.21	<.001	<.002	4.2
Blood/g *	0.195 ± 0.124	1.879 ± 0.88	<.001	<.002	9.6
Whole body without gut	0.646 ± 0.124	3.277 ± 1.108	<.001	<.002	5.1

* ($\bar{x}$ ± S.D.) x 10^2

Table 28

Uptake of Lead from 20% Fat Diet

Organ	5% Fat (Control)	20% Fat	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	2.82 ± 0.49	15.50 ± 3.64	<.001	<.002	5.5
Femur *	2.07 ± 0.51	9.62 ± 2.72	<.001	<.002	4.6
Liver *	6.61 ± 1.20	28.86 ± 9.15	<.001	<.002	4.4
Blood/g *	0.195 ± 0.124	1.534 ± 0.645	<.001	<.002	7.9
Whole body without gut	0.646 ± 0.124	2.555 ± 0.539	<.001	<.002	4.0

*$(\bar{x} \pm \text{S.D.}) \times 10^2$

Table 29

Uptake of Lead from 40% Fat Diet

Organ	5% Fat (Control)	40% Fat	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	2.82 ± 0.49	40.14 ± 14.07	<.001	<.002	14.2
Femur *	2.07 ± 0.51	22.51 ± 7.70	<.001	<.002	10.8
Liver *	6.61 ± 1.20	47.08 ± 14.84	<.001	<.002	7.1
Blood/g *	0.195 ± 0.124	2.643 ± 0.706	<.001	<.002	13.6
Whole body without gut	0.646 ± 0.124	5.912 ± 1.932	<.001	<.002	9.2

*($\bar{x} \pm$ S.D.) x 10^2

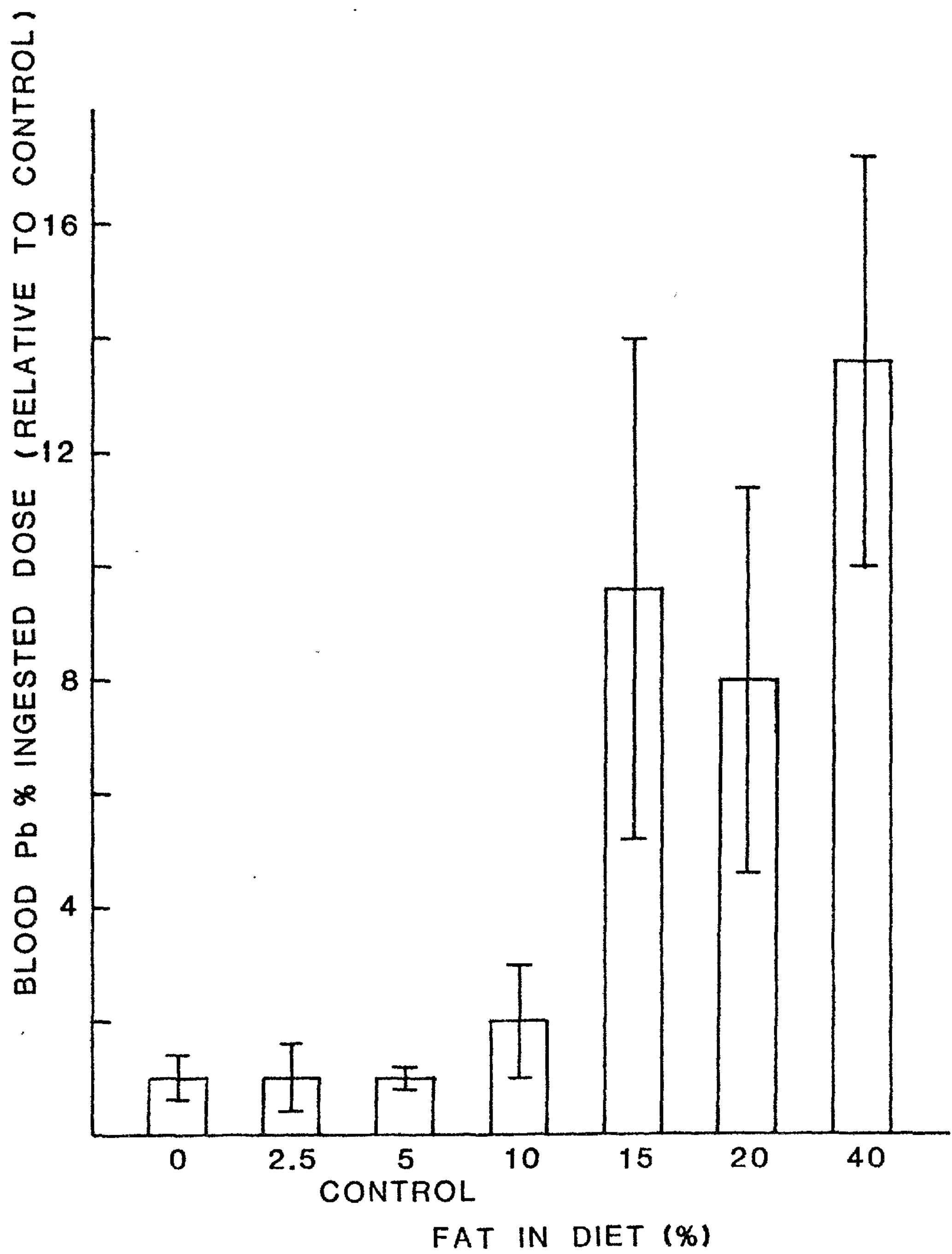


Figure 20

Mean Blood Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Fat Composition Relative to Control Diet (n = 6).

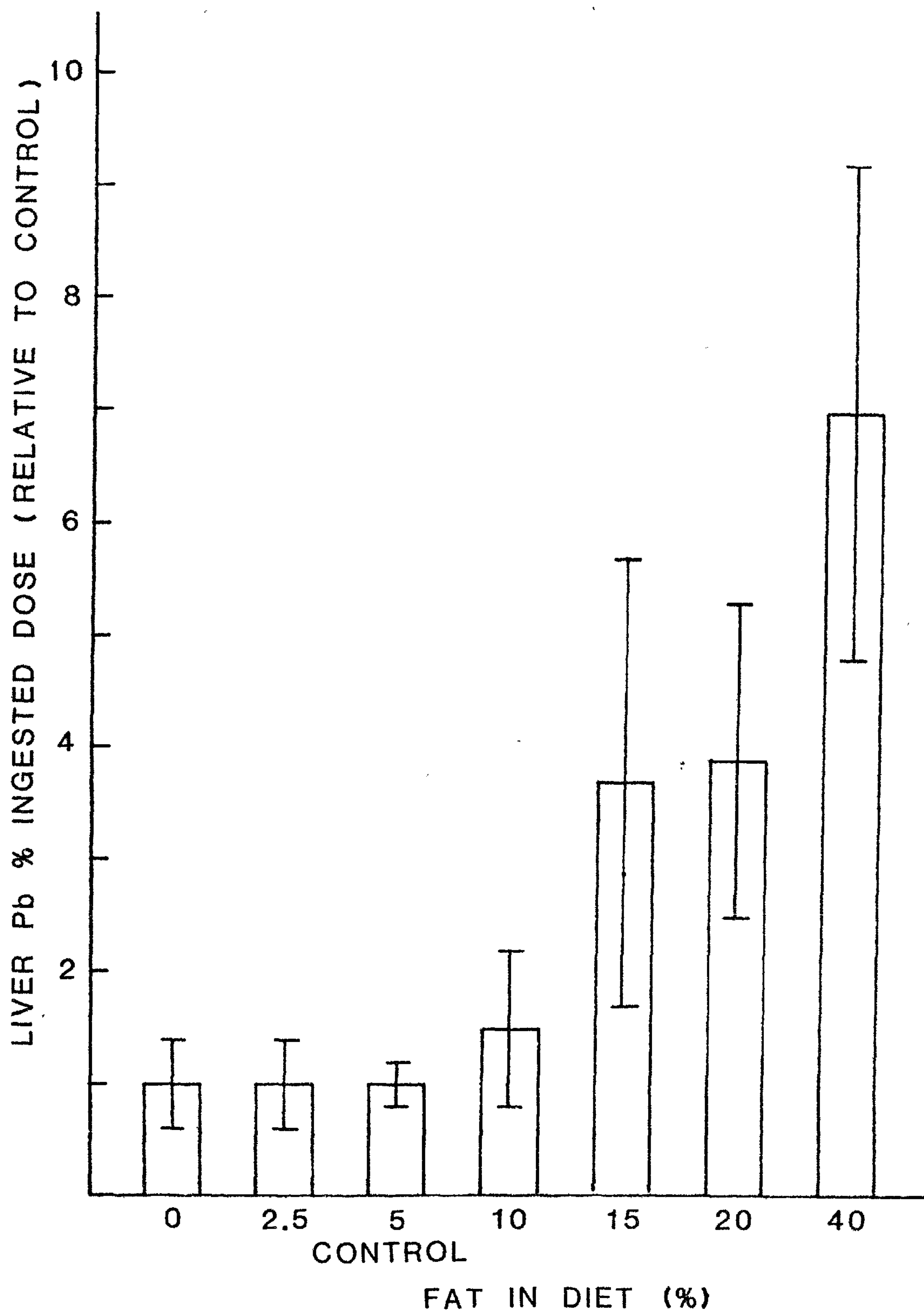


Figure 21

Mean Liver Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Fat Composition Relative to Control Diet (n = 6).

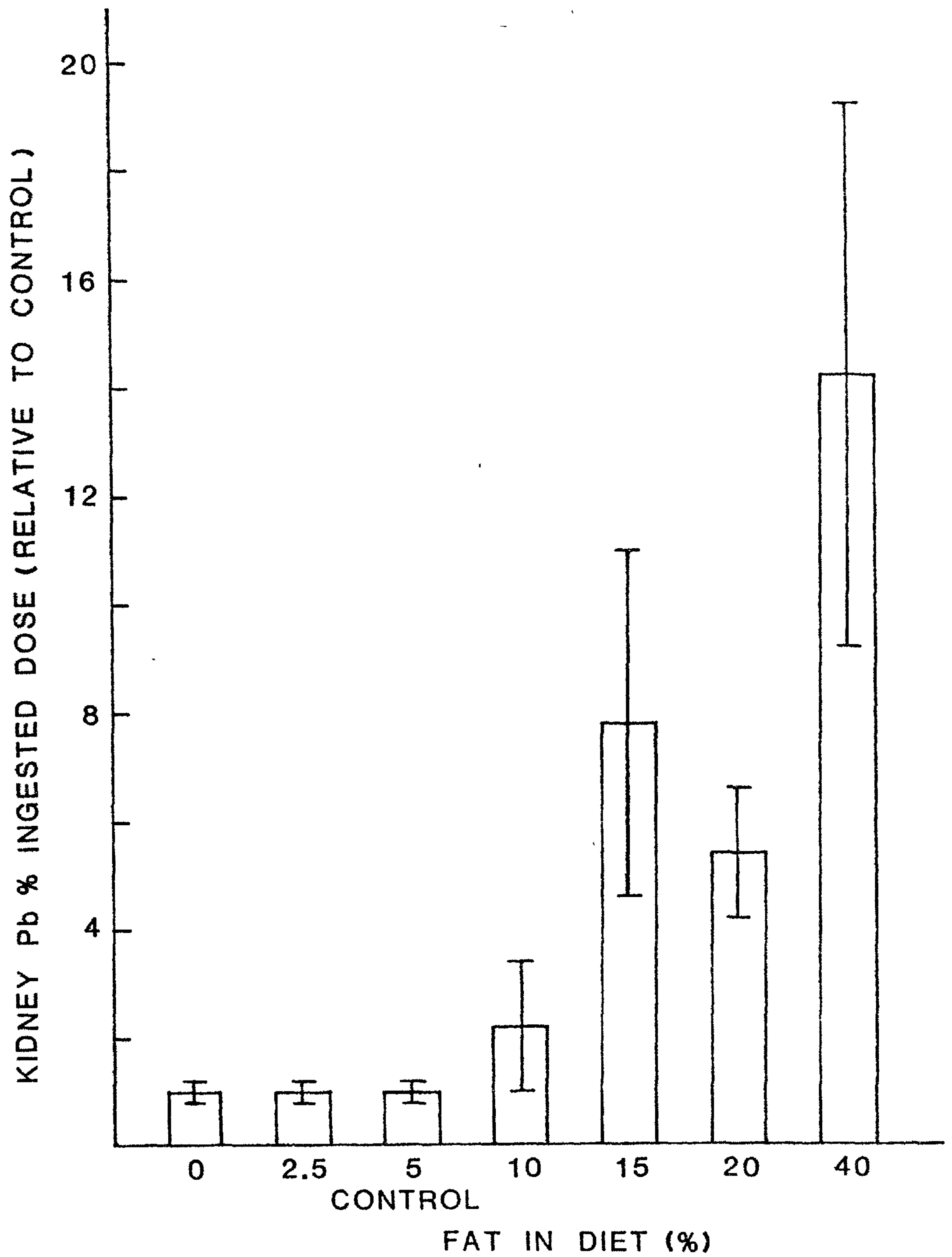
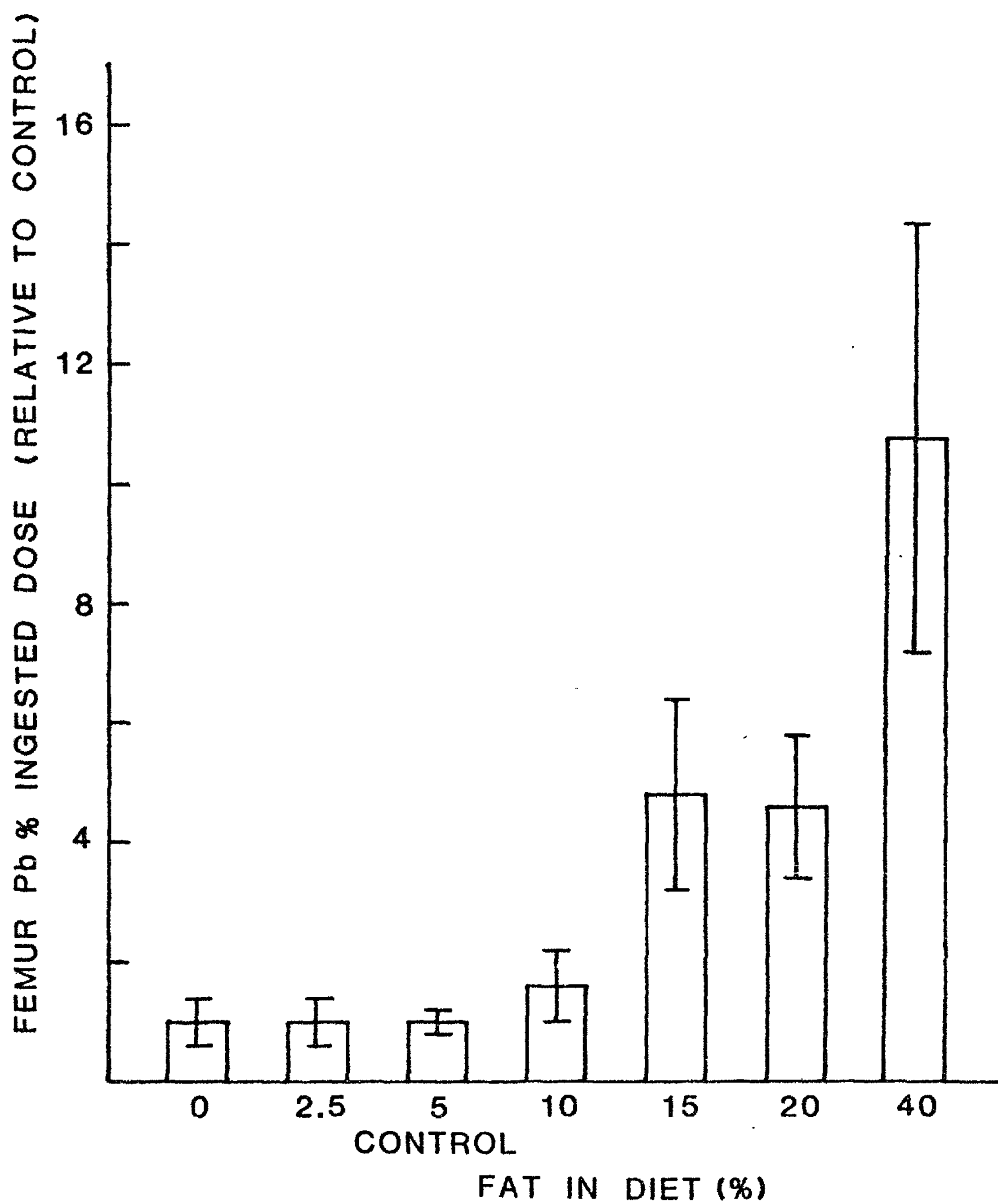


Figure 22

Mean Kidney Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Fat Composition Relative to Control Diet (n = 6).



<u>Figure 23</u>

Mean Femur Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Fat Composition Relative to Control Diet (n = 6).

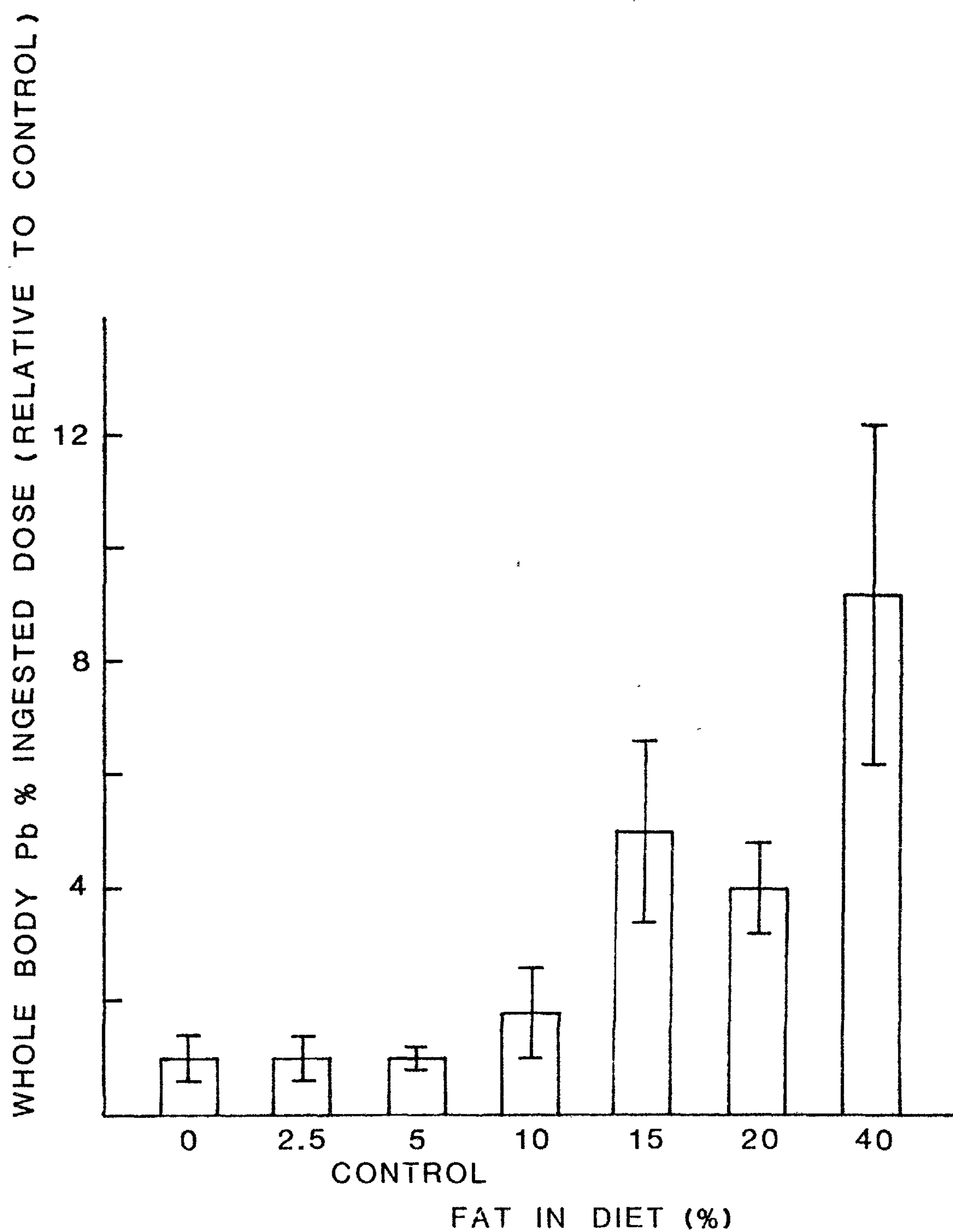


Figure 24

Mean Whole Body Lead Concentration as % Ingested Dose $\pm$ S.D. for Diets of Varying Fat Composition Relative to Control Diet (n = 6).

respect. A series of vegetable oils and butterfat were studied. The oils were selected on the basis of the nature of their constituent fatty acids. All the oils were analysed by gas-liquid chromatography for their component fatty acid content (Table 30).

The first study compared absorption of lead from animals fed diets containing rapeseed oil as a substitute for corn oil in the control diet. As seen in Table 30, rapeseed oil contains only 3.6% saturated fatty acid with a marked excess of unsaturated fatty acid of carbon chain length 18, 20 and 22. Corn oil contains 14% saturated fatty acid but a high proportion of unsaturates. There was no significant difference by the Student's "t" test and the Mann-Whitney 'U' test for any of the tissues studied (Table 31).

In the next experiment, sunflower oil was substituted for corn oil. Sunflower oil contains 10% saturated fatty acid in the medium carbon chain group (16:0 and 18:0). However, it contains 87% medium chain unsaturated fatty acid (18:1 and 18:2). The findings are reported in Table 32. No significant differences were observed for any of the tissues studied by means of the "t" test. Only the liver uptake of lead in the sunflower oil diet was diminished significantly ($p < 0.04$) to half that of the corn oil diet by means of the non-parametric "U" test. No significant difference was observed for the remaining tissues.

The third oil studied was coconut oil which contains 89% saturated short chain fatty acid. 67% of these are of carbon chain length of 12 and less with contents of 17%

Table 30

Composition of Dietary Fat

Fatty Acid	Corn	Rapeseed	Sunflower	Coconut	Butterfat
12:0 and below	-	-	-	66.7	-
14:0	-	-	-	16.8	10
16:0	12	-	5	8.6	25
16:1	-	6.3	-	-	-
18:0	2	3.6	5	2.5	10
18:1	25	25	22	5.2	32
18:2	60	18	65	-	5
18:3	1	10	-	-	-
20:0	-	-	-	-	trace
20:1	-	10	-	-	-
22:1	-	31	-	-	-

Table 31

Uptake of Lead from Rapeseed Oil Diet

Organ	Control (Corn Oil)	Rapeseed Oil	"t" Test (p)	"U" Test (p)	Test/Control
Kidneys*	4.38 ± 1.84	3.61 ± 1.70	N.S.	N.S.	-
Femur*	2.52 ± 1.83	2.09 ± 1.62	N.S.	N.S.	-
Liver*	9.79 ± 4.10	5.84 ± 1.21	N.S.	N.S.	-
Blood/g*	0.272 ± 0.141	0.169 ± 0.113	N.S.	N.S.	-
Whole body without gut	0.878 ± 0.457	0.704 ± 0.437	N.S.	N.S.	-

*$(\bar{x} \pm S.D.) \times 10^2$

Table 32

Uptake of Lead from Sunflower Oil Diet

Organ	Corn Oil (Control)	Sunflower Oil	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	4.38 ± 1.84	3.28 ± 1.55	N.S.	N.S.	-
Femur *	2.52 ± 1.83	1.48 ± 1.18	N.S.	N.S.	-
Liver *	9.79 ± 4.10	4.90 ± 2.83	N.S.	<.04	0.5
Blood/g *	0.272 ± 0.141	0.176 ± 0.136	N.S.	N.S.	-
Whole body without gut	0.878 ± 0.457	0.534 ± 0.341	N.S.	N.S.	-

*($\bar{x}$ ± S.D.) x 10^2

for 14:0, 8% for 16:0 and 2% for 18:0. Data in comparison with the control corn oil diet are given in Table 33. Decreased absorption of lead was observed in the femur, liver and carcass minus the gut. The ratio of lead uptake with coconut oil to corn oil diet was 0.5 in each case. The differences observed were only significant ($P < 0.04$, 0.01 and 0.004 respectively) when compared by the Mann-Whitney "U" test. The "t" test gave no significant differences in any of the tissues studied.

The final fat studied was butterfat which is characterized by a 72% content of medium chain fatty acid. Its major constituents include 10% of 14:0, 25% of 16:0, 10% of 18:0 and 32% of 18:1. These findings are given in Table 34. Enhanced absorption of lead from the butterfat diet was observed for all the tissues studied. The ratio of lead absorption for butterfat to control diets lie in the range 1.4 to 1.8. Significant differnces were observed for kidneys, femur and whole body without gut, but not for liver or blood when compared by means of the "t" test. However, significant differences were observed for all the tissues when the Mann-Whitney "U" test was used.

Minerals

Low mineral diets have been shown to increase lead uptake in all the organs studied (Table 35). The greatest increase was observed in the blood which had a 17.7-fold enhancement of lead uptake. Further studies were thus conducted to identify the individual minerals responsible for increasing the lead absorption from the gut. Each of

Table 33

Uptake of Lead from Coconut Oil Diet

Organ	Corn Oil (Control)	Coconut Oil	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	4.38 ± 1.84	2.81 ± 0.98	N.S.	N.S.	-
Femur *	2.52 ± 1.83	1.16 ± 0.57	N.S.	<.04	0.5
Liver *	9.79 ± 4.10	5.04 ± 2.02	N.S.	<.01	0.5
Blood/g *	0.272 ± 0.141	0.176 ± 0.136	N.S.	N.S.	-
Whole body without gut	0.878 ± 0.457	0.439 ± 0.164	N.S.	<.004	0.5

*($\bar{x}$ ± S.D.) x 10^2

Table 34

Uptake of Lead from Butterfat Diet

Organ	Corn Oil (Control)	Butterfat	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	2.92 ± 0.98	4.58 ± 0.58	<.01	<.001	1.6
Femur *	1.60 ± 0.57	2.79 ± 0.85	<.02	<.008	1.7
Liver *	5.08 ± 1.30	6.92 ± 1.49	N.S.	<.03	1.4
Blood/g *	0.177 ± 0.057	0.312 ± 0.149	N.S.	<.02	1.8
Whole body without gut	0.601 ± 0.263	0.914 ± 0.151	<.05	<.02	1.5

*($\bar{x}$ ± S.D.) x 10^2

Table 35

Uptake of Lead from 0% Mineral Diet

Organ	Control (5% Mineral)	0% Mineral	"t" Test (p)	"U" Test (p)	Test/Control
Kidneys *	2.55 ± 0.64	30.39 ± 13.72	<.001	<.002	11.9
Femur *	1.39 ± 0.31	19.05 ± 8.76	<.001	<.002	13.7
Liver *	4.53 ± 0.80	39.65 ± 16.31	<.001	<.002	8.8
Blood/g *	0.188 ± 0.054	3.320 ± 1.526	<.001	<.002	17.7
Whole body without gut	0.448 ± 0.078	5.132 ± 2.266	<.001	<.002	11.5

$*(\bar{x} \pm S.D.) \times 10^2$

the minerals selected was omitted sequentially from the diet. Initially, the response to the omission of major and minor components was determined. Diets deficient in calcium, phosphate, magnesium, sodium, potassium and chloride resulted in a marked increase in lead absorption. There was a 15-fold increase in the kidney, 11-fold in the femur, 8-fold in the liver, 18-fold in the blood and 11-fold in the carcass lead retention (Table 36). However, exclusion of the minor components, iron, manganese, copper, zinc, iodine and molybdenum from the diet had no effect on lead absorption from the gut under these experimental conditions (Table 37).

Further studies showed that sodium, potassium and chloride-deficient diets did not affect lead absorption (Table 38). Diets without calcium resulted in increased lead retention in all tissues (Table 39) ranging from a 3.5-fold enhancement in carcass and femur to a 7.4-fold enhancement in blood. Diets without phosphate also enhanced lead absorption but to a lesser degree. There was a 2-fold increase in lead uptake by the femur, liver and whole-body and a 3-fold increase in the kidneys and blood (Table 40). When both calcium and phosphate were omitted from the diet, an additive effect was observed in the increased lead retention of all the tissues (Table 41). Blood lead uptake was increased 11.5 times with the other tissues having increases of 7.2 times in kidney, 5.2 times in femur, 3.6 times in liver and 4.9 times in the whole-body. The exclusion of magnesium from the diet resulted in significant increases in lead uptake by kidneys, liver and blood

Table 36

Uptake of Lead from 0% Macromineral Diet

Organ	Control	0% Macromineral (Without Ca, PO_4, Mg, Na, K, Cl)	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	2.55 ± 0.64	37.99 ± 8.77	<.001	<.002	14.9
Femur *	1.39 ± 0.31	14.91 ± 3.14	<.001	<.002	10.7
Liver *	4.53 ± 0.80	37.23 ± 5.87	<.001	<.002	8.2
Blood/g *	0.188 ± 0.054	3.326 ± 1.641	<.001	<.002	17.6
Whole body without gut	0.448 ± 0.078	4.391 ± 0.640	<.001	<.002	11.0

$*(\bar{x} \pm S.D.) \times 10^2$

Table 37

Uptake of Lead from 0% Micromineral Diet

Organ	Control	0% Micromineral (Without Fe, Mn, Cu, Zn, Mo, I)	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	2.55 ± 0.64	2.18 ± 0.34	N.S.	N.S.	-
Femur*	1.39 ± 0.31	1.52 ± 0.26	N.S.	N.S.	-
Liver*	4.53 ± 0.80	4.53 ± 1.08	N.S.	N.S.	-
Blood/g*	0.188 ± 0.054	0.139 ± 0.036	N.S.	N.S.	-
Whole body without gut	0.448 ± 0.078	0.468 ± 0.067	N.S.	N.S.	-

*($\bar{x}$ ± S.D.) x 10^2

Table 38

Uptake of Lead from 0% K, Na, Cl Diet

Organ	Control	0% K, Na & Cl	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	3.24 ± 1.20	3.47 ± 1.74	N.S.	N.S.	-
Femur*	2.31 ± 0.90	2.57 ± 1.59	N.S.	N.S.	-
Liver*	7.28 ± 1.81	8.60 ± 3.45	N.S.	N.S.	-
Blood/g*	0.220 ± 0.094	0.266 ± 0.183	N.S.	N.S.	-
Whole body without gut	0.698 ± 0.216	0.758 ± 0.390	N.S.	N.S.	-

*($\bar{x}$ ± S.D.) x 10^2

Table 39

Uptake of Lead from 0% Calcium Diet

Organ	Control	0% Calcium	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	3.24 ± 1.20	13.66 ± 6.99	<.001	<.002	4.2
Femur *	2.31 ± 0.90	8.12 ± 3.04	<.001	<.002	3.5
Liver *	7.28 ± 1.81	27.84 ± 12.54	<.001	<.002	3.8
Blood/g *	0.220 ± 0.094	1.619 ± 0.807	<.001	<.002	7.4
Whole body without gut	0.698 ± 0.216	2.464 ± 0.847	<.001	<.002	3.5

* $(\bar{x} \pm S.D.) \times 10^2$

Table 40

Uptake of Lead from 0% Phosphate Diet

Organ	Control	0% Phosphate	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	3.24 ± 1.20	9.51 ± 3.41	<.005	<.002	2.9
Femur *	2.31 ± 0.90	5.03 ± 1.79	<.005	<.002	2.2
Liver *	7.28 ± 1.81	13.09 ± 3.31	<.005	<.002	1.8
Blood/g *	0.220 ± 0.094	0.712 ± 0.254	<.005	<.002	3.2
Whole body without gut	0.698 ± 0.216	1.388 ± 0.258	<.005	<.002	1.9

* $(\bar{x} \pm S.D.) \times 10^2$

Table 41

Uptake of Lead from 0% Ca and PO_4 Diet

Organ	Control	0% Calcium And 0% Phosphate	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	3.24 ± 1.20	23.18 ± 8.19	<.001	<.002	7.2
Femur*	2.31 ± 0.90	11.93 ± 3.93	<.001	<.002	5.2
Liver*	7.28 ± 1.81	26.79 ± 9.52	<.001	<.002	3.6
Blood/g*	0.220 ± 0.094	2.531 ± 1.502	<.001	<.002	11.5
Whole body without gut	0.698 ± 0.216	3.389 ± 1.074	<.001	<.002	4.9

$*(\bar{x} \pm S.D.) \times 10^2$

(Mann-Whitney 'U' test). Using the "t" test, the liver, blood and whole-body showed significant increases. However, the enhancements were small ranging from 1.6 to 2.1. The femur showed no significant difference (Table 42).

Half the recommended amounts of calcium added to the diet resulted in approximately 2-fold increases in all the tissues studied (Table 43). Similarly, half the recommended content of dietary phosphate increased lead uptake but to a slightly lesser degree (Table 44). Diets containing half the recommended concentration of calcium and phosphate again showed an additive effect on lead uptake. The kidney lead uptake was enhanced 3.3 times, femur 3.8 times, liver 2.6 times, blood 4.1 times and whole-body 3.6 times (Table 45). A summary of the effect of decreased dietary calcium and phosphate is presented in Table 46.

Animals fed extra minerals had decreased uptake of lead in all their tissues (Table 47). Kidney and blood lead uptake was decreased 70% while the values for femur and liver were decreased by 90 and 80% respectively. The response to the addition of major and minor component minerals was determined. Diets with 4 times the normal concentration of calcium, phosphate, magnesium, sodium, potassium and chloride resulted in a marked decrease in lead absorption (Table 48). Kidney and blood lead uptake was reduced by 60%, femur and whole-body by 70% and liver by 80% However, collectively increasing iron, manganese, copper, zinc, iodine and molybdenum to 4 times the normal concentration had no effect on kidneys, femur, blood and whole-body while liver lead

Table 42

Uptake of Lead from 0% Magnesium Diet

Organ	Control	0% Magnesium	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	3.24 ± 1.20	5.24 ± 1.94	N.S.	<.02	1.6
Femur *	2.31 ± 0.90	3.43 ± 1.33	N.S.	N.S.	-
Liver *	7.28 ± 1.81	12.60 ± 2.49	<.001	<.002	1.7
Blood/g *	0.220 ± 0.094	0.458 ± 0.231	<.01	<.02	2.1
Whole body without gut	0.698 ± 0.216	1.104 ± 0.433	<.02	N.S.	1.6

* ($\bar{x}$ ± S.D.) x 10^2

Table 43

Uptake of Lead from 0.35% Ca and 0.5% PO_4 Diet

Organ	Control	½ x Ca	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	3.16 ± 1.09	6.52 ± 4.29	<.005	<.002	2.1
Femur*	1.71 ± 0.87	3.68 ± 1.70	<.001	<.002	2.2
Liver*	6.25 ± 2.15	13.35 ± 4.64	<.001	<.002	2.1
Blood/g*	0.188 ± 0.099	0.364 ± 0.187	<.005	<.002	1.9
Whole body without gut	0.522 ± 0.203	1.215 ± 0.468	<.001	<.002	2.3

*$(\bar{x} \pm S.D.) \times 10^2$

Table 44

Uptake of Lead from 0.7% Ca and 0.25% PO_4 Diet

Organ	Control	$\frac{1}{2}$ x PO_4	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	3.16 ± 1.09	5.66 ± 2.49	<.001	<.002	1.8
Femur*	1.71 ± 0.87	3.17 ± 1.39	<.005	<.002	1.9
Liver*	6.25 ± 2.15	9.03 ± 3.67	<.02	<.002	1.4
Blood/g*	0.188 ± 0.099	0.382 ± 0.230	<.005	<.002	2.0
Whole body without gut	0.522 ± 0.203	1.174 ± 0.367	<.001	<.002	2.2

$*(\bar{x} \pm S.D.) \times 10^2$

Table 45

Uptake of Lead from 0.35% Ca and 0.25% PO_4 Diet

Organ	Control	½ x Ca & ½ x PO_4	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	3.16 ± 1.09	10.40 ± 4.30	<.001	<.002	3.3
Femur*	1.71 ± 0.87	6.47 ± 2.62	<.001	<.002	3.8
Liver*	6.25 ± 2.15	16.07 ± 5.43	<.001	<.002	2.6
Blood/g*	0.188 ± 0.099	0.778 ± 0.364	<.001	<.002	4.1
Whole body without gut	0.522 ± 0.203	1.873 ± 0.756	<.001	<.002	3.6

*($\bar{x}$ ± S.D.) x 10^2

Table 46

Effect of Decreased Minerals on Lead Absorption

(Ratio of Mean Retention Experimental : Control)

Dietary Minerals						
Calcium (%)	Phosphate (%)	Whole body without gut	Blood	Kidneys	Femur	Liver
*0.7	0.5	1	1	1	1	1
0	0.5	-	6.2	4.4	3.4	4.0
0.7	0	-	2.7	3.0	2.1	1.9
0	0	-	9.7	7.4	4.9	3.8
0.35	0.5	2.3	1.9	2.1	2.2	2.1
0.7	0.25	2.2	2.0	1.8	1.9	1.4
0.35	0.25	3.6	4.1	3.3	3.8	2.6

*Control

Table 47

Uptake of Lead from High Mineral Diet

Organ	Control (5% Mineral)	High Mineral (20% Mineral)	"t" Test (p)	"U" Test (p)	Test/Control
Kidneys*	2.23 ± 0.57	0.58 ± 0.36	$<.001$	$<.001$	0.3
Femur*	1.10 ± 0.27	0.15 ± 0.05	$<.001$	$<.001$	0.1
Liver*	4.85 ± 0.95	0.85 ± 0.17	$<.001$	$<.001$	0.2
Blood/g*	0.173 ± 0.052	0.051 ± 0.017	$<.001$	$<.001$	0.3

*($\bar{x}$ ± S.D.) x 10^2

Table 48

Uptake of Lead from High Macromineral Diet

Organ	Control	4 x Macro Mineral	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	3.33 $\pm$ 0.72	1.22 $\pm$ 0.42	<.001	<.001	0.4
Femur*	1.72 $\pm$ 0.72	0.50 $\pm$ 0.30	<.02	<.004	0.3
Liver*	6.28 $\pm$ 1.65	1.57 $\pm$ 0.81	<.001	<.001	0.2
Blood/g*	0.185 $\pm$ 0.070	0.068 $\pm$ 0.028	N.S.	<.02	0.4
Whole body without gut	0.561 $\pm$ 0.192	0.205 $\pm$ 0.086	N.S.	<.004	0.3

*$(\bar{x} \pm \text{S.D.}) \times 10^2$

retention was significantly enhanced 1.4 times by means of the "t" test (Table 49).

Diets with increased calcium and phosphate concentration were fed to further groups of 6 animals. Diets containing twice the recommended concentration of calcium did not produce any significant change in lead uptake (Table 50). Doubling the dietary phosphate did not have any effect on lead uptake by the different organs except for a 30% decrease in the liver lead retention significant by means of the "t" test (Table 51). However, when both calcium and phosphate were doubled simultaneously, lead retention in all the organs studied was halved except in the blood which did not show any significant difference (Table 52).

Increasing calcium and phosphate content individually and simultaneously to 4 times normal values reduced lead uptake in all the organs. A 4-fold dietary calcium increase decreased lead uptake in the liver, blood and whole-body by 50%, the kidneys by 30% and the femur by 60% (Table 53). The diet containing 4 times the normal concentration of phosphate resulted in a 60% decrease in femur, liver and blood, a 70% decrease in kidneys and a 50% decrease in the whole-body (Table 54). Increasing calcium and phosphate content 4 times simultaneously decreased lead retention in the kidneys (60%), femur (80%), liver (50%) and the whole-body (60%). The blood lead uptake was not reduced significantly, possibly because of the large standard deviation obtained (Table 55). Table 56 summarizes the effect of increased dietary calcium and phosphate on lead absorption

Table 49

Uptake of Lead from High Micromineral Diet

Organ	Control	4 x Micro-Mineral	"t" Test (p)	"U" Test (p)	Test/Control
Kidneys*	3.33 ± 0.72	4.79 ± 1.90	N.S.	N.S.	-
Femur*	1.72 ± 0.72	1.89 ± 0.74	N.S.	N.S.	-
Liver*	6.28 ± 1.65	8.97 ± 3.78	<.05	N.S.	1.4
Blood/g*	0.188 ± 0.070	0.347 ± 0.227	N.S.	N.S.	-
Whole body without gut	0.561 ± 0.192	0.681 ± 0.270	N.S.	N.S.	-

*$(\bar{x} \pm S.D.) \times 10^2$

Table 50

Uptake of Lead from 1.4% Ca and 0.5% PO_4 Diet

Organ	Control	2 x Ca	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys *	3.16 ± 1.08	3.00 ± 1.16	N.S.	N.S.	-
Femur *	1.70 ± 0.87	1.35 ± 0.74	N.S.	N.S.	-
Liver *	6.24 ± 2.15	5.75 ± 1.85	N.S.	N.S.	-
Blood/g *	0.188 ± 0.099	0.153 ± 0.062	N.S.	N.S.	-
Whole body without gut	0.522 ± 0.203	0.476 ± 0.204	N.S.	N.S.	-

*$(\bar{x} \pm S.D.) \times 10^2$

Table 51

Uptake of Lead from 0.7% Ca and 1.0% PO_4 Diet

Organ	Control	2 x PO_4	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	3.16 ± 1.08	2.28 ± 0.75	N.S.	N.S.	-
Femur*	1.70 ± 0.87	1.10 ± 0.59	N.S.	N.S.	-
Liver*	6.24 ± 2.15	4.17 ± 1.16	<.05	N.S.	0.7
Blood/g*	0.188 ± 0.099	0.165 ± 0.060	N.S.	N.S.	-
Whole body without gut	0.522 ± 0.203	0.355 ± 0.161	N.S.	N.S.	-

*($\bar{x}$ ± S.D.) x 10^2

Table 52

Uptake of Lead from 1.4% Ca and 1.0% PO_4 Diet

Organ	Control	2 x Ca and 2 x PO_4	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	3.16 $\pm$ 1.08	1.38 $\pm$ 0.47	<.02	<.002	0.6
Femur*	1.70 $\pm$ 0.87	0.82 $\pm$ 0.29	<.05	<.002	0.5
Liver*	6.24 $\pm$ 2.15	3.53 $\pm$ 1.06	<.02	<.002	0.6
Blood/g*	0.188 $\pm$ 0.099	0.151 $\pm$ 0.048	N.S.	N.S.	-
Whole body without gut	0.522 $\pm$ 0.203	0.281 $\pm$ 0.053	<.02	<.002	0.5

*$(\bar{x} \pm S.D.) \times 10^2$

Table 53

Uptake of Lead from 2.8% Ca and 0.5% PO_4 Diet

Organ	Control	4 x Ca	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	3.16 ± 1.08	2.16 ± 0.95	<.05	<.002	0.7
Femur*	1.70 ± 0.87	0.64 ± 0.30	<.01	<.002	0.4
Liver*	6.24 ± 2.15	3.27 ± 1.29	<.005	<.002	0.5
Blood/g*	0.188 ± 0.099	0.089 ± 0.042	<.02	<.002	0.5
Whole body without gut	0.522 ± 0.203	0.267 ± 0.099	<.01	<.002	0.5

*$(\bar{x} \pm$ S.D.$) \times 10^2$

Table 54

Uptake of Lead from 0.7% Ca and 2.0% PO_4 Diet

Organ	Control	4 x PO_4	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	3.16 ± 1.08	1.68 ±	<.01	<.002	0.3
Femur*	1.70 ± 0.87	0.60 ±	<.01	<.002	0.4
Liver*	6.24 ± 2.15	2.72 ±	<.005	<.002	0.4
Blood/g*	0.188 ± 0.099	0.083 ±	<.02	<.002	0.4
Whole body Without gut	0.522 ± 0.203	0.280 ±	<.02	<.002	0.5

*($\bar{x}$ ± S.D.) x 10^2

Table 55

Uptake of Lead from 2.8% Ca and 2.0% PO_4 Diet

Organ	Control	4 x Ca and 4 x PO_4	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	3.16 ± 1.08	1.23 ± 0.82	<.005	<.002	0.4
Femur*	1.70 ± 0.87	0.41 ± 0.34	<.01	<.002	0.2
Liver*	6.24 ± 2.15	2.93 ± 2.40	<.01	<.002	0.5
Blood/g*	0.188 ± 0.099	0.091 ± 0.047	N.S.	N.S.	-
Whole body without gut	0.522 ± 0.203	0.204 ± 0.125	<.01	<.002	0.4

*($\bar{x}$ ± S.D.) x 10^2

Table 56

Effect of Increased Minerals on Lead Absorption

(Ratio of Mean Retention Experimental : Control)

Calcium (%)	Phosphate (%)	Whole body without gut	Blood	Kidneys	Femur	Liver
*0.7	0.5	1	1	1	1	1
1.4	0.5	1	1	1	1	1
0.7	1.0	1	1	1	1	0.7
1.4	1.0	0.5	1	0.6	0.5	0.6
2.8	0.5	0.5	0.5	0.7	0.4	0.5
0.7	2.0	0.5	0.4	0.3	0.4	0.4
2.8	2.0	0.4	1	0.4	0.2	0.5

*Control

and distribution in the different organs studied. Figures 25 and 26 illustrate the effect of varying dietary mineral content on blood and kidney lead uptake respectively. Fig. 27 illustrates the effect of varying dietary calcium and phosphate on kidney lead uptake.

Minerals and Fat

High fat diets were shown to increase lead absorption (Fig. 20 to 24). Combination of a high fat (15% corn oil) and mineral-deficient (0% minerals) diet resulted in enhanced lead uptake suggesting a synergistic effect (Table 57). Thus lead uptake by the whole animal was increased to 19.4 times control values. Similarly, the kidney and femur lead uptake were increased 26.6 and 18.9 times respectively. The blood lead showed the greatest effect with a 49.6-fold increase. These increases were the greatest that have been obtained with any of the experimental diets studied (Fig. 28).

Other Dietary Components

The effects of dietary alginates, pectins and phytates on lead retention were studied because alginates and pectins are being increasingly utilized in the food industry as thickening agents. The alginates and pectins are both complex polysaccharides whereas the phytates are derivatives of inositol. Both alginates and pectins are used as thickening agents in the food industry and are found in such foodstuffs as ice-cream and jam. The phytates are naturally occurring substances present mainly in cereals. These substances have been studied extensively with respect to

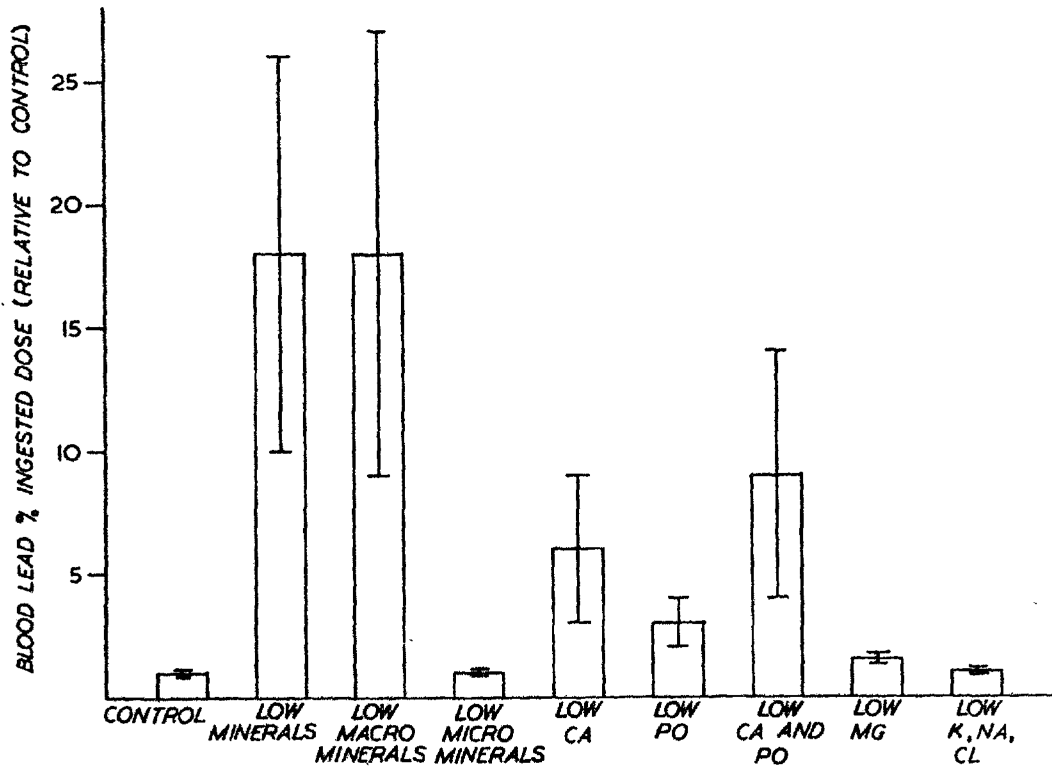


Figure 25

Mean Blood Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Mineral Content Relative to Control Diet (n = 6).

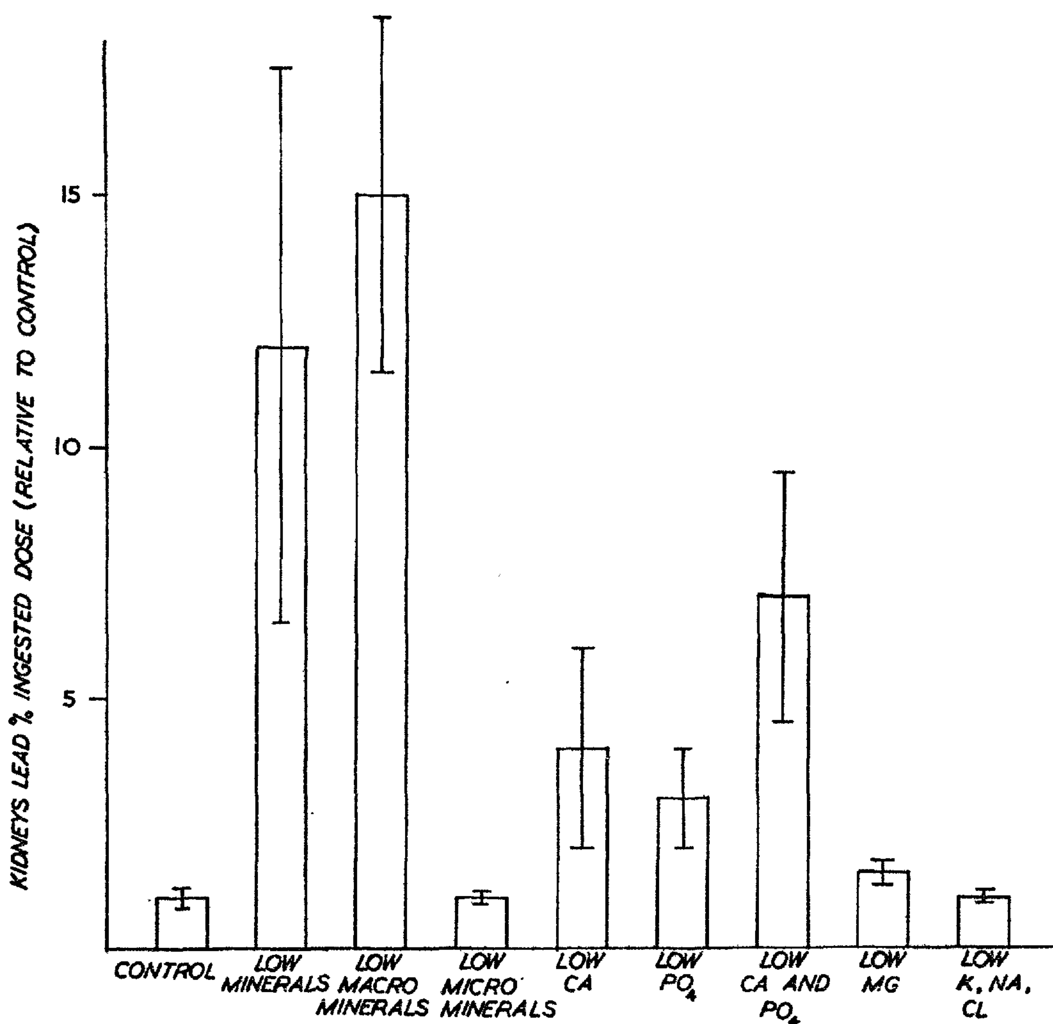


Figure 26

Mean Kidney Lead Concentration as % Ingested Dose $\pm$ S.D. for Diets of Varying Mineral Content Relative to Control Diet (n = 6).

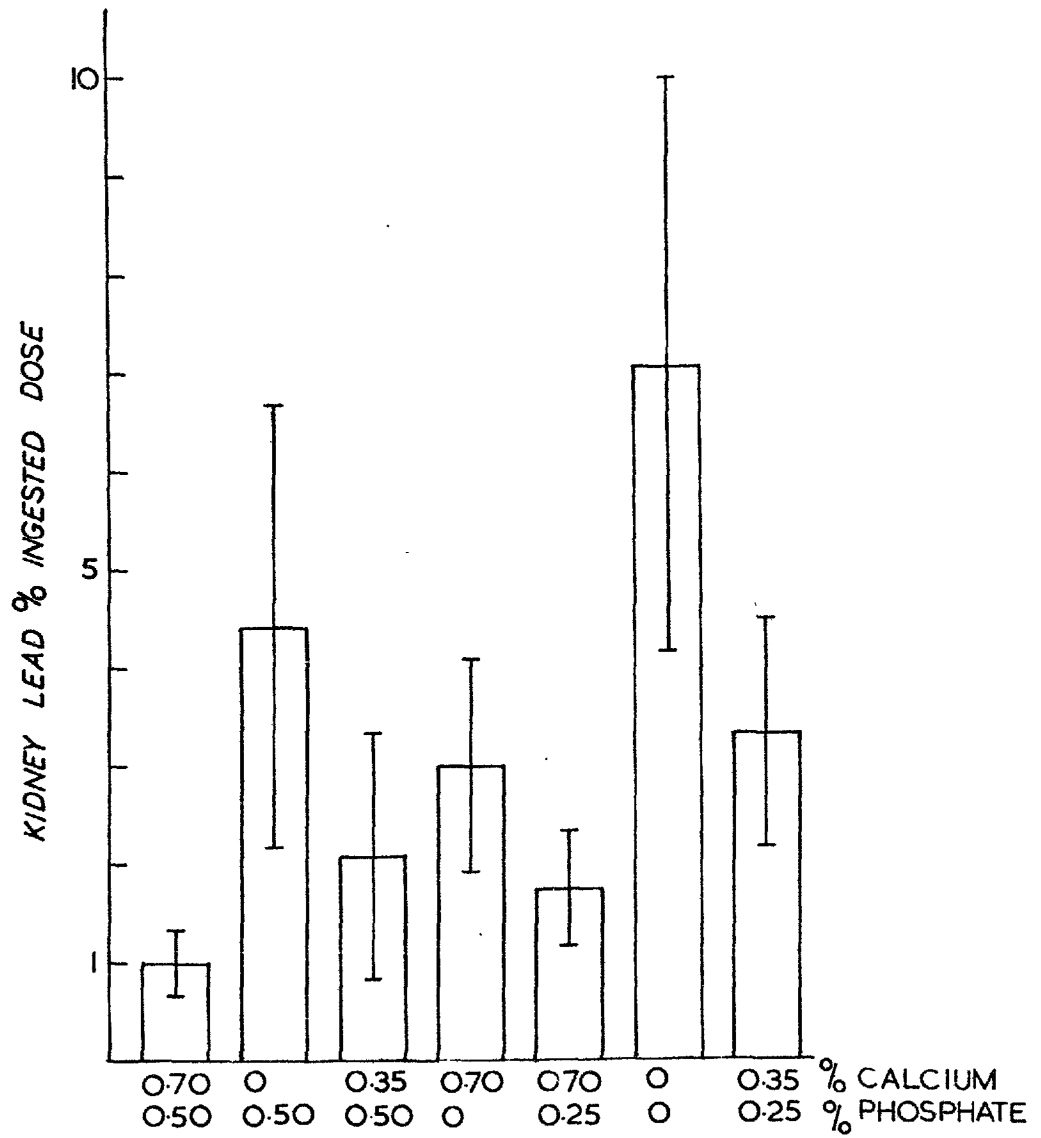


Figure 27

Mean Kidney Lead Concentration as % Ingested Dose ± S.D. for Diets of Varying Calcium and Phosphate Content (n = 6).

Table 57

Uptake of Lead from 0% Mineral and 15% Fat Diet

Organ	Control (5% Fat, 5% Mineral)	15% Fat and 0% Mineral	"t" Test (p)	"U" Test (p)	Test/ Control
Kidneys*	3.62 ± 2.30	96.34 ± 37.57	<.001	<.001	26.6
Femur*	1.76 ± 0.53	33.26 ± 9.35	<.001	<.001	18.9
Liver*	4.95 ± 1.01	72.66 ± 27.78	<.001	<.001	14.7
Blood/g*	0.119 ± 0.039	5.904 ± 3.186	<.001	<.001	49.6
Whole body without gut	0.547 ± 0.207	10.603 ± 2.834	<.001	<.001	19.4

*($\bar{x}$ ± S.D.) x 10^2

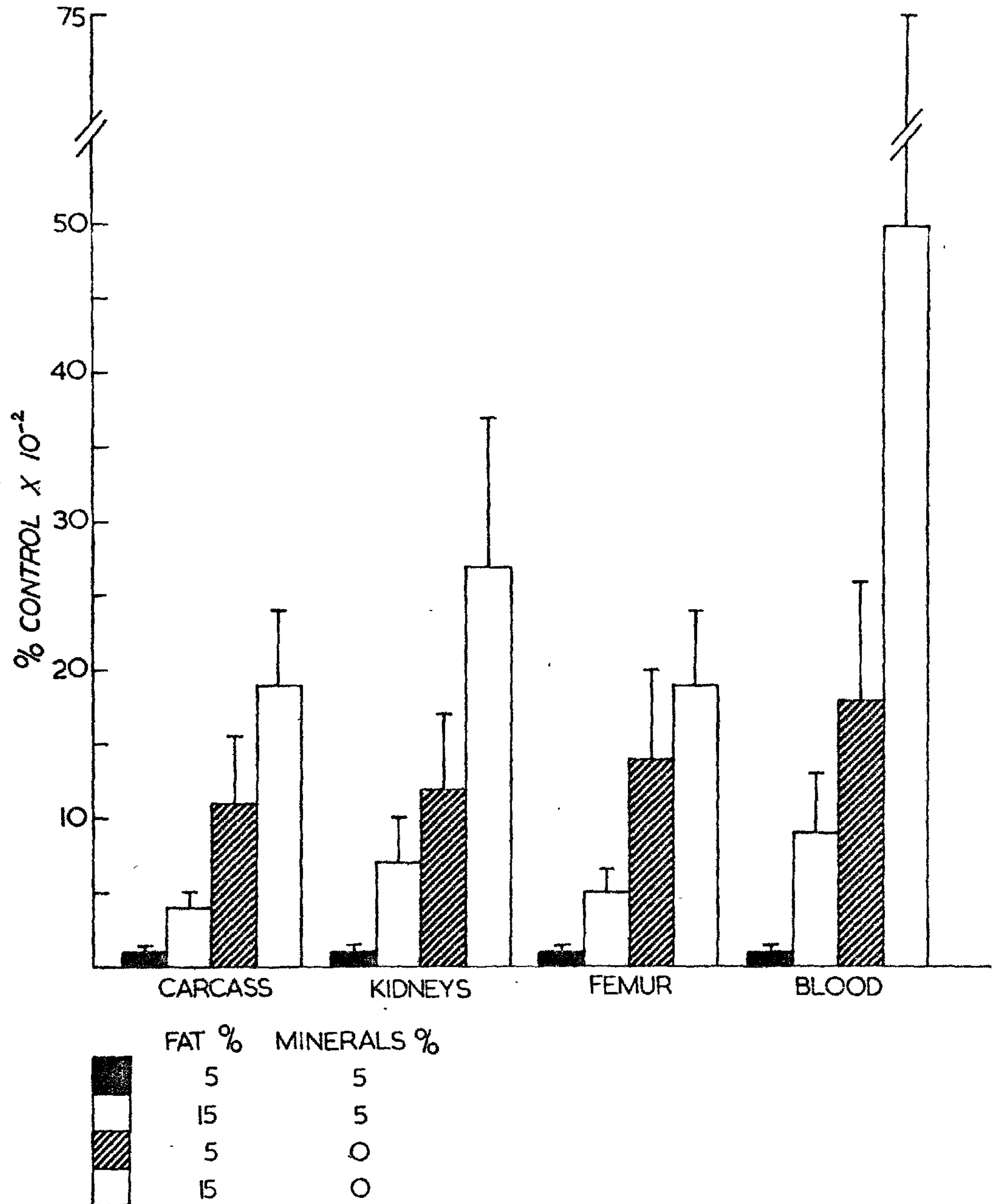


Figure 28

Lead Uptake in Rats fed Diets of Varying Fat and Mineral Content. Mean + S.D. (n = 6).

calcium absorption. Alginates have also been recommended for reducing gastrointestinal absorption of radioactive strontium (Kostial et al, 1969; Sutton et al 1971).

Alginates

Each of the 3 different types of alginates was added to Oxoid 41B to a concentration of 10% by weight of the total diet. Manucol/LK, Manujel DJ and C/BSF were tested (See Table 58 for their composition). Significant differences were seen in the uptake of lead by blood and kidneys (Table 59, Fig. 29). Although the rats fed Manucol/LK diet ingested less lead, both the blood and kidneys had increased lead concentrations compared to control animals fed alginate-free diets containing lead. The 10% Manujel DJ diet also increased the blood and kidney lead concentration. However, the 10% C/BSF diet did not increase blood and kidney lead concentration significantly except by means of the "t" test for kidney lead. The increases in lead concentration were unexpected since the metal-binding capacity of these compounds, for example, to calcium and strontium is well known and it might have been expected that lead would behave similarly.

Since normal dietary conditions would not necessarily be reflected by a 10% alginate concentration in the diet, the studies were repeated using 1% of the test compounds. Significant enhancement of lead in the blood and kidneys was only found with the 1% Manucol/LK diet. The other diets showed slight enhancement but the differences were not significant (Table 60, Figure 30).

Table 58

Composition of Alginates

1. Manucol LK -

Sodium Alginate - mainly mannuronic acid residues and viscosity approximately 90 cps. It is used as a food additive and in pharmaceuticals as a thickening agent.

2. Manujel DJ -

Sodium Alginate - mainly guluronic acid residues and viscosity approximately 110 cps. It is used in gel formation.

3. C/BSF -

Calcium alginate has a very high content of guluronic acid residues and low viscosity. Used for prevention of radioactive strontium uptake.

Table 59

Uptake of Lead from 10% Alginate Diet

Diet	Pb Ingested (mg)	Mean Blood Pb $\bar{x} \pm$ S.D. (µg/100g)	"t" Test (p)	"U" Test (p)	Total Kidney Pb $\bar{x} \pm$ S.D. (µg)	"t" Test (p)	"U" Test (p)
Control	26	50.2 $\pm$ 24.7			11.98 $\pm$ 6.22		
10% Manucol/LK	16	128.8 $\pm$ 47.3	<.005	<.02	27.40 $\pm$ 3.79	<.001	<.004
10% Manujel	20	101.2 $\pm$ 17.7	<.005	<.02	24.53 $\pm$ 4.11	<.005	<.02
10% $\bar{C}$/BSF	26	75.3 $\pm$ 14.2	N.S.	N.S.	20.75 $\pm$ 3.07	<.02	N.S.

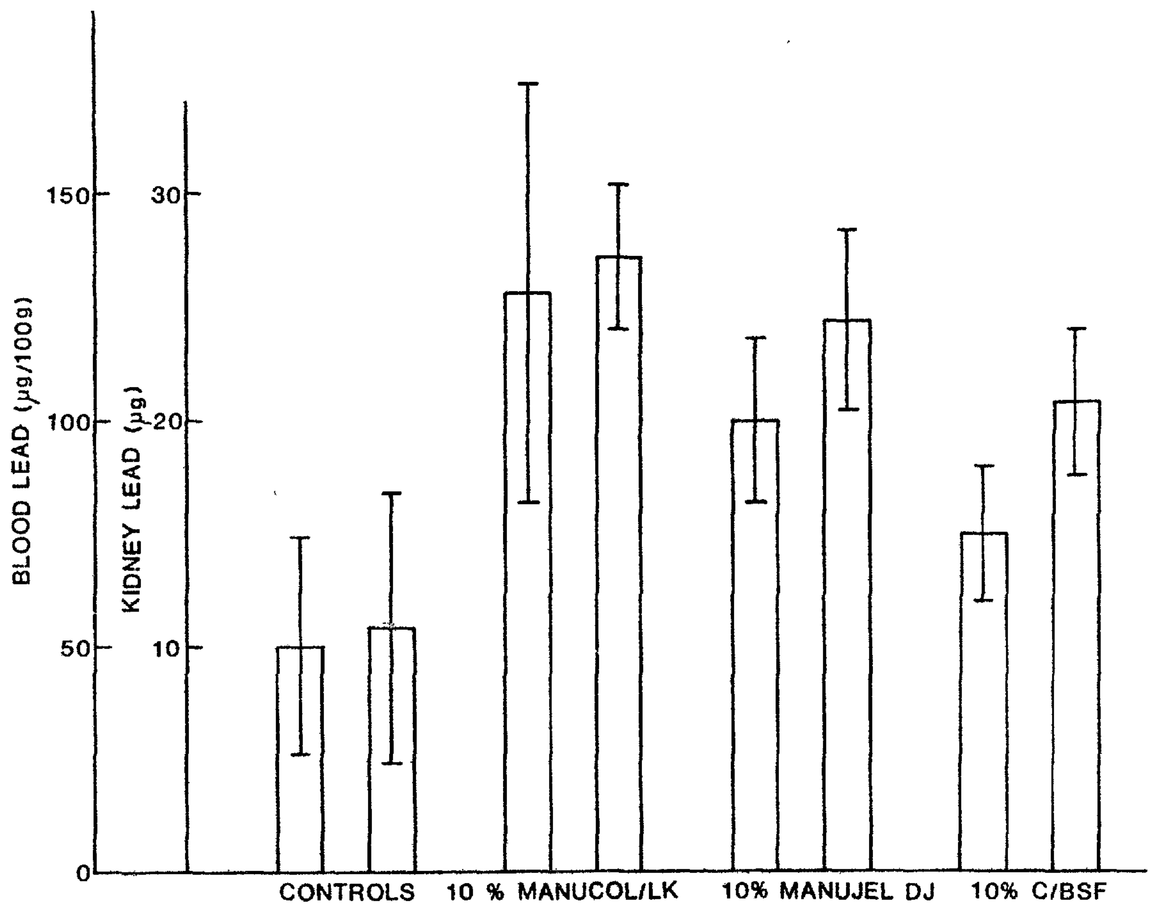


Figure 29

Blood and Kidney Lead Uptake from 10% Alginate Diets. Mean ± S.D. (n = 6).

Table 60

Uptake of Lead from 1% Alginate Diet

Diet	Mean Blood Pb $\bar{x} \pm$ S.D. (μg/100g)	"t" Test (p)	"U" Test (p)	Total Kidney Pb $\bar{x} \pm$ S.D. (μg)	"t" Test (p)	"U" Test (p)
Control	56.2 ± 8.9			17.84 ± 4.20		
1% Manucol/LK	84.2 ± 20.6	<.02	<.04	29.80 ± 6.75	<.005	<.008
1% Manujel	68.7 ± 12.3	N.S.	N.S.	20.40 ± 8.90	N.S.	N.S.
1% C/BSF	65.3 ± 13.1	N.S.	N.S.	22.76 ± 6.58	N.S.	N.S.

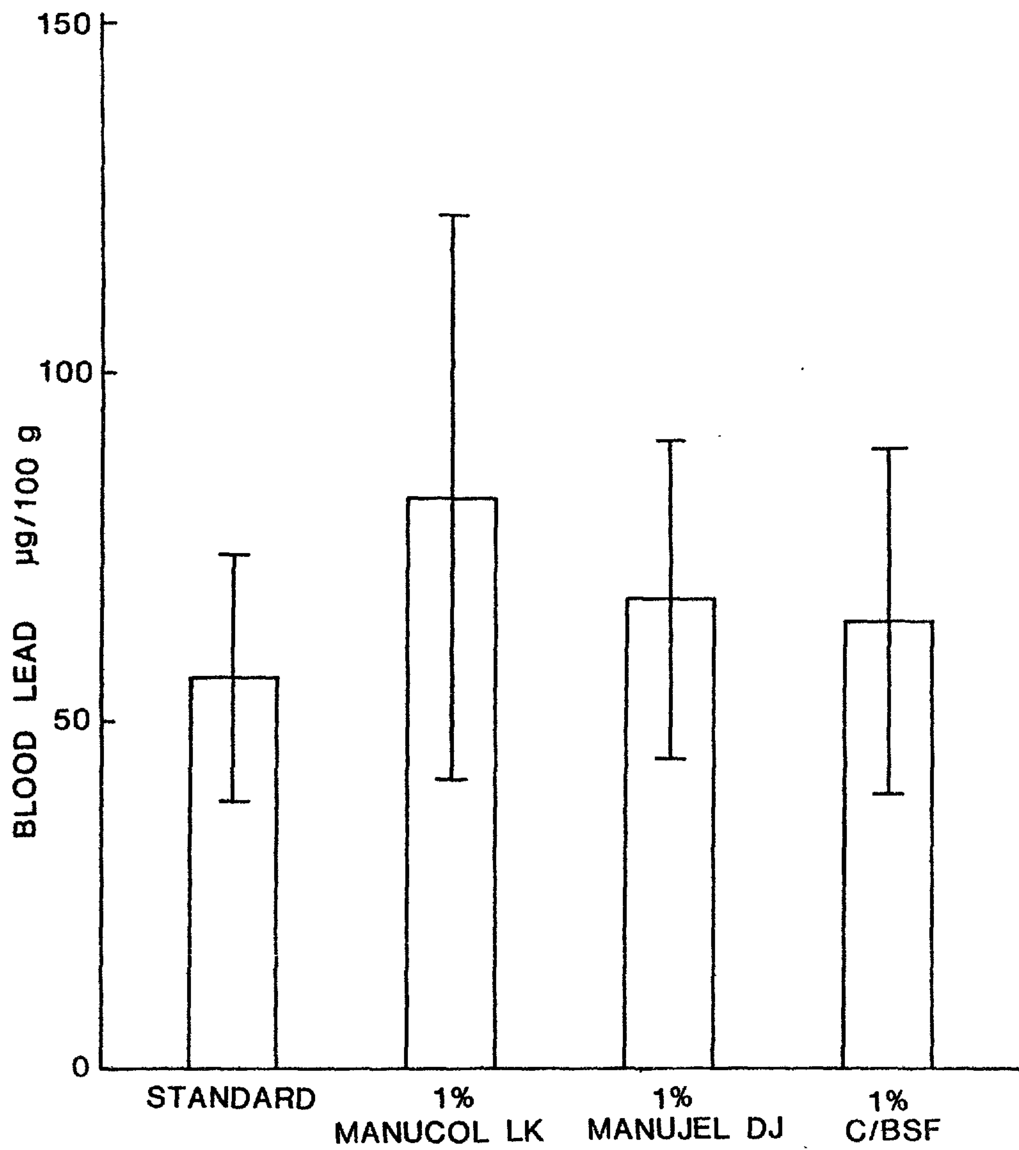


Figure 30

Blood Lead Uptake from 1% Alginate Diets.
Mean ± S.D. (n = 6).

Studies on pectins and phytates were also initiated by incorporating them into the diet to a concentration of 1%. The sodium salt of phytate was used to prevent unwanted effects from other minerals. Two sets of studies were undertaken using lead acetate and lead chloride. A significant increase was observed only for blood lead by means of the "t" test in the case of control versus 1% pectin. All other comparisons were not significant (Table 61).

Diet Consistency

Preliminary studies were conducted using 3 groups of 6 animals each. One group was fed a solid control diet without added lead, the second group was fed a solid diet containing 0.075% lead and the third group was fed a liquid diet consisting of the solid diet with 0.075% lead homogenised with 150ml of water for 100g of solid food. Blood lead concentration was increased 4 times and kidney lead content was also enhanced 5-fold in animals fed the liquid diet compared to animals receiving the solid diet with 0.075% lead (Table 62 and Fig. 31).

Further studies were conducted with diets of varying consistency to extend the range of results obtained. 12 rats were kept in all glass "Metabowl" metabolism cages. The metabolism cages were necessary to contain the liquid food without spillage and to enable the measurement of water intake. One control and 5 lead diets were prepared with varying proportions of water, each of the lead diets containing 0.075% lead in the dry diet.

Table 61

Uptake of Lead from 1% Phytate Diet and 1% Pectin Diet

Diet	Mean Blood Pb $\bar{x} \pm$ S.D. (µg/100g)	"t" Test (p)	"U" Test (p)	Total Kidney Pb $\bar{x} \pm$ S.D. (µg)	"t" Test (p)	"U" Test (p)
Control + PbAc	53.8 ± 7.9			8.75 ± 1.77		
1% Pectin + PbAc	86.0 ± 27.2	<.02	N.S.	9.97 ± 2.48	N.S.	N.S.
1% Phytate + PbAc	67.8 ± 16.5	N.S.	N.S.	10.58 ± 2.61	N.S.	N.S.
Control + $PbCl_2$	62.2 ± 6.5			8.18 ± 1.90		
1% Pectin + $PbCl_2$	69.8 ± 15.8	N.S.	N.S.	10.51 ± 3.45	N.S.	N.S.
1% Phytate + $PbCl_2$	51.8 ± 9.8	N.S.	N.S.	7.85 ± 1.80	N.S.	N.S.

Table 62

Effect of Water Content on Lead Absorption

Diet	Blood Pb (µg/100g)	"t" Test (p)	Total Kidney Pb (µg)	"t" Test (p)
Solid control without lead	8.2 ± 1.3		0.52 ± 0.9	
Solid control with 0.075% Pb	65 ± 34		8.36 ± 3.73	
Liquid lead diet (150 ml water : 100g lead diet)	250 ± 71	< .001	42.8 ± 15.2	< .001

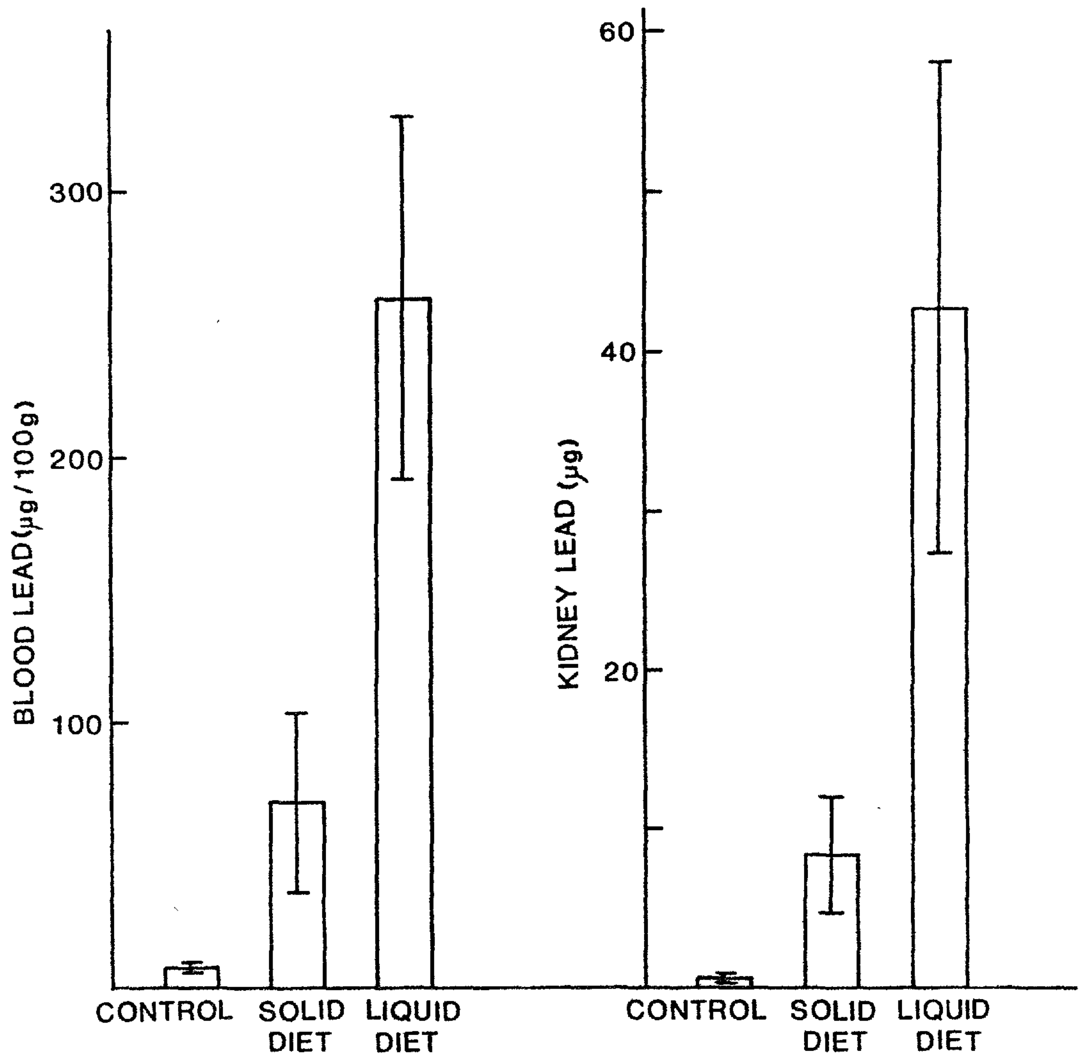


Figure 31

**Effect of Additional Water (150 ml to 100g Food) on Blood and Kidney Lead Concentration.
Mean ± S.D. (n = 6).**

2 rats were fed each diet for 48 hours while water was given ad libitum. The liquid diets were constantly stirred by magnetic stirrers to prevent the solids from settling down on the bottom of the feeder. After 48 hours these rats were killed and blood and kidneys were removed for analysis. Then another 12 rats were placed in the metabolism cages for the same experimental regime. This was repeated to give a total of 8 animals in each diet group. Results in Table 63 show that water intake decreased with added water to the diet. Although a large volume of liquid food was ingested by the animals fed the diets containing more water, approximately the same weight of dry diet and lead was ingested by all the groups. There was increasing lead uptake by the blood as the water content of the diet was increased (Fig. 32). However, comparison by means of the "t" test gave significant differences in lead uptake between only the diets with 1:1.5 and 1:3 diet : water ratio and the solid lead diet (Table 63).

Ligated Gut Loops

Varying Concentrations

There was a directly proportional relationship between lead transferred from the lumen across the gut wall and the concentration of lead in the initial perfusate up to a concentration of 10 μg/ml (Table 64, Fig. 33). Approximately half the initial concentration of lead was transferred up to the limiting value of 10 μg/ml. Subsequently only 15% and 2.3% of the initial perfusates containing 30 μg/ml and

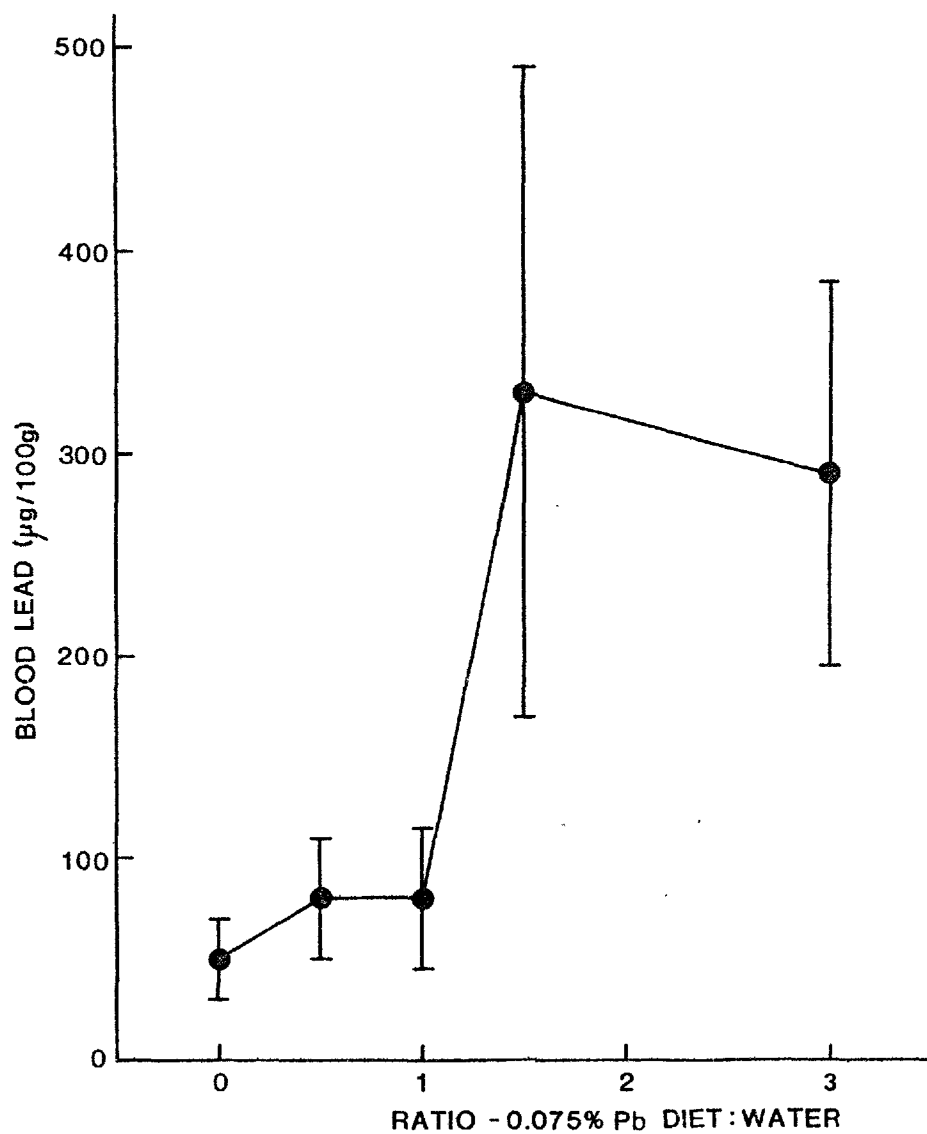


Figure 32

Effect of Varying Dietary Water Content on Blood Lead Concentration. Mean ± S.D. (n = 8).

Table 63

Effect of Diet Consistency on Lead Absorption

Diet	Water Ingested (ml)	Blood Pb (µg/100g)	"t" Test (p)	Total Kidney Pb (µg)	"t" Test (p)
Solid control without added lead	37.9	7.0 ± 3.0		0.40 ± 0.07	
Solid control with 0.075% lead	40.5	48.6 ± 17.7		9.68 ± 1.91	
Lead diet: Water					
1 : 0.5	23.5	80.3 ± 31.8	N.S.	14.96 ± 7.59	N.S.
1 : 1	13.4	83.1 ± 36.9	N.S.	14.30 ± 6.77	N.S.
1 : 1.5	10.9	354.0 ± 165.0	<.001	77.16 ± 37.29	<.001
1 : 3	6.0	289.0 ± 91.0	<.001	51.23 ± 28.90	<.001

Table 64

Effect of Varying Lead Concentrations on Lead Transfer Across the Intestinal Wall of Ligated Gut Loops

ug Pb/ml initial dose	Pb transported/hr % initial dose*	Pb on gut wall % initial dose*
0	9.4 ± 1.9	52.2 ± 16.2
0.1	36.5 ± 13.7	41.4 ± 5.4
0.5	35.5 ± 9.6	50.0 ± 7.8
1.0	49.4 ± 4.5	39.4 ± 3.5
2.0	49.0 ± 6.5	37.1 ± 3.9
6.0	41.7 ± 8.5	30.6 ± 8.3
10.0	51.9 ± 5.8	24.0 ± 3.4
30.0	14.8 ± 7.5	45.5 ± 21.4
200.0	2.3 ± 1.1	34.6 ± 6.5

* Mean ± S.D. (n = 6)

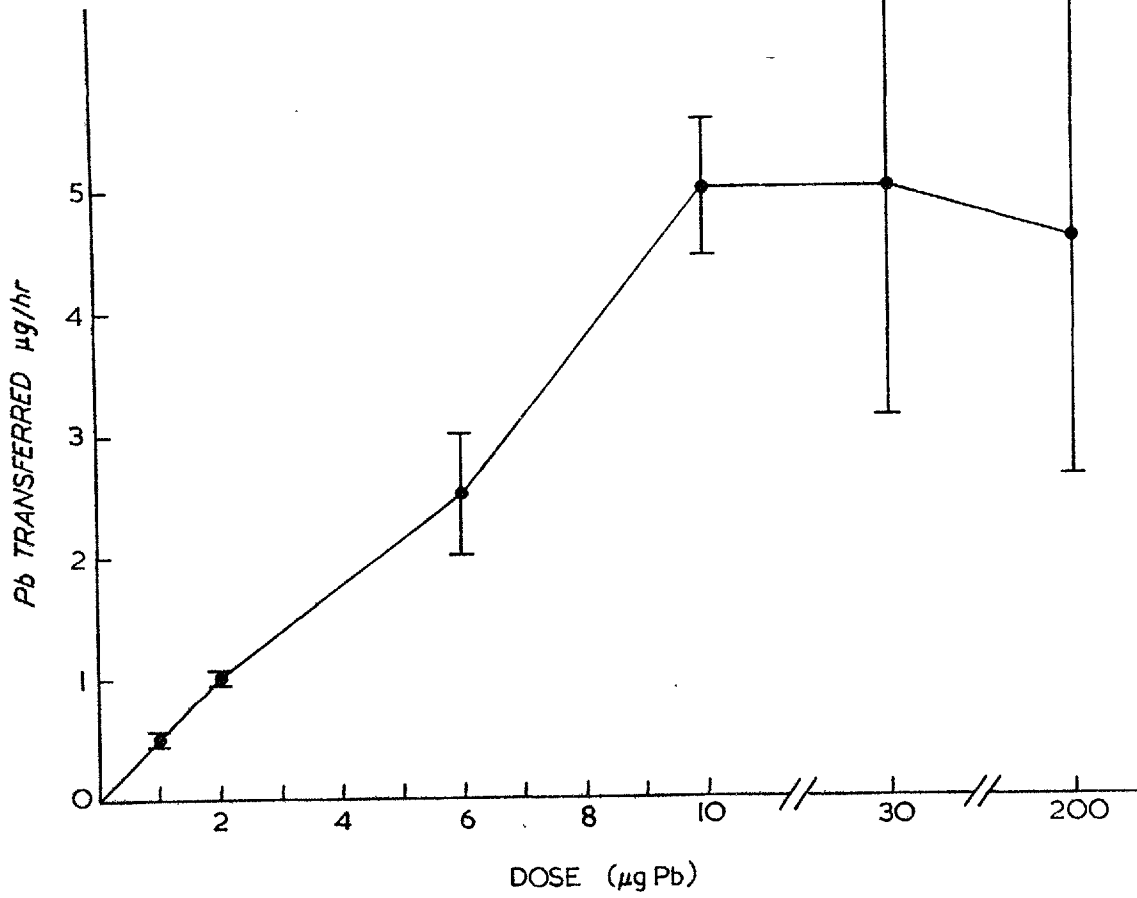


Figure 33

Rate of Lead Transport from Intestinal Loops in vivo as a Function of Initial Dose. Mean ± S.D. (n = 6).

200 µg/ml were transferred (Table 64). Thus the amount of lead transferred remained at a constant value of 5 µg/hr with inital perfusate concentrations of 10 µg/ml or greater. There appeared to be a rate-limiting process in the transfer of lead across the gut wall with a maximum rate of transfer of 0.3 µg Pb/cm/hr.

Varying Times

A constant amount of lead (1 µg/ml) was allowed to be transferred for varying periods of time (Fig. 34). The amount of lead transferred increased proportionally with time up to 40 minutes after which it remained relatively constant for periods lasting up to 2 hours (Table 65). Conversely, the gut wall appeared to be saturated with 0.4 to 0.5 µg lead regardless of the duration the perfusate remained in the intestinal loop.

Varying Calcium

Adding calcium to the 1 µg/ml lead solution reduced the transfer of lead from 28.3% to 8.5% (Table 66). Increasing the concentration of calcium in the perfusate resulted in progressive reduction of lead transferred from the lumen and a corresponding decrease in the amount of lead associated with the gut wall (Table 66). The maximum reduction of 70% in the amount of lead transferred was observed at a calcium concentration of 300 µg/ml (7.5 mM) and remained at that level up to 1000 µg/ml (25 mM) (Fig. 35). The lead associated with the gut wall showed a similar relationship with the maximum reduction occurring with the addition of

Table 65

Effect of Varying Incubation Time on Lead Transfer Across the Intestinal Wall of Ligated Gut Loops

Time (mins)	Pb transported % initial dose*	Pb on gut wall % initial dose*
10	11.2 ± 3.7	49.0 ± 6.0
20	18.2 ± 8.0	48.4 ± 13.5
30	28.3 ± 9.8	41.9 ± 2.1
40	43.0 ± 6.3	43.2 ± 3.3
50	45.8 ± 6.9	44.6 ± 3.6
60	49.4 ± 4.5	39.4 ± 3.5
120	42.8 ± 5.4	35.7 ± 3.5

* Mean ± S.D. (n = 6)

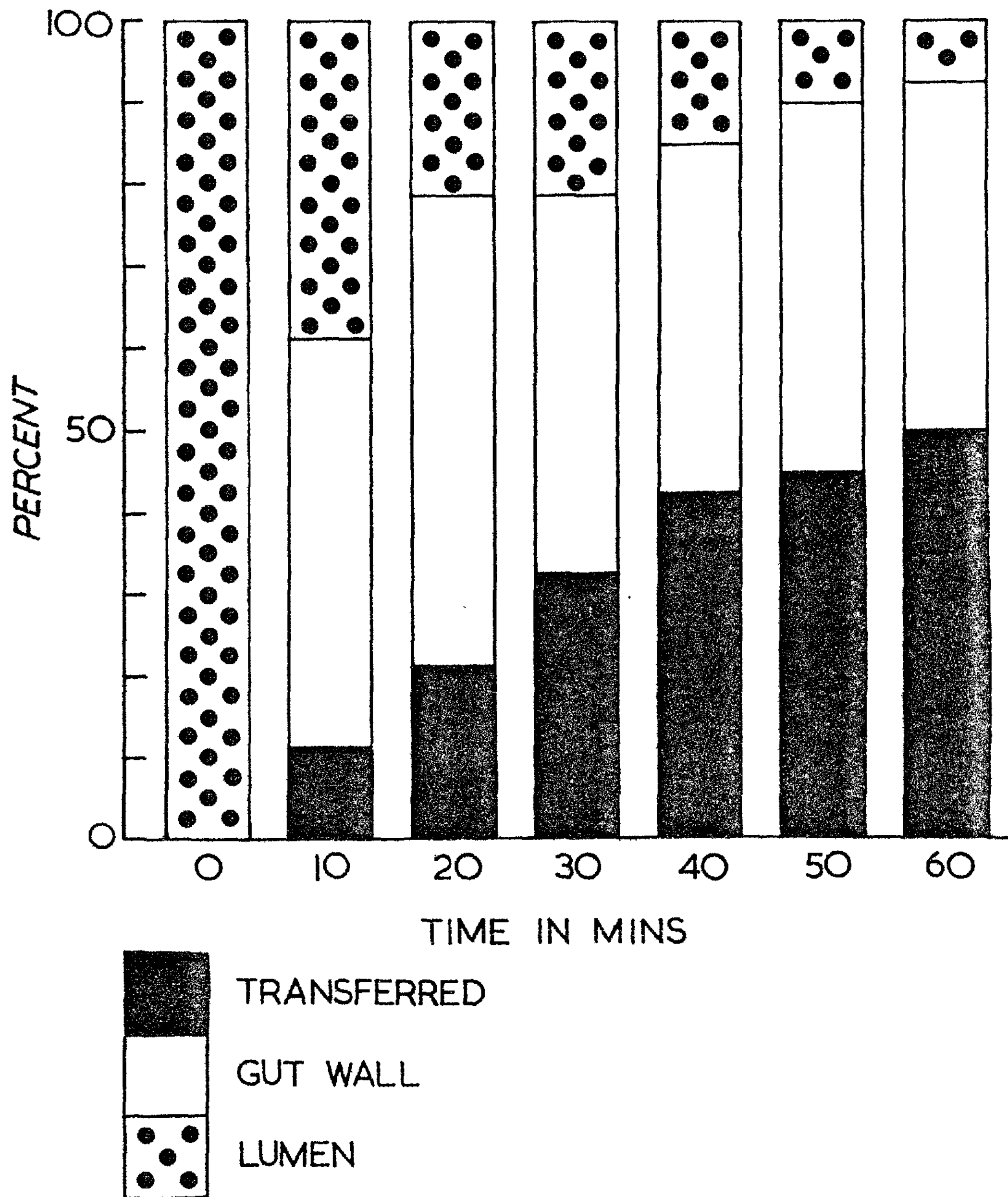


Figure 34

Distribution of 1 μg Lead in Intestinal Loops in vivo as a Function of Time. (n = 6).

Table 66

Effect of Varying Concentrations of Calcium Added to 1 μg Pb/ml Incubated for 30 Minutes in Ligated Gut Loops

Ca μg/ml		Pb transported %initial dose*	"t" Test (p)	Pb on gut wall % initial dose*	"t" Test (p)
0		28.3 ± 9.8		41.9 ± 2.1	
100	(2.5 mM)	21.7 ± 8.1	<.001	37.5 ± 3.8	<.001
200	(5.0 mM)	10.6 ± 5.8	<.001	27.7 ± 8.0	<.001
300	(7.5 mM)	8.5 ± 4.2	<.001	24.6 ± 3.8	<.001
700	(17.5 mM)	8.2 ± 4.7	<.001	24.0 ± 4.8	<.001
1000	(25.0 mM)	5.8 ± 1.6	<.001	20.1 ± 3.4	<.001

* $\bar{x}$ ± S.D. (n = 6)

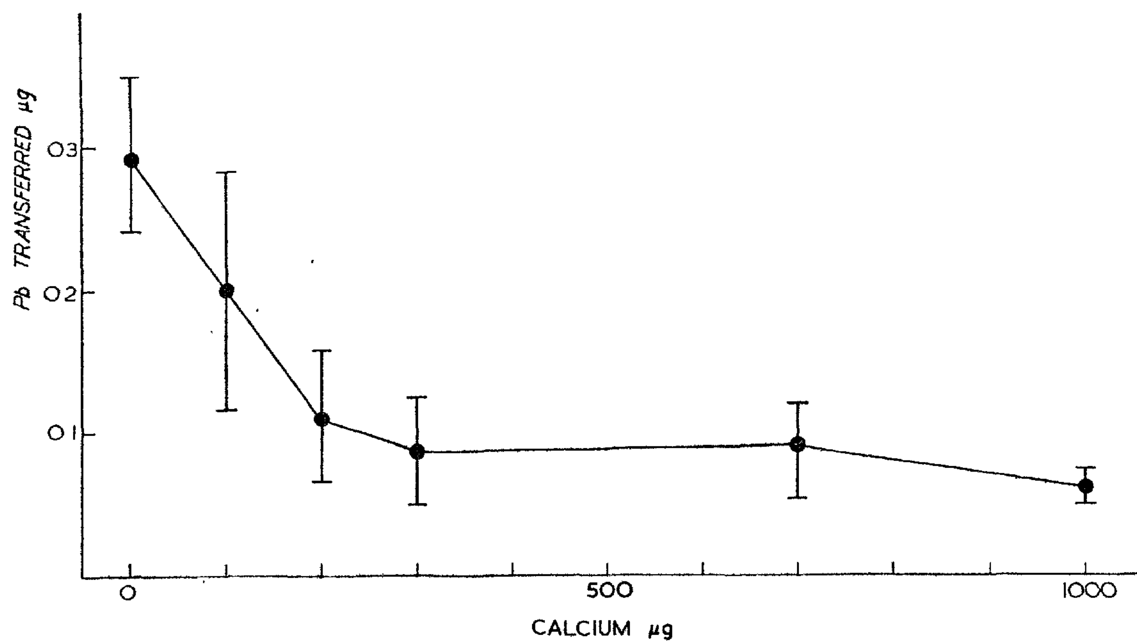


Figure 35

Influence of Varying Concentrations of Calcium on Lead (1 µg) Transport from Intestinal Loops in vivo Incubated for 30 Mins. Mean ± S.D. (n = 6).

300 μg Ca/ml and greater (Fig. 36).

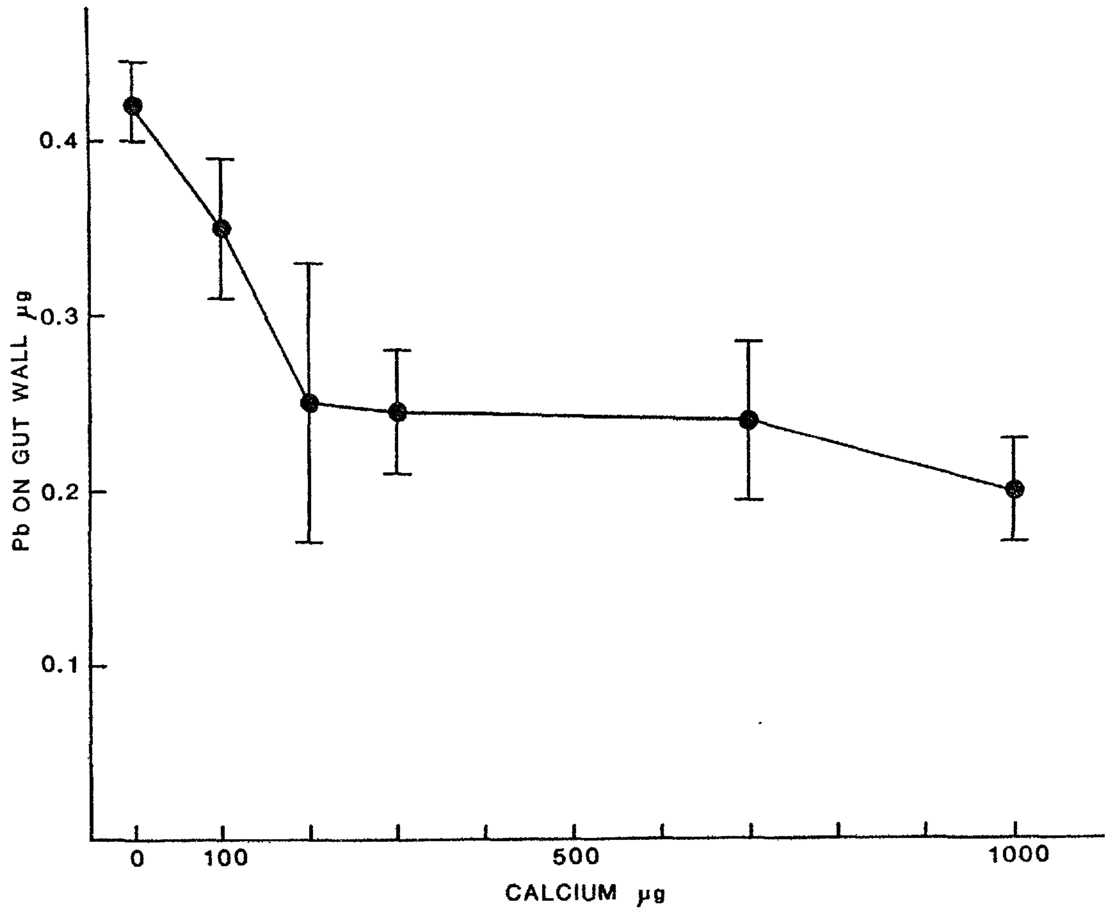
To confirm the results obtained with 1 μg Pb/ml, calcium was added to 10 μg Pb/ml (Table 67). Similar reductions in the amount of lead transferred (Fig. 37) and the amount of lead associated with the gut wall (Fig. 38) were observed with increasing calcium concentration. The maximum reduction of 74% in the amount of lead transferred was observed at a calcium concentration of 400 μg/ml (10 mM) and remained at that level up to 1000 μg/ml (25 mM) (Fig. 37). The amount of lead associated with the gut wall was diminished by 25% at a calcium concentration of 100 μg/ml (2.5 mM) and 52% at 1000 μg/ml (25 mM) (Fig. 38).

Varying Phosphate

The addition of phosphate to 1 μg Pb/ml (Table 68) reduced lead transport at very low concentrations of phosphate (0.025 mM). No relationship between phosphate concentration and reduction in the amount of lead transferred was observed. The presence of phosphate, however, had no effect on the amount of lead associated with the gut wall suggesting that the mechanism for the inhibition of lead transport by phosphate differs from that of calcium.

With increasing lead concentration, the inhibition of lead transport by a constant dose of phosphate (0.025 mM) was decreased from 87% at 1 μg Pb/ml to 34% at 10 μg Pb/ml (Table 69). The amount of lead associated with the gut wall was not affected by the presence of phosphate.

Confirmation of these results was provided by incorporating lead phosphate into the rat diets. Blood and



<u>**Figure 36**</u>

Influence of Varying Concentrations of Calcium on Lead (1 µg) Associated with the Gut Wall of Intestinal Loops in vivo Incubated for 30 Mins. Mean ± S.D. (n = 6).

Table 67

Varying Concentrations of Ca added to 10μg Pb/ml incubated for 30 mins

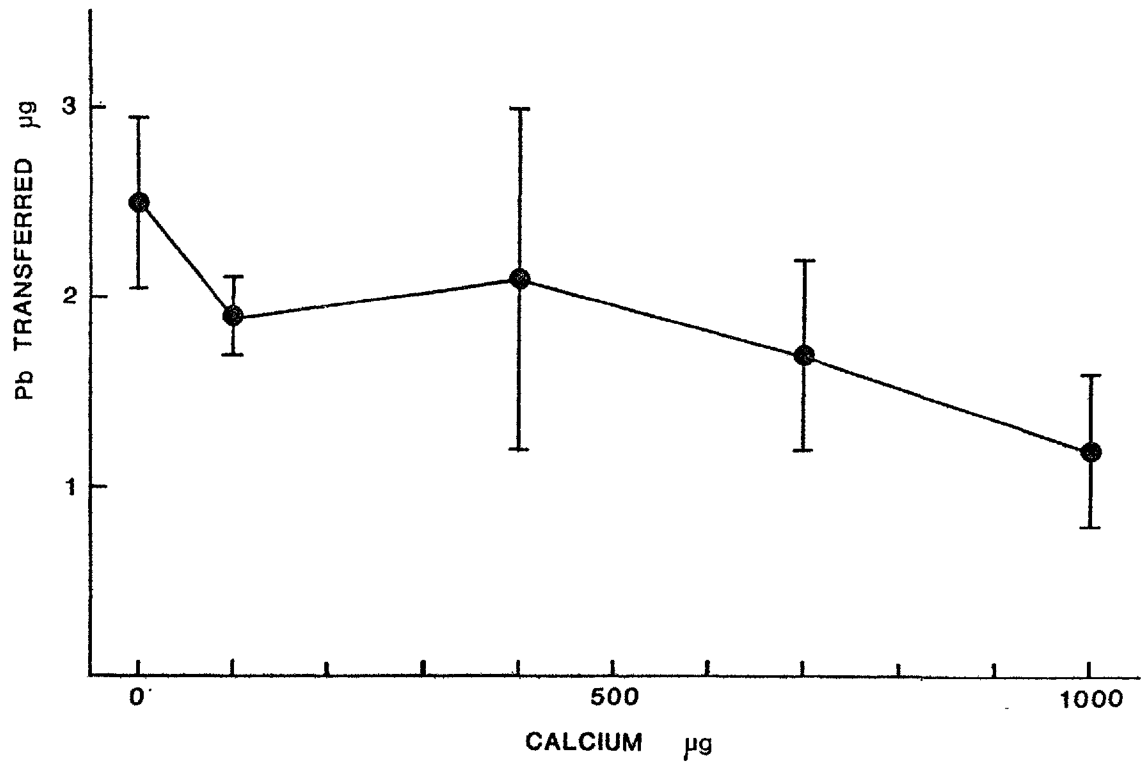
Ca μg/ml	Pb Transported % Initial Dose	Pb on Gut Wall % Initial Dose
0*	26.5 ± 11.6	25.1 ± 4.3
100**	23.5 ± 8.2	18.9 ± 2.1
400*	6.9 ± 1.9	21.6 ± 9.5
700+	7.6 ± 4.9	17.1 ± 4.9
1000+	8.0 ± 4.0	12.1 ± 4.2

* n = 17

** n = 5

+ n = 6

All values are mean ± S.D.



<u>Figure 37</u>

Influence of Varying Concentrations of Calcium on Lead (10 µg) Transport from Intestinal Loops in vivo Incubated for 30 Mins. Mean ± S.D. (n = 6).

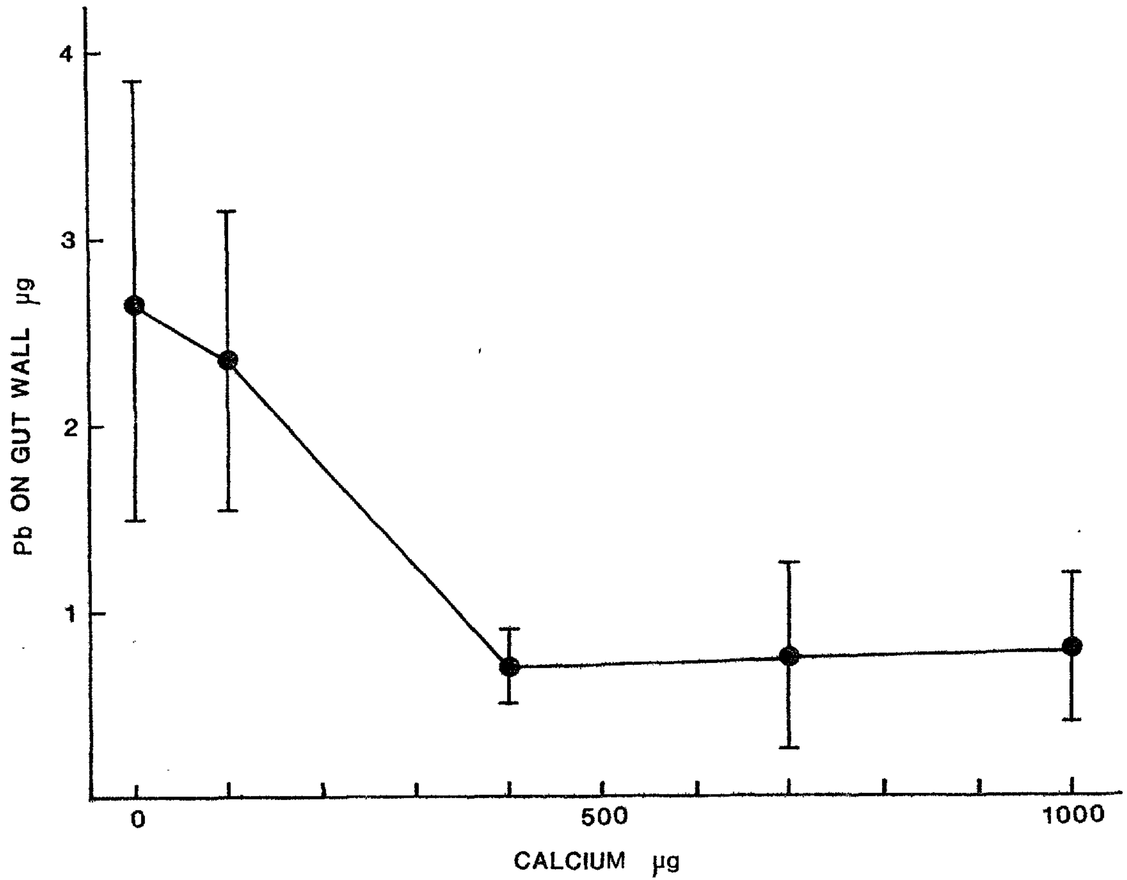


Figure 38

Influence of Varying Concentrations of Calcium on Lead (10 µg) Associated with the Gut Wall of Intestinal Loops in vivo Incubated for 30 Mins. Mean ± S.D. (n = 6).

Table 68

Varying Concentrations of PO_4 added to 1μg Pb/ml incubated for 30 mins

mM PO_4	Pb Transported % Initial Dose	Pb on Gut Wall % Initial Dose
0**	23.3 ± 7.8	40.7 ± 8.8
0.025*	3.1 ± 2.5	35.2 ± 6.8
0.25*	8.7 ± 6.2	45.8 ± 8.8
2.5*	1.4 ± 0.5	34.0 ± 9.5
10.0*	4.2 ± 1.5	39.6 ± 10.9

** n = 33

* n = 6

Table 69

Effect of 0.025 mM PO_4 on Lead Transfer Across the Intestinal Wall of Ligated Gut Loops

Initial Perfusate	Pb Transported % Initial Dose	% Reduction of Pb Transport by PO_4	Pb on Gut Wall % Initial Dose
1 μg Pb/ml**	23.3 ± 7.8		40.7 ± 8.8
1 μg Pb/ml + 0.025 mM PO_4*	3.1 ± 2.5	87	35.2 ± 6.8
5 μg Pb/ml*	29.8 ± 10.5		31.1 ± 5.1
5 μg Pb/ml + 0.025 mM PO_4*	15.1 ± 4.5	49	37.1 ± 12.8
10 μg Pb/ml*	26.9 ± 15.8		27.8 ± 4.5
10 μg Pb/ml + 0.025 mM PO_4 *	17.8 ± 6.7	34	32.5 ± 6.8

** n = 33

* n = 6

kidney lead concentrations of animals fed lead phosphate were half and two-thirds those of animals fed lead chloride (Table 70).

Inhibitors

The presence of 5×10^{-5} M 2, 4 dinitrophenol (an uncoupler of oxidative phosphorylation) resulted in diminished lead transport and a slight decrease in the lead associated with the gut wall (Table 71). Addition of 0.5 mM ouabain (a specific inhibitor of Na^{+} transport which acts by competing with K^{+} for access to the enzyme-phosphate-Na^{+} complex) resulted in similar diminution of lead transport but an increase in the lead associated with the gut wall. These findings suggest that lead transport may be an active transport process requiring energy from oxidative phosphorylation or it may be coupled to the sodium transport pump. By contrast, the mechanism by which lead is bound to the gut wall does not appear to be coupled to the sodium pump.

Intestinal Segments

The ileal segment only transported 15% of the amount of lead transported by the 15 cm duodenal-jejunal segment. The amount of lead associated with the gut wall, however, remained constant (Table 72).

Vitamin D

The intravenous adminstration of 25ng of 1,25 dihydroxycholecalciferol 4 hours prior to the lead transport studies resulted in a 29% increase in lead transport. However, no effect was observed in the amount of lead associated with the gut wall (Table 73).

Table 70

Comparison of Lead Absorption for Lead Phosphate and Lead Chloride

	Blood Pb µg/100g*	"t" Test (p)	Kidney Pb µg/g*	"t" Test (p)
Lead chloride	49.4 ± 5.3		9.67 ± 1.24	
Lead phosphate	35.5 ± 5.3	$<.005$	5.03 ± 0.57	$<.001$

* $\bar{x} \pm$ S.D. (n = 6)

Table 71

Effect of Various Inhibitors on Lead (1μg/ml) Transport from Ligated Gut Loops Incubated for 30 Mins

Active Transport Inhibitors	Pb Transported % Initial Dose	"t" Test (p)	Pb on Gut Wall % Initial Dose	"t" Test (p)
0 (n = 33)	23.3 ± 7.8		40.7 ± 8.8	
5×10^{-5} M 2, 4 dinitrophenol (n = 11)	17.5 ± 7.0	<.001	38.1 ± 4.1	<.025
0.5 mM Ouabain (n = 6)	16.6 ± 4.3	<.001	50.7 ± 6.6	<.001

Table 72

Comparison of Pb Transport Across Two 15 cm Intestinal Segments

	Pb Transport % Initial Dose	"t" Test (p)	Pb on Gut Wall % Initial Dose	"t" Test (p)
Duodenum and jejunum	23.3 ± 7.8	-	40.7 ± 8.8	-
Ileum	3.4 ± 2.1	< .001	46.3 ± 9.1	N.S.

Table 73

Effect of Parenteral 1, 25 Dihydroxycholecalciferol on Lead (1 ug/ml) Transport from Ligated Gut Loops Incubated for 30 Mins

	Pb Transported % Initial Dose	"t" Test (p)	Pb on Gut Wall % Initial Dose	"t" Test (p)
1 ug Pb/ml + 95% ethanol (n = 6)	18.7 ± 10.5	-	40.5 ± 7.5	-
1 ug Pb/ml + 25 ng 1, 25 di-OH Vitamin D (n = 5)	24.1 ± 7.2	$< .02$	39.5 ± 5.1	N.S.

DISCUSSION

The results obtained show that certain nutritional factors have a marked effect on the acute absorption of lead from the gastrointestinal tract. There are 3 possible mechanisms by which nutritional factors could affect the absorption of lead from the gut. Firstly, by the binding of lead to a poorly absorbed factor or its derivatives thus rendering the lead unavailable for absorption; secondly, by the interaction of the factor with the cellular processes regulating lead absorption; thirdly, modification of the metabolic status of tissues with an affinity for absorbed lead. Since the experimental period used in this study was acute and the diets isocaloric, metabolic adaptation is unlikely to account for the results obtained. The effects observed are more likely to be due to the nutritional factors acting directly on lead absorption from the gut.

It is not known whether lead absorption involves single or multiple pathways. The results from the ligated gut loop studies and the protein studies seem to indicate that multiple routes might be involved. Both low and high protein intake enhanced the absorption of lead. Similar results were obtained by Milev et al (1970) although they found increased lead in all the organs of animals on a high protein diet, whereas in this study an increased lead content was found in only the kidneys and femurs. Gontzea et al (1970) suggested that a low protein diet might result in impaired detoxication mechanisms of the liver thus allowing more lead to be accumulated. This hypothesis, however, does not explain the

enhanced lead uptake associated with a high protein diet. The increased lead absorption from a high protein diet may represent a different mechanism of interaction which may be unrelated.

Since a high protein diet markedly increases the absorption of calcium (Harper, 1971), presumably through the formation of soluble complexes with amino-acids, it might be possible that the same process could increase the absorption of lead, an effect which would be at odds with the apparent ability of protein to protect against the toxic effects of lead (Baernstein and Grand, 1942; MacDonald et al, 1953). However, the defence mechanism associated with protein probably involves sulphydryl groups which facilitate excretion of lead like standard chelating agents. The overall dynamics of such a system may explain the observations of Milev et al (1970) and the present data that both high and low protein concentrations increase intestinal lead absorption.

The role of increased dietary fat in enhancing the absorption of lead and potentiating the effect of low dietary minerals has not previously been described and is difficult to explain. Low fat diets (0% and 2.5%) had no effect while high fat diets increased lead uptake. Thus it may be possible that the increased fat in the lumen could form a more soluble complex of lead-bile-fatty acid similar to the bile-fatty acid-calcium complexes described by French (1942). Fatty acids could also interact with the cellular proceesses regulating lead absorption. Confirmation of this could be obtained with gut loop studies by measuring transport across

the gut wall from fatty acid and ^{203}Pb preparations.

In contrast to the results presented here, Kello and Kostial (1975) stated that they did not find a clear correlation between fat and gastrointestinal absorption of lead. However, there were many variables involved in their study. Their data showed that the group of animals fed on cows' milk and which also absorbed most lead, ingested 3 times more water, less calcium, less iron and more fat than the other 3 groups fed on solid food and water, solid food and milk and powdered milk and water. All of these factors would have resulted in increased lead absorption. The excess water, decreased calcium and increased fat have all been demonstrated in this study to increase lead absorption (Barltrop and Khoo, 1975). Decreased dietary iron has also resulted in increased lead uptake in studies of 10 weeks' duration (Six and Goyer, 1972). Thus the excess dietary fat intake of the animals ingesting cows' milk must have contributed partly to the increased lead uptake that was observed.

The studies with fats of differing fatty acid content suggest that there may be a relationship between lead absorption and the dietary content of medium chain fatty acids (butterfat) whereas by contrast, little enhancement was observed from fats containing an excess of unsaturated fatty acids (rapeseed oil). However, this conclusion can only be tentative because of the limited range and complex nature of the different fats studied. Although the enhanced absorption associated with butterfat would seem to be of

particular relevance to the normal human diet, further studies are required for a more detailed evaluation of the effects of individual fatty acids. This would require the use of pure triglycerides containing fatty acids of varying type incorporated into the diet or alternatively into the gut loop to study the interaction of fatty acids and lead in the lumen and the mechanism by which increased dietary fat increases lead absorption.

The 50-fold increase in blood lead retention by rats ingesting a low mineral/high fat diet is remarkable and strongly supports the contention that dietary factors may be important in determining the response of individuals to a given environmental exposure. Since the animals receiving the low mineral/high fat diets had blood lead retention which were 50 times control values, then very modest changes in the diet could result in marked changes in blood lead. In human populations a blood lead concentration of less than twice the control value would be regarded as significant.

The finding that increased dietary fat potentiates the effect of low minerals on lead uptake is consistent with the study of Boyadzhiev (1959) who showed that increased dietary fat also potentiates the effect of low protein on lead poisoning. However, the mechanism by which these effects occur is at present unknown. It is possible that the lead, fatty acids and perhaps bile salts could form a soluble diffusible complex in the intestine which might be more readily absorbed in the absence of other ions or amino acids in the lumen. Fatty acids could also interact with the cellular processes regulating lead absorption more effectively

in the absence of other ions or amino acids. Further work should therefore involve the use of fatty acids, amino acids, ions like calcium and phosphate and lead in ligated gut loop preparations to elucidate their possible interactions.

Both low and high vitamin diets did not have any effect on lead uptake. This is contrary to the findings in the relatively long-term studies where dietary deficiency in vitamin C (Pillemer et al, 1940) and vitamin E (Levander et al, 1975) enhanced lead toxicity. Increased dietary vitamin D resulted in enhanced lead absorption (Sobel et al, 1940). Vitamin B_6 and B_{12} have also been applied to reduce clinical lead poisoning symptoms (Harada et al, 1955; Pokotilenko, 1964). However, the findings of this study would agree with those of Dannenberg et al (1940) and Evans et al (1943) who found no effects of vitamin C on lead toxicity. Kao and Forbes (1973) have also reported that a ten-fold dietary excess of niacin provided no significant protection against lead poisoning.

The short-term nature of the present experiments means it is unlikely that the vitamin status of the animals would have been significantly changed during an interval of only 48 hours. Thus these findings suggest that the effect of vitamins do not result from an action on the gut. While it would be possible to precondition the animals by maintaining them on diets of modified vitamin content for relatively long periods before the study, such a method might result in adaptive changes in the animals. Any observed differences then might not be due to changes at the gut level

but might represent differences in the affinity of lead for particular tissues after absorption. Since the purpose of this study was to investigate the direct effect of different nutritional factors on lead absorption from the gastrointestinal tract, dietary preconditioning of the experimental animals was not attempted.

The cellulose studies suggested that there was no effect of fibre on the absorption of lead. The high fibre diet containing 12% cellulose (Table 12) would have been expected to decrease lead absorption by the extra fibre providing intraluminal sites for lead-binding. However, since cellulose might not necessarily act like the natural fibre found in the normal rat diet, phytates were also studied. Animals ingesting a diet containing 0.075% lead and 1% phytates did not show any difference in blood and kidney lead from animals ingesting a diet containing 0.075% lead alone. This was unexpected because high phytate diets decrease gastrointestinal absorption of calcium by the extra phytate acting as a chelating agent in the lumen (Watney et al, 1971; Wills et al, 1972; Holmes et al, 1973; Reinhold et al, 1973). The same mechanism might have been expected to decrease lead uptake by binding lead in the lumen thus rendering it less available for absorption. However, the decreased availability of calcium might have lead to increased absorption of lead, an effect which might balance any decreased lead uptake as a result of the added phytate.

Enhancement of lead absorption was observed with 10% alginate-supplemented diets. This finding was again unexpected since the metal-binding capacity of the alginates

is well known in the case of calcium and strontium, and lead was expected to behave similarly. The alginates had been shown to decrease calcium and strontium uptake (Kostial et al, 1969; Sutton et al, 1971). Since a 10% alginate diet was too high to be realistic, the studies were repeated with 1% alginate diets. Enhancement of lead absorption was again observed with the most marked increase in the case of Manucol.

Although comparison of blood and kidney lead concentrations of animals ingesting 1% pectin and unmodified diets did not result in any significant difference, there appeared to be a slight increase in blood lead with the pectin diet. This conflicts with the previous report by Arkhipova (1964) which claimed that injected pectins might serve as prophylactics in the prevention of lead poisoning.

The findings obtained with phytates, alginates and pectins are unusual because they are among the few agents which enhance lead absorption. The mechanism of this action is unknown but might reflect changes in the physical and chemical properties of the intestinal contents thus changing the viscosity and transit time of the contents and perhaps the solubility of the lead compound.

Although increased lead absorption has been demonstrated to occur with the addition of alginates and pectins to the diet, these are not major and are insufficient to constitute a hazard to the human population. In these studies a dosage level of 1% was used and although this might be attained in the case of individual items in the human dietary intake, it

is unlikely that this value would be attained for the total dietary intake. However, the findings indicate that alginates which can be used in the reduction of radiostrontium absorption cannot be used for the same purpose in the case of lead.

The results from the mineral studies have identified the principal minerals which modify the absorption of lead as calcium, phosphorus and magnesium. The calcium and phosphate effects were continuous over the whole range of concentrations studied from 0 to 4 times control values and appeared to be additive. These findings are consistent with those reported by Six and Goyer (1970) for calcium-deficient diets in long-term animal studies. In the present work, the dietary exposures were relatively brief (48 hours), therefore excluding the possibility of metabolic adaptation in the animal. The effects induced would appear to be occurring at the gut level rather than by modification of the animals' mineral metabolism which would operate in long-term studies. This would be consistent with the observations of Lederer and Bing (1942) that lead retention after intraperitoneal injection of lead into rats was not affected by dietary calcium and phosphorus levels. Only the uptake of orally ingested lead was affected by the calcium content of the diet.

A low calcium diet is known to modify the absorption of other minerals, for example magnesium (Morris and O'Dell, 1963) and strontium (MacDonald et al, 1952, 1955). It is therefore possible that a common pathway exists for the absorption of calcium and other minerals including lead from

the gut. This hypothesis agrees with Schroeder's (1965) proposal that as a general rule the absorption of trace metals by plants, lower animals, fish and mammals is inversely related to the concentration of calcium in the medium from which it is being absorbed. He suggested that there might be a cellular transport mechanism common to all living things which is saturated by calcium ions and which regulates the exchange of other cations from the immediate environment. The work of Tidball (1964) provides some support for this hypothesis by showing that magnesium and calcium loosely bound in the structure of the mucosal membrane can regulate the aqueous permeability of the intestinal epithelium of the rat.

The significance of dietary calcium deficiency is increased by the possibility that calcium deficiency may be involved in the production of a preference for lead-containing water in rats. Weanling rats on a calcium deficient diet were found to voluntarily ingest toxic amounts of lead offered in their drinking water (Snowdon and Sanderson, 1974). A nutrient deficiency induces an animal to seek other food substances, sampling until one source relieves the aversive symptoms produced by the deficiency. Rozin and Kalat (1971) suggested that such an animal would thus continue to ingest this symptom-relieving food. Since calcium (Snowdon and Sanderson, 1974) and iron (Watson et al, 1958) deficiencies have been suggested to be responsible for pica, it is possible that the search for materials containing these minerals may inadvertently result in the simultaneous ingestion of lead by the child.

Clarkson and Kench (1958) suggested that phosphate lowers plasma lead levels by promoting increased binding of peptized lead phosphate to erythrocytes so rendering it inert. The lead integration process proposed by McRoberts (1973) would be due to a similar mechanism. In the gut lumen however, the phosphate probably forms an insoluble lead phosphate which would render the lead less readily absorbed. The gut loop studies have shown this to be the case. Increasing phosphate in the intestinal loop decreased the amount of lead transferred across the gut wall but did not affect the association of lead with the gut wall. In contrast, increasing calcium decreased both the lead associated with, as well as transferred across the gut wall, which would suggest the existence of a competitive mechanism by which calcium affects lead absorption. Such a mechanism does not appear to exist for phosphate.

Although the total omission of magnesium from the diet showed some effect, the enhancement of lead absorption was small. The lack of effect of iron is of interest and conflicts with a previous report in long-term studies (Six and Goyer, 1972). These authors reported an increase in lead absorption with iron-deficient diets over a period of 10 weeks. Kochen and Greener (1975) also found that iron-supplemented rats had a decreased intestinal absorption of lead. The evidence suggests that the enhancement of lead uptake in iron-deficiency is the consequence of metabolic adaptation rather than a direct effect at the gut level.

The results of the study involving liquid diets show that lead is absorbed to a greater extent from liquid diets than from solid diets. This observation is important because it partly explains the increased lead absorption in rats fed a milk diet (Kello and Kostial, 1973). There was a significant difference in the amount of water ingested by the 4 experimental groups of animals. The group with the greatest lead uptake had also ingested the most water. As the present results indicate, enhancement of lead absorption up to 7 times in the kidneys and blood (Table 63) can be obtained with liquid diets. The role of water in the modification of lead absorption has not previously been studied. The finding that ingestion of a more liquid diet without any corresponding increase in the weight of solid food or volume of water ingested can result in increased lead absorption raises questions about the validity of feeding lead to experimental animals in their drinking water to study the effects of nutritional factors which are incorporated in their solid diets.

This finding also supports Patterson's (1965) suggestion that lead in water was more readily absorbed than lead in solid food.

From the maximum rate of transfer of 0.3 µg Pb/cm/hr in the gut loop, the daily maximum total uptake of lead into the body can be estimated. Assuming the small intestine has similar rates of transfer along its whole length of 80 cm, the maximum uptake would be of the order of 500 µg Pb/day. Since a 100g rat consumes 15g of food per day and there is 0.6% absorption of dietary lead (Table 9), the greatest

effective dose in the diet would be 0.6g Pb/100g food. Other investigators have used greater doses but it is uncertain whether the greater dietary lead dose increased lead uptake substantially. There would appear to be a rate-limiting step involved in the process of lead transfer across the gut wall.

Using the low dosage of 1 µg Pb there was a maximum of 50% uptake from the gut loop (Fig. 37). In the studies involving dietary lead, there was only 0.6% uptake. This difference may have been due to the other nutritional components present in the normal diet since the intestinal loop only contained lead in isotonic saline.

The proportion of lead taken up by the gut wall of the intestinal loop in vivo was a constant of 50% of the initial dose and was achieved within the first 10 minutes of incubation (Fig. 34). This finding in gut loop preparations in which the blood supply has been preserved intact differs from that of Cikrt (1970) in which increasing lead uptake in duodenal and ileal segments in vitro up to 60 minutes was demonstrated. The differences might be due to the different techniques used.

The decreased amount of lead transferred across the gut wall of the ileum (Table 71) contrasts with the data reported by Cikrt (1970) and Gruden and Stantic (1975). Cikrt (1970) observed that lead transport across the ileal wall was greater than across the duodenal wall. Gruden and Stantic (1975) reported that there was no difference in lead transport through and lead uptake by the gut wall throughout

the whole length of the gut. Data presented by Gerber and Derov (1974) showed that absorption of lead was higher in the jejunum than the duodenum or colon but they did not investigate lead absorption across the ileum.

In contrast to the studies by Gruden and Stantic (1975) which indicated that active transport of lead in the small intestine was negligible, the present results suggest that lead transport is an active process while lead binding to the gut wall is not. These conflicting reports mean that more studies have to be performed before the kinetics of lead transport across the intestinal wall can be defined.

The effect of calcium on lead transfer from the gut loop confirms the results from dietary studies where added calcium had been shown to reduce lead uptake. The corresponding decrease in the lead associated with the gut wall suggests a competitive mechanism for the effect of calcium on lead transport across the gut wall. Calcium is also known to modify the absorption of magnesium (Morris and O'Dell, 1963) and strontium (MacDonald et al, 1955). Thus it is possible that the effects of added calcium on lead transfer is due to the existence of a common pathway for the absorption of calcium and other minerals including lead from the gut. Such a pathway might also explain the effects of other nutritional factors on lead absorption.

Gruden et al (1974) found that oral doses of lead acetate decreased the transfer of both calcium and strontium across the duodenal wall using everted gut sacs. This observation may also be due to the existence of a competitive

mechanism for calcium, lead and strontium transport across the gut wall. However, they found that the retention of calcium and strontium on the intestinal wall was not affected by previous oral lead treatment.

Phosphate appeared to affect lead transport by a mechanism which differs from that of calcium. The data suggest that phosphate forms an insoluble complex with lead in the lumen which does not affect the binding of lead to the gut wall but which decreases the amount of lead available for transport.

25ng of 1,25 dihydroxycholecalciferol administered intravenously 4 hours before the gut loop experiments increased lead transferred across the gut wall, thus confirming studies in whole animals in which increased vitamin D was shown to increase lead absorption (Sobel et al, 1938; Rapoport and Rubin, 1941; Thawley, 1975). This finding also suggests that lead absorption shares a similar mechanism with calcium absorption which has also been shown to be modified by vitamin D treatment (Omdahl and DeLuca, 1973; Freund and Bronner, 1975). Since the transport of other cations such as magnesium, zinc, cadmium, strontium, barium, iron and cobalt have all been reported to be increased by vitamin D, its effect may be on a general transport pathway for these cations (Migicovsky and Jamieson, 1955; Wasserman, 1962; Migicovsky, 1963).

These results from gut loop experiments indicate that lead transport across the gut wall is an active process which can be modified by the presence of calcium and phosphate in the lumen and vitamin D. The binding of lead to the gut wall, however, was decreased only by increasing calcium in the lumen.

This suggests that calcium-binding-protein (CaBP) which is found in the gut wall may also be responsible for binding lead. The increase in lead absorption after the intravenous administration of 1,25 dihydroxycholecalciferol may also be the result of the ability of CaBP to bind lead because it is known that vitamin D treatment of rachitic rats (Drescher and DeLuca, 1971) and chicks (Wasserman and Taylor, 1966) increases the synthesis of CaBP thus increasing calcium absorption and perhaps lead absorption too. Verification of this hypothesis requires further experiments to study the properties of CaBP or other lead-binding sites on the gut wall and the effect of dietary factors such as vitamin D and phosphate on these sites.

The present findings suggest that the lead status of exposed individuals may be modified by their nutritional experience. The findings may explain the differences in response to lead exposure which have been observed for various population groups, for example Negroes versus Puerto Ricans (Guinee, 1972) and children versus adults (Alexander et al, 1972; Barltrop et al, 1975). They may also partly explain the wide range of blood lead concentrations in community studies. Thus the differences in lead absorption which have been attributed to differences in race or age may just be a reflection of each group's different nutritional experience. Further studies will have to investigate which factor or factors are responsible for each group's differing response.

The greatest intake of lead in young children is usually associated with pica and the highest incidence of pica

overlaps that of lead poisoning, which occurs between the ages of 18 and 30 months (Byers, 1959; Barltrop, 1966; Lin-Fu, 1973). However, Sayre et al (1974) suggested that ingestion of leaded paint may not be the main cause of the elevated body burdens of lead found in inner city children. They reported that lead on inner-city household surfaces and on the hands of children living in such houses at levels high enough to afford a significant alternative source of exposure. In contrast, Barltrop et al (1975) could not find any consistent relationship between blood lead values and pica for lead-contaminated soil. They concluded that lead in soil in this situation did not constitute a hazard although it provided a small additional burden for children.

The body burdens of lead of some children may also be increased by inadequate intakes of calcium or phosphorus resulting in enhanced absorption. This could be related to the possibility that calcium deficiency may be a cause of pica thus increasing the child's intake of lead from the ingestion of lead-containing substances. However, this has not been confirmed in humans.

Although these findings in animals may not readily be extrapolated to humans it is suggested that the nutritional determinants of lead absorption in human populations should be considered in relation to permissible limits of lead intake.

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Appendix A

Rats were fed diets without added lead for 48 hours as controls. The values of lead concentration in their organs are presented in Table A-1. The mean values of lead concentration in the organs of the two animals sacrificed before each experiment to confirm no previous exposure to lead are presented in Table A-2.

Table A-1

Lead Concentration of Control Rats

Kidney Pb (µg)	Blood Lead (µg/100g)	Femur Pb (µg)	Liver Pb (µg)	Spleen Pb (µg)	Heart Pb (µg)	Muscle Pb (µg)
0.48 ± 0.09	9.2 ± 1.9	0.60 ± 0.17	1.83 ± 0.37	0.16 ± 0.07	0.06 ± 0.02	0.10 ± 0.02
(18)*	(18)	(12)	(6)	(6)	(6)	(5)

Rats were fed diets without added lead for 48 hours.

* (n)

All values are mean ± S.D.

Table A-2

Lead Concentration of Control Rats

Kidney Pb (µg)	Blood Lead (µg/100g)	Femur Pb (µg)	Liver Pb (µg)	Spleen Pb (µg)	Heart Pb (µg)	Muscle Pb (µg)
0.44 ± 0.21	8.9 ± 3.4	0.58 ± 0.23	2.30 ± 0.62	0.14 ± 0.06	0.07 ± 0.02	0.17 ± 0.07
(32)*	(31)	(32)	(32)	(32)	(32)	(32)

Rats were sacrificed before each experiment

* (n)

All values are mean ± S.D.

Appendix B

Radioactivity Measurement

^{203}Pb was supplied by the M.R.C. Cyclotron Unit at Hammersmith Hospital, London. The energy of gamma-photons and the absence of particle emission in the decay of ^{203}Pb makes it particularly suitable for both in vivo and in vitro applications. ^{203}Pb decays with a half-life of 52.1 hr. by electron capture followed by emission of gamma-photons of energies 0.279 MeV (81%), 0.401 MeV (5%) and 0.680 MeV (0.9%), going to the ground state of stable thallium-203. Unlike some other radionuclides of lead, ^{203}Pb has no daughter radiations to cause complications in its measurement (Horlock et al, 1975).

It is produced by bombarding a thallium target with charged particles. The chemical separation of ^{203}Pb does not use any lead carrier thus keeping the ^{203}Pb "carrier-free."

In these studies, the gamma energy of ^{203}Pb was measured in two different counters: a Hewlwett Packard Auto-Gamma (Model 5204 Spectrometer) and the Armac whole-body counter (Model 3002 Spectrometer). Graphs are presented for trial counts to select the range appropriate for counting ^{203}Pb. Figure B-1 shows results from the Auto-Gamma where a range of 50 to 300 was chosen on 10% gain (full scale 3 MeV), that is, from 10 to 600 KeV. Similarly, the range selected on the Armac was 5 to 300 KeV using window range 50 to 300 on 10% gain (full scale 1 MeV) (Fig. B-2). There is no full energy peak in the spectrum of ^{203}Pb taken in the

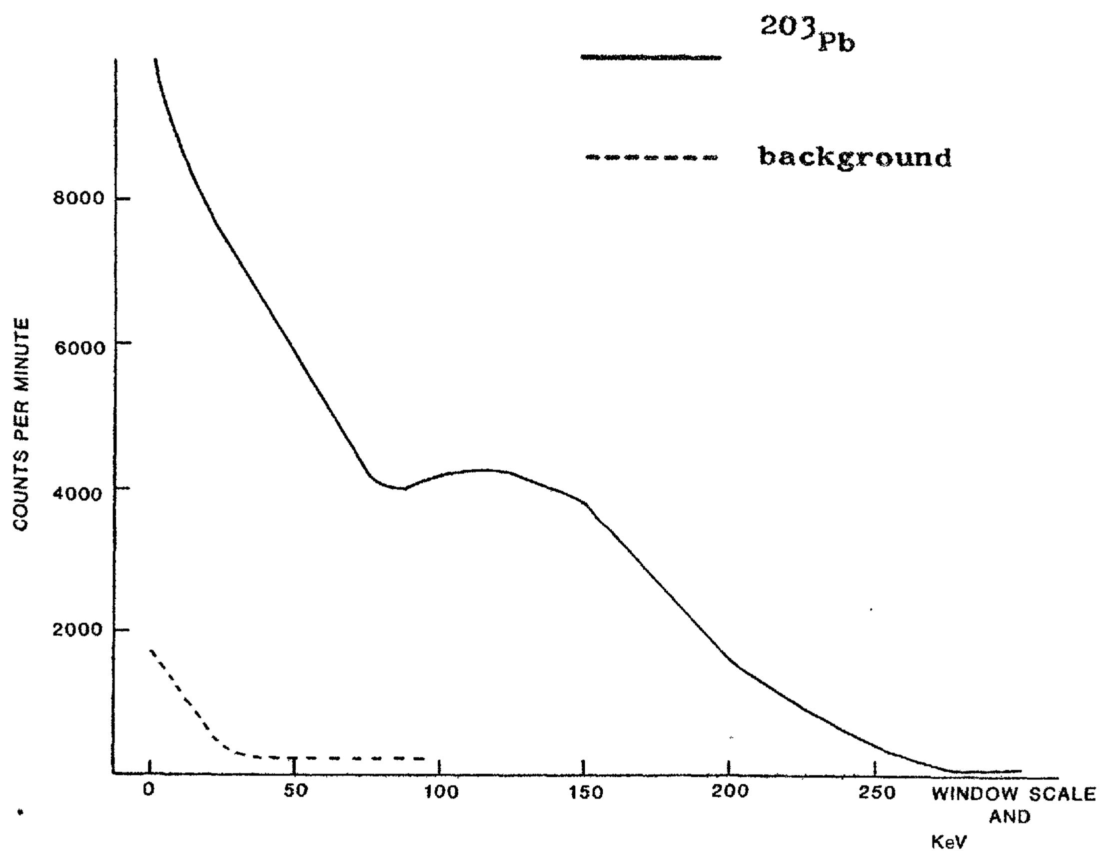
Armac because the principal mode of interaction in the liquid scintillator is the Compton effect rather than the photoelectric effect in the NaI crystal of the Auto-Gamma. The maximum energy of the Compton-scattered electron for ^{203}Pb is 0.146 MeV calculated from the equation:-

$$E_{max} = \frac{E_{\alpha}}{1 + \frac{0.51}{2\,E_{\alpha}}}$$

where $E_{\alpha} = 0.279$ MeV

A whole animal injected intraperitoneally with a solution containing ^{203}Pb was counted at different positions within the counting chamber of the Armac. There were some differences in counting rates. Holders were instructed to hold the animals in the same position to ensure counting reproducibility.

The efficiency of the Armac whole-body counter was 30% and that of the Auto-Gamma was 69%.



<u>Figure B-1</u>

Gamma-Energy Spectrum of ^{203}Pb in the Armac Whole-Body Counter at 2% Gain.

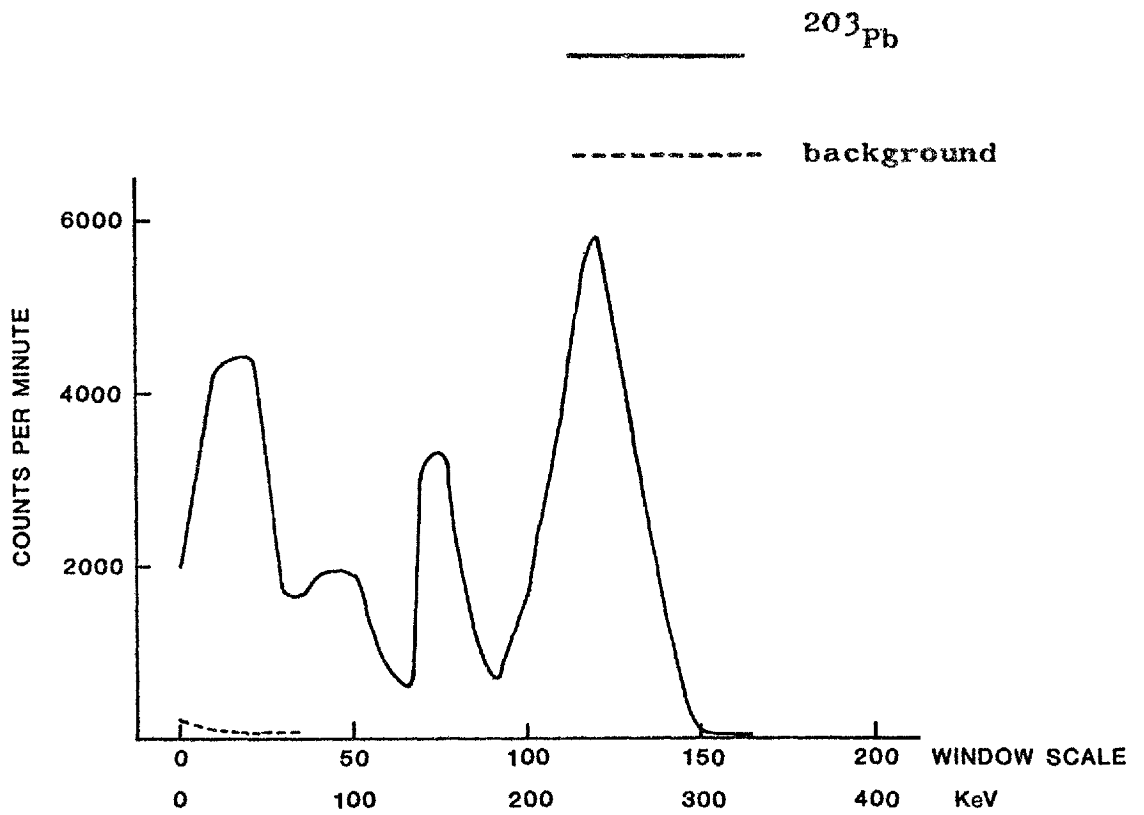


Figure B-2

Gamma-Energy Spectrum of ^{203}Pb in the Auto-Gamma Counter at 2% Gain.

Appendix C

Table C-1

Composition of Oxoid Diet 41B

Basic Ingredients	% in diet
Sussex ground oats	40
Wholemeal flour	46
White fish meal	8
Dried skim milk	3
Dried yeast	1
Mineral supplement*	1
Vitamin supplement**	1

Nutritional Components	
Carbohydrate	48.8
Protein	15.9
Fibre	5.1
Fat	3.2

* The mineral supplement provides the following:
Fe 214 ppm; Mn 46 ppm; Cu 30 ppm; Co 1 ppm; Zn 6 ppm; Ca 0.84%; P 0.7%; Na 0.23%; Cl 0.34%; K 0.50%.

** The vitamin supplement provides the following/lb:
Vitamin A 2434 IU; Vitamin D_3 670 IU; Vitamin E 21.8 IU; Thiamine 2.6 mg; Riboflavin 3.4 mg; Niacin 24.5 mg; Pantothenic acid 8.2 mg; Choline 621.0 mg; Biotin 0.3 mg; Folic acid 0.4 mg; Pyridoxine 3.9 mg; Inositol 100.0 mg; Vitamin B_{12} 12.8 ug.

Appendix D

The composition of test diets is presented in Tables D-1 to D-3.

Table D-1

100g Food	Ca (g)	Su (g)	CS (g)	Mo (g)
Control	20.0	28.0	33.0	5.0
Kcal	90	105	135	13
Low Fibre	20.0	31.0	33.0	5.0
Kcal	90	116	135	13
High Fibre	20.0	23.0	26.0	5.0
Kcal	90	86	107	13
Low Vitamins	20.0	28.0	33.62	5.0
Kcal	90	105	138	13
High Vitamins	20.0	28.0	31.14	5.0
Kcal	90	105	128	13

Ca	-	Casein	(Protein)	4.5 kcal/g
Su	-	Sucrose	(Carbohydrate)	3.74 kcal/g
CS	-	Corn Starch	"	4.10 kcal/g
Mo	-	Molasses	"	2.57 kcal/g

Table D-1

CO (g)	Ce (g)	MM (g)	UM (g)	V (g)	Total Kcal
5.0	3.0	5.34	0.04	0.62	
44					387
	0	"	"	"	
					398
	12.0	"	"	"	
					340
	3.0	"	"	"	
					390
	3.0	"	"	"	
					380

CO - Corn Oil (Fat) 8.76 kcal/g
Ce - Cellulose (Fibre)
MM - Macrominerals
UM - Microminerals
V - Vitamins

Table D-2

100g Food	Ca (g)	Su (g)	CS (g)	Mo (g)
Control	20.0	28.0	33.0	5.0
Kcal	90	105	135	13
0% Protein	0	34.5	46.0	5.0
Kcal	0	129	189	13
5% Protein	5.0	35.0	41.0	5.0
Kcal	23	131	168	13
10% Protein	10.0	35.0	36.0	5.0
Kcal	45	.131	148	13
15% Protein	15.0	33.0	33.0	5.0
Kcal	68	123	135	13
40% Protein	40.0	18.0	23.0	5.0
Kcal	180	67	94	13
50% Protein	50.0	13.0	18.0	5.0
Kcal	225	48	74	13
60% Protein	60.0	8.0	13.0	5.0
Kcal	270	30	53	13
80% Protein	80.0	1.0	0	5.0
Kcal	36	3.7	0	13

Ca - Casein (Protein) 4.5 kcal/g
Su - Sucrose (Carbohydrate) 3.74 kcal/g
CS - Corn Starch " 4.10 kcal/g
Mo - Molasses " 2.57 kcal/g

Table D-2

CO (g)	Ce (g)	MM (g)	UM (g)	V (g)	Total Kcal
5.0	3.0	5.34	0.04	0.62	
44					387
5.0	"	"	"	"	
44					375
5.0	"	"	"	"	
44					379
5.0	"	"	"	"	
44					381
5.0	"	"	"	"	
44					383
5.0	"	"	"	"	
44					398
5.0	"	"	"	"	
44					404
5.0	"	"	"	"	
44					410
5.0	"	"	"	"	
44					422

CO - Corn Oil (Fat) 8.76 kcal/g
Ce - Cellulose (Fibre)
MM - Macrominerals
UM - Microminerals
V - Vitamins

Table D-3

100g Food	Ca (g)	Su (g)	CS (g)	Mo (g)
Control	20.0	28.0	33.0	5.0
Kcal	90	105	135	13
0% Fat	20.0	28.0	38.0	5.0
Kcal	90	105	156	13
2.5% Fat	20.0	28.0	35.5	5.0
Kcal	90	105	146	13
10% Fat	20.0	28.0	28.0	5.0
Kcal	90	105	115	13
15% Fat	20.0	28.0	23.0	5.0
Kcal	90	105	94	13
20% Fat	20.0	28.0	18.0	5.0
Kcal	90	105	105	13
40% Fat	20.0	28.0	0	5.0
Kcal	90	105	0	13

Ca - Casein (Protein) 4.5 kcal/g
Su - Sucrose (Carbohydrate) 3.74 kcal/g
CS - Corn Starch " 4.10 kcal/g
Mo - Molasses " 2.57 kcal/g

Table D-3

CO (g)	Ce (g)	MM (g)	UM (g)	V (g)	Total Kcal
5.0	3.0	5.34	0.04	0.62	
44					387
5.0	"	"	"	"	
0					364
2.5	"	"	"	"	
22					376
10	"	"	"	"	
88					411
15	"	"	"	"	
131					433
20	"	"	"	"	
175					444
40	"	"	"	"	
550					550

CO - Corn Oil (Fat) 8.76 kcal/g
Ce - Cellulose (Fibre)
MM - Macrominerals
UM - Microminerals
V - Vitamins

Appendix E

Subsidiary Matter

Reprints of the following publications are presented here:-

1. Barltrop, D. and Khoo, H.E. 1975. The influence of nutritional factors on lead absorption. Postgrad. Med. J. 51 : 795-800.
2. Barltrop, D. and Khoo, H.E. 1975. Nutritional determinants of lead absorption. IXth Annual Conference on Trace Substances in Environmental Health, University of Missouri, U.S.A. pp 369-376.
3. Barltrop, D. and Khoo, H.E. 1976. The influence of dietary minerals and fat on the absorption of lead. Sci. Total Env. (In the press) (reprint not available).

Nutritional Determinants of Lead Absorption

Trace Substances in Environmental Health IX. A Symposium. D.D. Hemphill, Ed. University of Missouri. 1975.

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ABSTRACT

The nutritional factors influencing the absorption of lead from the gut have been studied using both intact animals and ligated gut loop preparation. Short-term feeding studies have been made in groups of 6 animals using diets of constant Pb content (0.075%) but in which the nutritional components were varied sequentially. Dietary Pb was labeled with ^{203}Pb. Absorption was determined in the carcass and individual organs by means of a small-animal whole-body counter. The results showed that absorption was enhanced to 20 times control value by diets deficient in minerals and 7-fold by diets of high fat content. Conversely, high mineral diets have been shown to result in a 2-fold reduction in Pb absorption. The interaction of Pb with individual dietary components has been further studied under controlled conditions using ligated gut loop preparations. Using this technique the relative roles of luminal interaction and tissue response for Pb absorption have been explored and the kinetics of Pb absorption determined.

INTRODUCTION

The importance of various nutritional factors in influencing the absorption of Pb from the gut has been documented. However, their mode of action is not understood and even the mechanism by which Pb is transferred from the lumen across the gut wall is not known.

Much of the evidence presented has been concerned with the enhanced absorption of Pb resulting from an increased or decreased ingestion of particular dietary factors. Thus, added milk (9), low intakes of Ca and P (14), low Fe (15), low and high protein (11) added Vitamin D (16), low Vitamin C (13) and high fat (18) have all been claimed to increase Pb absorption. Conversely, dietary Ca and P (14) and alginate supplements (8) to the diet have resulted in diminished Pb absorption in the rat. Although these studied have drawn attention to the effects of individual dietary components, their non-uniform experimental designs have rendered it difficult to compare the relative effects of each factor.

Many non-nutritional factors are also known to affect Pb absorption in the rat, including age and weight (5) and the chemical and physical form of the Pb administered (2). Some authors have also used carrier-free isotopes of Pb but it is not known whether the data thus obtained are valid under all experimental conditions. Consequently, before any comparison can be made careful control is required of the age and weight of the experimental animals, the chemical and physical form of the Pb administered and the specific activity of any isotope used.

A systematic study of the effect of individual dietary factors on Pb absorption from the gastro-intestinal tract of the rat has been carried out. In addition an *in vivo* technique has been applied to the study of the kinetics of Pb transfer across the gut wall.

MATERIALS AND METHODS

Feeding experiments were carried out on groups of 6 male Wistar albino rats of 100-115 g body weight, aged 32 days, as described previously (1). Each rat was housed individually and experimental diets and deionized water were given *ad libitum*. Food and water intake over the experimental period of 48 hr was recorded. The diets were compounded in the laboratory and the proportions of different constituents varied appropriately. Each diet contained 0.075% Pb as $PbCl_2$, labelled with ^{203}Pb to a specific activity of approximately 100 n Ci/mg Pb.

Ligated gut loop experiments were also made on similar animals in groups of 6. All the rats were allowed food and water *ad libitum* because overnight fasting might affect the transport of materials from the gut.

Each rat was anaesthetized with an intraperitoneal injection of 6 mg pentobarbitone per 100 g body weight. The duodenum and jejunum were located through a mid-line incision and the bile duct ligated. A loose ligature was made around the gut 2 cm from the distal end of the stomach and 2.5 ml of isotonic saline at 37°C was injected into the gut and the ligature tightened. The saline was moved along the gut gently for about 15 cm from the first ligature and a second ligature applied at this point. One ml of the perfusate ($PbCl_2$ labelled with ^{203}Pb in saline) was then injected into the gut loop at the proximal end through a No. 20 needle. After injection, the intestinal loop was returned to the abdomen with its blood supply intact and the skin incision closed. Absorption was allowed to proceed for a measured interval after which the rat was killed with pentobarbitone. The gut loop was removed, weighed and the length measured. The residual solution in the gut was collected and the gut flushed with isotonic saline. The amount of Pb transported was assessed from the loss of activity from the gut loop to carcass. To measure the amount of residual Pb in the gut wall the activity on the washed length of gut was also determined. All measurements of gamma-emission were carried out in a Packard Armac whole-body counter.

RESULTS

Ca and PO_4 have been identified as the 2 minerals responsible for major effects on Pb uptake. The results of more detailed studies on the varying content of dietary minerals are presented.

When Ca and PO_4 were reduced or omitted from the diet (Table I) the Pb content of all the organs increased. There appeared to be an additive effect in all organs when Ca and PO_4 were reduced simultaneously. The blood Pb concentration was increased to almost 10 times control values when either Ca or PO_4 was omitted from the diet. Even when half the normal amount of Ca and PO^4 was present, Pb concentrations in all the organs was still approximately 4 times greater than control values.

TABLE I. EFFECTS OF DECREASED MINERALS ON LEAD ABSORPTION
(RATIO OF MEAN RETENTION EXPERIMENTAL : CONTROL)

Diet	Whole Body Without Gut	Blood	Kidneys	Femur	Liver
(0.7% Ca 0.5% PO_4)	1	1	1	1	1
0 Ca	-	6.2	4.4	3.4	4.0
0 PO_4	-	2.7	3.0	2.1	1.9
0 Ca and 0 PO_4	-	9.7	7.4	4.9	3.8
½ x Ca	2.3	1.9	2.1	2.2	2.1
½ x PO_4	2.2	2.0	1.8	1.9	1.4
½ x Ca AND ½ x PO_4	3.6	4.1	3.3	3.8	2.6

Conversely, increasing the Ca or PO_4 content of the diet to twice the control value resulted in no difference in Pb uptake apart from a 30% decrease in the liver in the high PO_4 diet (Table II). However, simultaneously increasing dietary Ca and PO_4 two-fold resulted in a 40% reduction in the Pb uptake of both kidneys and liver and a 50% decrease in the carcass and femur. The blood Pb uptake was not affected.

TABLE II. EFFECTS OF INCREASED MINERALS ON LEAD ABSORPTION (RATIO OF MEAN RETENTION EXPERIMENTAL : CONTROL)

Diet	Whole Body Without Gut	Blood	Kidneys	Femur	Liver
(0.7% Ca 0.5% PO_4)	1	1	1	1	1
2 x Ca	1	1	1	1	1
2 x PO_4	1	1	1	1	0.7
2 x Ca AND 2 x PO_4	0.5	1	0.6	0.5	0.6
4 x Ca	0.5	0.5	0.7	0.4	0.5
4 x PO_4	0.5	0.4	0.3	0.4	0.4
4 x Ca AND 4 x PO_4	0.4	0.4	0.4	0.2	0.5

Increasing Ca and PO_4 content individually or simultaneously to 4 times normal values reduced Pb uptake in all organs. The femur showed the greatest effect as Pb uptake was reduced by 80% compared with control values.

High fat diets increased Pb uptake (Figure 1). By combining a high fat (15% corn oil) and mineral-deficient diet there was a synergistic effect; Pb uptake in the whole animal after the gut had been removed was increased by 19 times control values. In the kidneys and femur Pb uptake was increased 27 and 19 times control values respectively. The blood Pb showed the greatest effect with a 50-fold increase. These increases are the greatest that have been obtained with any of our experimental diets.

Ligated Gut Loops. There was a direct relationship between lead transferred from the lumen across the gut wall and the concentration of lead up to 10 μg, in the initial perfusate (Figure 2). After that level the transferred lead is limited to 5 μg regardless of the amount of Pb in the initial dose. There thus appeared to be a rate-limiting process in the transfer of Pb across the gut wall. The maximum rate of transfer was calculated to be 0.3 μg/cm/hr.

When a constant amount of Pb (1 μg) was introduced into the lumen and allowed to be transferred for varying periods of time (Figure 3) a similar relationship was observed. The amount of Pb transferred increased proportionately with time up to 40 min after which it remained constant for periods lasting up to 1 hr. Conversely, the gut wall appeared to be saturated with 0.5 μg Pb regardless of the length of time the perfusate remained in the intestinal loop.

Adding Ca to the one μg Pb solution reduced the transfer of Pb (Figure 4). Increasing amounts of Ca resulted in progressively smaller amounts of Pb transferred from the lumen.

DISCUSSION

When half the recommended intake of Ca and PO_4 is added to the diets there is a 4-fold increase in Pb uptake. By doubling the dietary Ca and PO_4 content Pb uptake can be halved. There is thus a possibility that these 2 minerals may have a prophylactic application in human populations in high risk situations. When dietary fat content is increased and the mineral content is decreased simultaneously, a marked increase in Pb uptake is induced.

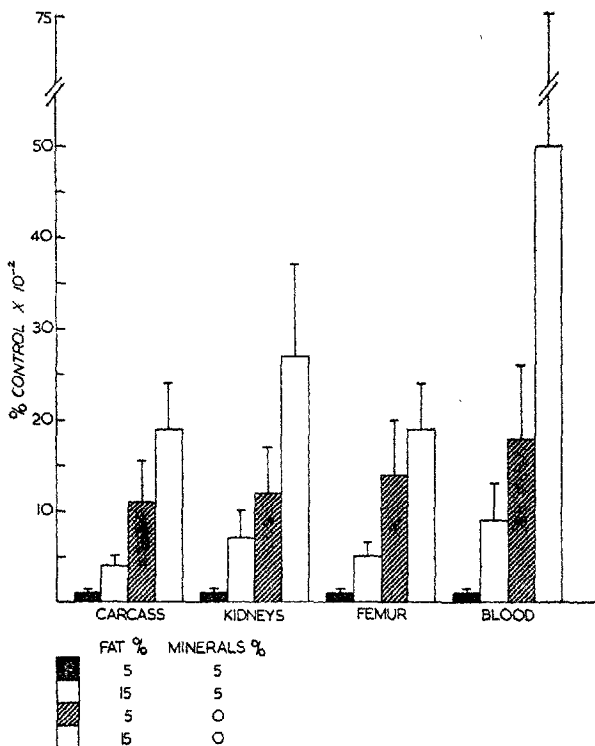

FIGURE 1—LEAD UPTAKE IN DIETS OF VARYING FAT AND MINERAL CONTENT.

From the maximum rate of transfer of 0.3 µg Pb/cm/hr the daily maximum total uptake of Pb into the body can be estimated. Assuming the small intestine has similar rates of transfer along its whole length of 80 cm, the daily maximum uptake would be 580 µg Pb. Since a 100 g rat consumes 15 g of food per day and there is 0.5% absorption (1) the greatest effective dose in the diet would be 0.8%. A greater dietary Pb dose would not increase Pb uptake substantially. There would appear to be a rate-limiting step involved in the process of intestinal Pb transfer.

Using a low dosage of 1 µg Pb there is a maximum of 50% uptake from the gut loop (Figure 3). In studies involving dietary Pb, there is only 0.5% uptake (1). This difference is probably due to the other nutritional components present in the normal diet, as the intestinal loops only contained Pb in isotonic saline.

The proportion of Pb taken up by the gut wall is a constant of 50% and is achieved within the first 10 min of incubation (Figure 3). This differs from Cikrt's studies (4) in which increasing uptake in duodenal and ileal segments *in vitro* up to 60 min was demonstrated. The difference might be due to the different techniques used.

The effect of Ca on Pb transfer from the gut loop confirms the results from dietary studies where added Ca had been shown to reduce Pb uptake. Ca is also known to modify the absorption of Mg (12) and Sr (10). Thus it is possible that the effects of added Ca on Pb transfer is due to the existence of a common pathway for the absorption of Ca and other minerals including Pb from the gut. Such a pathway might also explain

the effects of other nutritional factors on Pb absorption. High protein diets have been demonstrated to decrease Ca uptake (7) and to enhance Pb uptake (1). High fat diets are known to reduce the availability of Ca (3) and increase Pb absorption from the gut (1). Thus the role that these nutritional factors play in reducing Ca uptake could be contributing to increased Pb absorption. More work is required to determine the mechanism of Pb transfer across the gut wall and the mode of action of nutritional factors upon this.

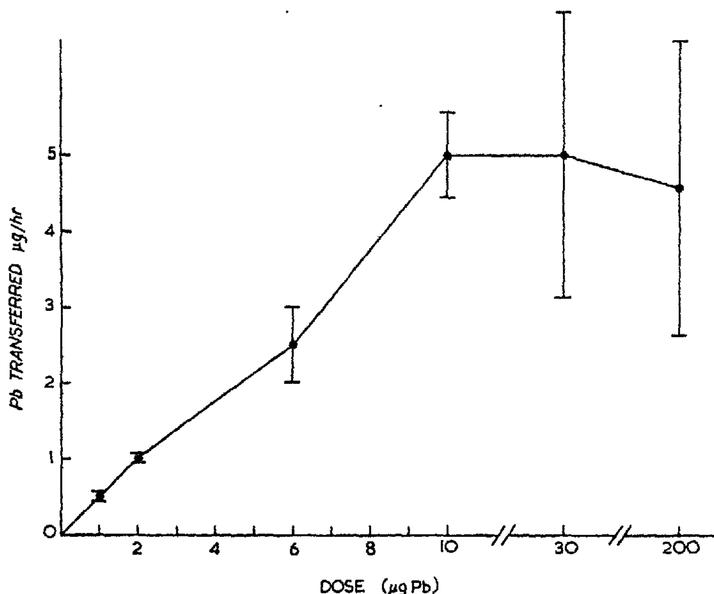

FIGURE 2—RATE OF Pb TRANSPORT AS A FUNCTION OF INITIAL CONCENTRATION.

CONCLUSION

These studies in animals may not be readily extrapolated to human populations. However, they raise the possibility that nutritional factors may affect the response of human populations to environmental exposure to Pb. Thus the results may partly explain the wide range of blood Pb concentration in community studies. The differences in susceptibility to Pb in 2 different racial groups (6) may also partly be explained by their different nutritional habits. It is suggested that the nutritional determinants of Pb absorption in human populations should be considered in relation to permissible limits of Pb intake.

ACKNOWLEDGMENTS

This work was supported by grants from the Buttle Trust and the National Institute for Occupational Safety and Health, U.S. Department of Health, Education and Welfare, Contract No. HSM 99-73-28.

^{203}Pb was supplied by the MRC Cyclotron Unit, Hammersmith Hospital, London.

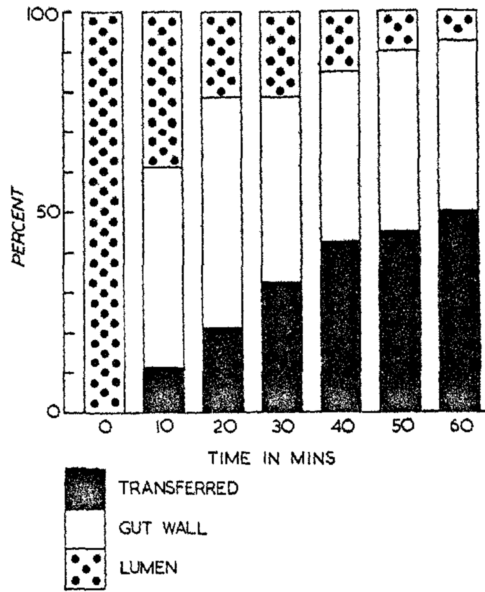

FIGURE 3—FATE OF ^{203}Pb IN THE GUT AS A FUNCTION OF TIME.

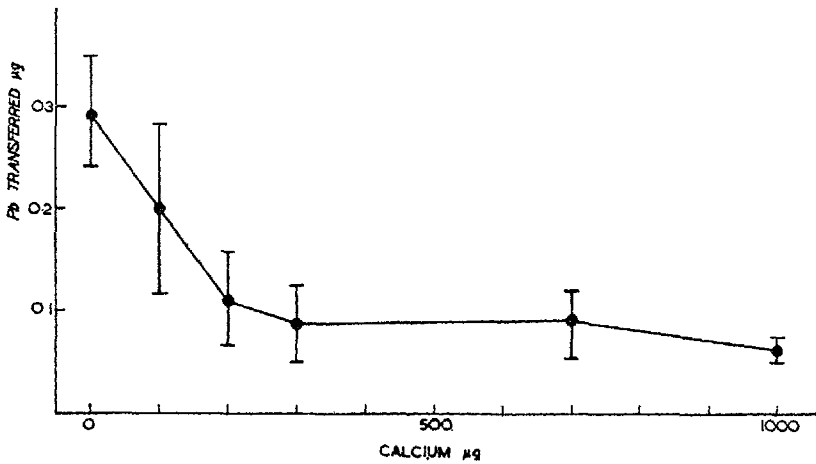

FIGURE 4—INFLUENCE OF Ca^{2+} ON Pb TRANSPORT.

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DISCUSSION

Inquirer: Margaret Elmes, Department of Medicine, Queen's University, Belfast, Northern Ireland

Q. Did you gut wall sample include the whole wall from the serosal coat through to the lumen?

A. Yes.

Inquirer: E. T. Kaminski, Northwestern University, Chicago, IL

Q. Which part of intestine was used?

A. The 15 cm of jejunum proximal to the stomach.

Q. Did you investigate the energy required for transport, i.e., is it active, passive diffusion, etc.?

A. No.

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The influence of nutritional factors on lead absorption

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Summary

The nutritional factors which affect lead absorption have been studied. Synthetic diets of known composition were compounded to contain 0·075% Pb as $PbCl_2$ labelled with ^{203}Pb. Rats were exposed to lead for periods of 48 hr. The dietary intake was then measured and the absorption of lead determined by means of a whole-body counter. Lead absorption was increased by high fat, low mineral, low protein and high protein diets but was decreased by high mineral diet. Low fat, low fibre, high fibre, low vitamin and high vitamin diets had no effects on lead absorption.

Introduction

The major source of lead for the unexposed human population is its diet. It has been calculated that the average daily intake of lead of an adult is 300 μg from food and beverages (Barry and Mossman, 1970) and of this, about 10% is absorbed (Kehoe, 1961). There is evidence that various dietary factors will influence the absorption of lead from the gastrointestinal tract. Milk has been found to increase lead absorption from the gastrointestinal tract in rats (Kello and Kostial, 1973). Calcium and phosphorus added to rat diets decreased the accumulation of lead in femurs and kidneys (Sobel *et al.*, 1940; Six and Goyer, 1970). Conversely, low dietary iron has been shown to enhance lead uptake from the gastrointestinal tract in rats (Six and Goyer, 1972). Protein has also been found to affect lead absorption and toxicity (Baernstein and Grand, 1942) so that rats fed both low-protein and high-protein diets had an increased lead uptake (Milev, Satler and Minden, 1970). Vitamins C and D may each modify the absorption of lead. Thus a low intake of Vitamin C may enhance the severity of plumbism (Pillemer *et al.*, 1940). Vitamin D added to the diet will increase lead toxicity (Sobel *et al.*, 1940) although this has been denied (Tompsett, 1939). Conflicting reports exist about the effect of increased dietary fat on lead absorption. Tompsett (1939) found no relationship between dietary fat and lead absorption but Weyrauch and Necke (1932) reported an increase in lead absorption with increased dietary fat. The common food additives, 'alginates', have been reported to diminish lead uptake from the intestine of newborn rats (Kostial, Simonović and Pisonić, 1971a) but this has not been confirmed (Harrison *et al.*, 1969; Carr, Nolan and Duraković, 1969).

The experimental designs used in previous studies have not been uniform and interpretation of their data is difficult. Many variables will influence lead absorption. Using carrier-free ^{212}Pb, Forbes and Reina (1972) showed that age and weight affect lead absorption in rats, with immature rats absorbing more lead than do mature animals. The chemical and physical form of lead has been reported to modify its absorption and toxicity (Fairhall and Sayers, 1940). Recently this has been confirmed in our laboratory (Barltrop and Meek, 1975). Carrier-free isotopes of lead have been used in some studies (Milev *et al.*, 1970; Kello and Kostial, 1973; Kostial *et al.*, 1971a, 1971b) but it is not known whether carrier-free lead behaves like stable lead in the gut under all experimental conditions. Comparison therefore requires careful control of the age and weight of the experimental animals, the chemical and physical form of the lead supplied and specific activity of any isotope used.

In this paper, a systematic study of the effect of individual dietary factors on lead absorption from the gastrointestinal tract of the rat is reported. This work was undertaken using diets compounded in this laboratory and containing 0·075% lead as $PbCl_2$ labelled with ^{203}Pb. The effects of varying fibre, vitamin, protein, fat and mineral content of a synthetic diet on the absorption of lead are presented.

Materials and methods

Male Wistar albino rats of 100–115 g body weight, aged 30–32 days, were used. Each experimental group comprised six animals but each rat was housed individually. The experimental diets and de-ionized water were given *ad libitum* for 48 hr but food and water intake over the experimental period was recorded.

The control diet was compounded in the laboratory as in Table 1. Other diets were prepared by varying the proportion of different constituents. The protein and fat content was varied by adjusting

TABLE 1. Composition of control diet

		%
Protein	Casein	20·0
Fat	Corn oil	5·0
Fibre	Cellulose	3·0
Carbohydrate	Molasses	5·0
	Sucrose	28·0
	Corn starch	33·0
	Minerals*	5·35
	Vitamins†	0·62
	$PbCl_2$	0·101
		100 0

* The mineral mix provided the following quantities of nutrient in g/kg of diet: $NaH_2PO_4 . 2H_2O$, 25·13; $CaCO_3$, 17·5; $MgCl_2 . 6H_2O$, 5 43; KCl, 3·43; NaCl, 1·27; $MnSO_4 . H_2O$, 0·168; $FeSO_4 . 7H_2O$, 0·105; $ZnCO_3$, 0 050; $CuSO_4$, 0·020; $(NH_4)_6 Mo_7O_{24} . 4H_2O$, 0·005 and KIO^8, 0·0003.

† The vitamin mix provided the following per kilogram of diet: vitamin A, 19,800 i.u.; vitamin D, 2300 i.u.; vitamin E, 7 i.u.; choline chloride, 4·65 g; ascorbic acid, 991 mg; *p*-aminobenzoic acid, 110 mg; inositol, 110 mg; niacin, 99·1 mg; calcium pantothenate, 68 mg; menadione, 49 mg; thiamine hydrochloride, 22 mg; pyridoxine hydrochloride, 22 mg; riboflavin, 22 mg; folic acid, 1·98 mg; D-biotin, 0·44 mg; vitamin B_{12}, 0·029 mg.

the content of casein and corn oil in the diet. Fibre was varied using different proportions of cellulose. Corn starch and sucrose content were then adjusted to make the diets isogravic and isocaloric. With the exception of diets containing fat in the range 15–40%, feeds were prepared by moulding into sticks and heating at 100°C for 20 min. The high fat diets were fed in powder form to the rats kept in individual glass metabolic chambers. Each diet contained 0·075% Pb as $PbCl_2$, labelled with ^{203}Pb to a specific activity of approximately 100 nCi/mg Pb. The experimental period of 48 hr, age and weight of experimental animals and lead concentration of 0·075% in the food were determined in preliminary studies (Barltrop and Meek, 1975).

After the end of the experimental period, the rats were killed by ether anaesthesia and counted in a Packard Armac small-animal whole-body counter. Approximately 3 ml of blood was obtained by venepuncture and collected into heparinized vials. After counting, each carcass was dissected and the gastrointestinal tract, kidneys, spleen, liver and femur were removed. The lead content of residual food, carcass without the gastrointestinal tract, carcass without any organs, whole liver, kidneys, spleen and femur were determined by measurement of the gamma-emission of ^{203}Pb. Residual food, carcass and liver were counted in the Packard Armac whole-body counter. Specimens of blood, kidneys, spleen and femur were counted in a Hewlett-Packard Auto-Gamma counter. The ingested dose was determined by the difference between the food given and the residual food. Lead content of the carcass and individual organs was expressed as percentage of ingested dose.

Results

The values for lead retention by animals fed on the control diet are given in Table 2. Whole-body retention was only 0·65% of the ingested dose compared with the value of 10% that has been reported for human adults (Kehoe, 1961). Of the organs, liver had the greatest retention of lead with progressively lesser values in kidney, femur and blood respectively.

TABLE 2. Controls*. Lead in organs as % ingested dose

	$\bar{x} \pm$s.d.
Blood	$(0·20 \pm 0·05) \times 10^{-2}$
Femur	$(2 08 \pm 0·51) \times 10^{-2}$
Kidneys	$(2·82 \pm 0·50) \times 10^{-2}$
Liver	$(6·62 \pm 1·20) \times 10^{-2}$
Whole body without gut	$0·65 \pm 0·12$

* $n = 11$.

TABLE 3. Effects of different diets on lead absorption (ratio of mean retention experimental : control)

Diet	Blood	Kidneys	Femur	Liver
Low protein	5 1	2·5	2·8	2·2
High protein	1	3·7	2·6	1
Low fat	1	1	1	1
High fat	9·6	7·6	4·8	4·2
Low minerals	17·7	11·9	13·7	8·8
High minerals	0·2	0·2	0·1	0·1
Low fibre	1	1	1	1
High fibre	1	1	1	1
Low vitamins	1	1	1	1
High vitamins	1	1	1	1

The effects of various experimental diets on lead distribution between the organs are given in Table 3. All results which are not significantly different from the control are presented with the experimental : control ratio as 1. In the low factor diets, the nutritional factor concerned was omitted while in the high factor diets it was increased three- to four-fold. Low protein, high fat and low mineral diets increased the blood lead concentration and high mineral diet decreased it (Fig. 1). The lead concentration of liver showed a similar relationship. In the kidneys and femurs, however, a high protein diet was also associated with an increased lead content. Low fat, low fibre, high fibre, low vitamin and high vitamin diets had no effect on lead absorption.

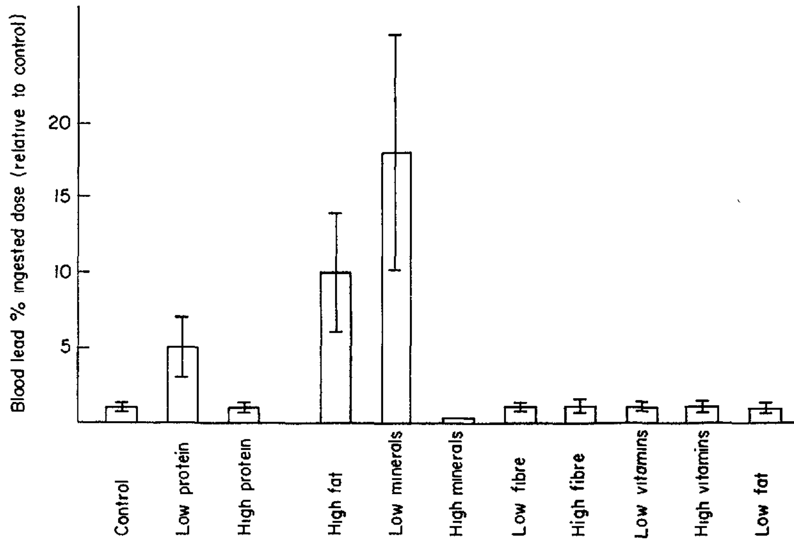

FIG. 1. Mean blood lead concentration as % ingested dose±s.d. for diets of varying composition relative to control diet (n = 6).

Protein

Detailed studies of the effects of varying dietary protein confirmed the findings in the preliminary investigations (Table 4). Diets containing 10% and 15% protein had no effect on lead absorption but diets containing 0% and 5% protein increased the lead concentration in all the organs studied. Conversely, doubling the recommended protein content to 40% by weight resulted in greater lead concentration in the kidneys (Fig. 3) but had no effect on blood, liver and femur. Further increases in dietary protein content to 80% increased the lead concentration in both kidneys and femur but did not produce any significant effects in the blood (Fig. 2) and liver.

Fat

Data showing the relationship between tissue lead and varying dietary fat are given in Table 5. Decreasing the dietary fat content from 5% to 0 and 2·5% did not affect the lead content of the tissues studied. By contrast, increasing the fat content to 10, 15, 20 and 40% resulted in increased lead content of the tissues. There was a progressively increasing effect on lead concentration in carcass and organs from 10% to 40% dietary fat. The effects of 15% and 20% dietary fat did not differ significantly from each other. The values for blood and kidneys are given in Figs 4 and 5.

TABLE 4. Effects of dietary protein on lead absorption (ratio of mean retention experimental : control)

Diet (% protein)	Blood	Kidneys	Femur	Liver
0	5·1	2·5	2·8	2·2
5	2·2	2·8	3·3	3·0
10	1	1	1	1
15	1	1	1	1
20 (control)	1	1	1	1
40	1	2·0	1	1
50	1	1·9	1·3	1
60	1	3·7	2·6	1
80	1	2·5	1·5	1

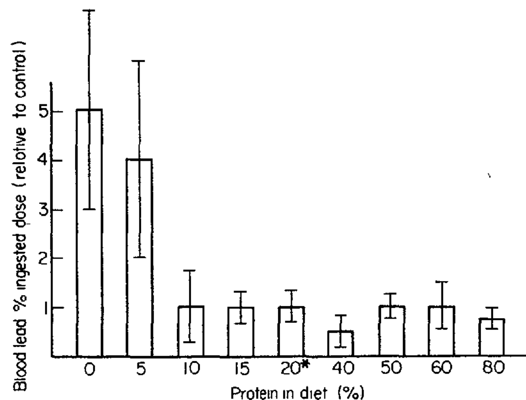

FIG. 2. Mean blood lead concentration as % ingested dose ±s.d. for diets of varying protein content relative to control diet (n = 6). * Control.

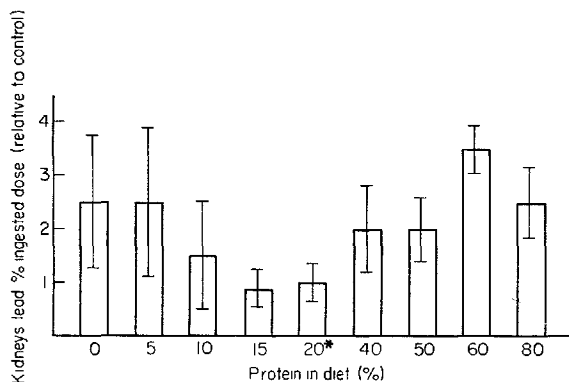

FIG. 3. Mean kidney lead concentration as % ingested dose ±s.d. for diets of varying protein content relative to control diet ($n = 6$). * Control.

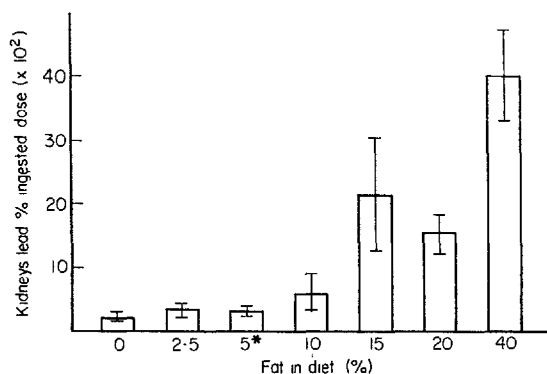

FIG. 5. Mean kidney blood lead concentration as % of ingested dose ±s.d. ($\times 10^2$) for diets of varying fat content ($n = 6$). * Control.

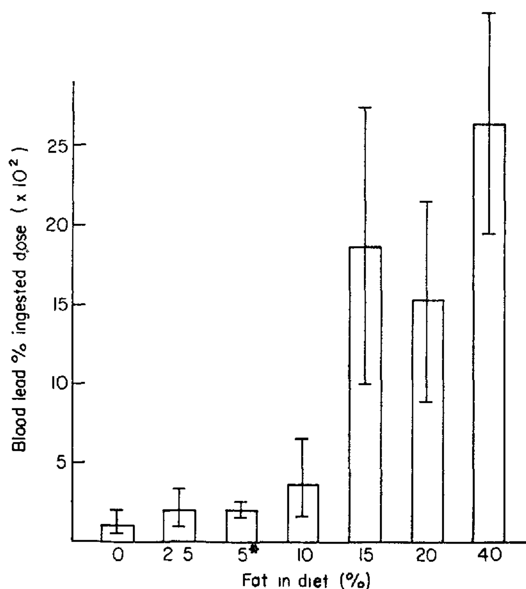

FIG. 4. Mean blood lead concentration as % ingested dose ±s.d. ($\times 10^2$) for diets of varying fat content. (n = 6). * Control.

Minerals

In order to identify the individual minerals responsible for increasing lead uptake from the gut, each of the minerals selected was omitted sequentially from the diet (Table 6). Initially, the response to the omission of major and minor components was determined. Diets deficient in calcium, phosphates, magnesium, sodium, potassium and chloride resulted in a marked increase in lead absorption but exclusion of iron, manganese, copper, zinc, iodine and molybdenum from the diet had no effect. Further studies showed that sodium, potassium and chloride had no effect on lead uptake. Diets without added calcium or phosphates resulted in increased tissue lead concentrations which appear to be additive. A low dietary magnesium resulted in increased lead retention in the tissues but to a lesser extent than either calcium or phosphates alone.

Increasing the total mineral content of the diet four-fold resulted in an 80% decrease in lead retention in blood, 50% in kidneys and 90% in femur and liver. The individual minerals responsible for this effect are currently under investigation in this laboratory.

TABLE 5. Effects of dietary fat on lead absorption (ratio of mean retention experimental : control)

Diet (% fat)	Blood	Kidneys	Femur	Liver	Carcass
0	1	1	1	1	1
2·5	1	1	1	1	1
5 (control)	1	1	1	1	1
10	1·9	2	1·5	1·5	1·8
15	9 6	7·5	4 8	4·2	5·2
20	7·9	5·5	4·6	4 4	4·2
40	13·6	14·2	10·8	7·1	8·9

TABLE 6. Effects of dietary minerals on lead absorption (ratio of mean retention experimental : control)

Diet	Blood	Kidneys	Femur	Liver	Carcass
Low total minerals	17·7	11·9	13·7	8·8	11·8
High total minerals	0·2	0·5	0·1	0·1	—
Low Ca, PO_4, Mg, K, Na, Cl	17·7	14·9	10·7	8·2	11·0
Low Fe, Mn, Cu, Zn, I, Mo	1	1	1	1	1
Low calcium	6·2	4·4	3·4	4·0	3·1
Low phosphate	2·7	3·0	2·1	1·9	1·8
Low calcium and phosphate	9·7	7·4	4·9	3·8	4·4
Low magnesium	2·7	1·6	1·6	1·7	1·7
Low K, Na, Cl	1	1	1	1	1

Discussion

The results show that nutritional factors have a marked effect on the absorption of lead from the gastrointestinal tract. Previous data on the apparent high absorption of lead from the gastrointestinal tract in suckling rats compared with adult rats (Pentschew and Garro, 1966; Kostial *et al.*, 1971a, b) may thus be partly explained by the nature of their diet rather than functional immaturity of the gut.

Cow's milk has been used as a prophylactic for lead poisoning but this view has been questioned by Kello and Kostial (1973) who found enhanced lead uptake in rats when milk was added to the animals' diet. The findings of this paper suggest that the high fat and protein content of milk might be expected to increase lead absorption but the effect would be counteracted by the high mineral content.

There are three possible mechanisms by which nutritional factors could affect the absorption of lead from the gut. Firstly, by the binding of lead to a poorly absorbed factor or its derivatives thus rendering the lead unavailable for absorption; secondly, by the interaction of the factor with the cellular processes regulating lead absorption; thirdly, modification of the metabolic status of tissues with an affinity for absorbed lead. At present, the data presented in this paper cannot distinguish between these possibilities. Since the experimental period used in this study was acute and the diets isocaloric, metabolic adaption is unlikely to account for the results obtained. The effects observed are more likely to be due to the nutritional factors acting directly on lead absorption from the gut.

It is not known whether lead absorption from the gut involves single or multiple pathways. The results from the protein studies seem to indicate that multiple routes might be involved since both low and high protein intake enhanced the absorption of lead. Similar results were obtained by Milev *et al.* (1970) although they found increased lead in all the organs of animals on a high protein diet, whereas in this study an increased lead content was found in only the kidneys and femurs. Gontzea *et al.* (1970) suggested the hypothesis that a low protein diet results in impaired detoxifying mechanisms of the liver thus allowing more lead to be accumulated. This hypothesis, however, does not explain the enhanced lead uptake associated with a high protein diet.

Using kidney lead retention as an index of lead uptake, the relative importance of individual nutritional factors can be determined. A regime containing 40% fat will increase uptake fifteen-fold. A mineral-deficient diet will result in a twelve-fold increase. However, the increases in lead absorption due to the lack of the individual minerals, calcium, phosphates and magnesium do not summate to a twelve-fold increase (Table 6) so that there would appear to be a synergistic effect. Since a low calcium diet is known to modify the absorption of other minerals, for example magnesium (Morris and O'Dell, 1963) and strontium (MacDonald *et al.*, 1952, 1955), it is probable that a common pathway exists for the absorption of calcium and other minerals including lead from the gut. Both low and high protein diets will also enhance lead uptake in the kidneys 2·5 and 3·5 times respectively.

Vitamins did not have any effect on lead uptake. This is contrary to the findings in relatively long-term studies where a dietary deficiency in vitamin C (Pillemar *et al.*, 1940) enhanced lead toxicity and an increase in vitamin D (Sobel *et al.*, 1940) resulted in enhanced lead absorption. The data reported in this paper suggest that the effect of vitamins do not result from an action on the gut.

The only dietary regime that decreased lead uptake was that with added minerals. These results suggest a potential application of minerals as prophylactics for lead workers and children living in high risk situations. The marked effects of the different nutritional factors demonstrated in this report might in part explain the wide range of blood lead concentrations found in community studies.

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