THE GASTROINTESTINAL ABSORPTION OF LEAD

STEPHEN PARTRIDGE

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Stephen Partridge

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The intestinal absorption of orally dosed lead was studied in the rat using radiotracers. The main target organs for lead were liver and kidneys, though lead was widely distributed throughout soft and hard tissues. The overnight fasted rat absorbed approximately 30% of an oral dose of lead, whereas the fed animal absorbed only 5%. This suggested that food exerts a protective effect against lead toxicity.

The kinetics of lead absorption were studied. The uptake of lead was seen to be directly proportional to the concentration of lead acetate dosed. No evidence of saturation of absorption was seen in any tissue. This suggests that lead enters the body via a diffusion mechanism.

Agar replicas and mucosal scrapes were used to study the interaction of lead with the intestinal tissue. Significant amounts of lead were associated with apical glycocalyx and muscle layers. Although several factors (glucose, EDTA, iron competition, and amount of lead dosed) altered this interaction, no significant change in the amount of lead absorbed into the animal was observed. In the young rat, increased amounts of lead were observed in the intestinal wall and organs of the body cavity. This suggests that alternative mechanisms to those in adults exist.

The small intestinal contents of lead dosed rats were partitioned into soluble and insoluble phases using ultracentrifugation.

Gel permeation chromatography of the soluble (supernatant) phase indicated that it was mainly inorganic lead and lead bile salt complexes. Another minor species (presently unidentified) was produced in the fasted rat. This species may be protective in nature. The pellet (insoluble) phase was investigated using a sequential leaching technique. The pellet was shown to contain mainly lead carbonates, although other species (e.g. phosphates) were also present.

Key Words: Lead, Intestine, Absorption, Speciation.

DECLARATION

This work was carried out between October (1982) and September (1985) in the Molecular Sciences Department, Aston University.

The work has been done independently and has not been submitted for any other degree.

Stephen Partridge

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CHAPTER 1

INTRODUCTION

1.1 LEAD IN THE ENVIRONMENT

Lead is widely distributed throughout the environment. It is toxic and has a long residence time within the biosphere. Lead poisoning remains an important public health problem. Lead poisoning has been reviewed by several authors (Chisholm, 1971; Waldron and Stofen, 1974; Singhal and Thomas, 1980; O'Brien, 1980; King, 1982; Rutter and Russell-Jones, 1983 and Southwood, 1983).

Lead enters the environment from natural and anthropogenic sources. The natural sources (leaching and volcanic eruptions) contribute less than 10% of the total input (Nriagu, 1979). Anthropogenic sources are motor vehicle emissions, lead smelting and refining and the burning of domestic refuse and coal (Patterson, 1980).

To exert a toxic effect lead must be absorbed into the body. The main absorption routes are ingestion and inhalation. Subcutaneous absorption is a minor route but this may be important in the case of organic lead species (Grandjean, 1978).

Ingested lead is composed of lead associated with food and water though these sources are interactive. Dietary lead is the main contributor to the body burden of lead (Mahaffey, 1977). Evidence suggests that dietary intake of lead is falling (Hilburn, 1979) and presently it is estimated to be approximately 100 μg/day in adults and 60 μg/day in children (Bander, Morgan and Zabik, 1983).

1.1.1 Lead in Food

Lead may enter food during the growing, storage and cooking processes.

In the growing cycle lead from air is precipitated onto the plants and soil (J.U.R.U.E., 1982). The treatment of land with sewage sludge containing high lead levels raises the soil lead level (Sterritt and Lester, 1981). Translocation of lead

from soil to the plant is poor (approximately 10%, M.A.F.F., 1982) but in areas with highly contaminated soils this may cause a significant increase in the crop lead level (Gallacher, Elwood, Phillips, Davies, Ginnever, Toothill and Jones, 1984)).

Leaching of lead from solder in cans during storage contaminates food. The amount of lead leached depends upon food type and whether the opened can is used for storage. This source of lead has been estimated to contribute 14 μg/day of the daily lead intake from food (Southwood, 1983). Legislation necessitates that solder containing no lead be used in cans containing baby foods. The U.K. food processing industry seems to be moving from soldered to welded steel cans in an attempt to reduce lead contamination of food during processing.

During cooking certain foods (vegetables and pasta) absorb lead from cooking water. Lead may also be absorbed onto food from cooking utensils (Moore, Hughes and Goldberg, 1979).

1.1.2. Lead in Water

Most of the lead in domestic water supplies arises from lead piping. The most recent survey of household water lead levels found that 20% of households in the United Kingdom had water lead levels in excess of the E.E.C. recommended level of 50 μg dl^{-1} (Pocock, 1980). Plumbosolvency is affected by several factors: pH, temperature and water hardness being most important (Moore, 1977), although the length of the lead piping, bends in the system, contact time and mechanical vibration are also involved (Oliver, 1980). Newer properties and modernised properties generally use copper piping; although this should eliminate lead from the domestic system the presence of lead as a sealant in joints can give rise to significant levels of lead in water (Moore, 1977). Acid soft water is plumbosolvent; since much of Scotland's water is of this type treatment is needed to raise pH and hardness and reduce plumbosolvency. Control of water lead has an effect on exposure to lead and this is reflected in a fall in blood lead levels (Sherlock,

Ashby, Delves, Forbes, Moore, Patterson, Pocock, Quinn, Richards and Wilson, 1984).

1.1.3 Lead in Air

The main source of lead in air is motor vehicle emissions. The D.H.S.S. working party (Lawther, 1980) estimated 90% of air lead was derived from the lead additives used as octane boosters in petrol with the remaining 10% from industrial sources.

Air lead levels are very variable depending on traffic density and local industrial sources (scrapyards, smelters and refineries). Airborne lead is known to influence blood lead levels but the data do not allow any conclusive relationship between the two factors to be drawn.

The isotopic lead experiment was used to try to quantify the contribution of petrol lead to the body lead burden. From 1975 to 1979 all the lead additives to petrol used in the Turin area were manufactured using lead from the Broken Hill mine in Australia. This lead has an isotopic ratio of $^{206}Pb : ^{207}Pb$ of 1.04. This isotopic ratio was distinct from that used before the experiment. Measurements of the isotopic lead ratio in blood enabled the contribution of petrol lead to intake to be determined (Falconetti, 1982). The results indicated that in areas with high traffic density up to 24% of blood lead was petrol derived whereas in rural areas only 11-12% of blood lead was from this source. The data must be treated with caution since airborne contamination of food and water may be involved in lead uptake.

1.1.4 Adventitious Sources of Lead

These sources include paint, dust, soil and the glaze used on certain utensils (Gloag, 1981). Paint, dust and soil may be particularly important lead sources in children who practice pica (a habit of ingesting non food items, Johnson,

1980). Adults who smoke take in more lead; this is reflected in a higher blood lead level (Tola and Nordman, 1977). Also D.I.Y. enthusiasts are exposed to high lead levels particularly when sanding down old paint (containing high levels of lead) from surfaces.

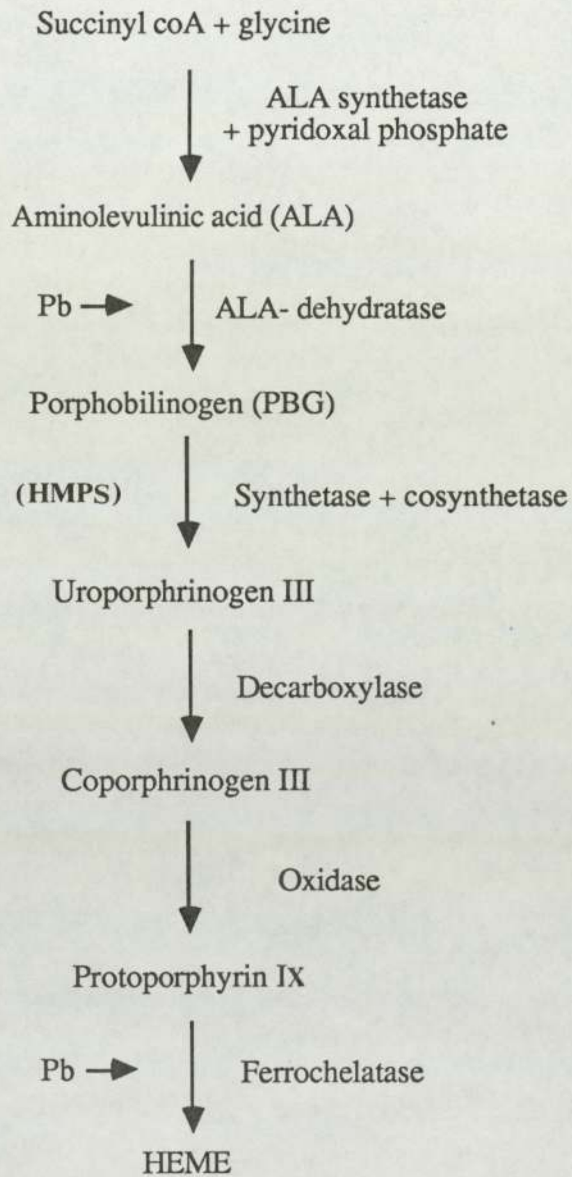
1.2 THE HEALTH EFFECTS OF LEAD

Human exposure to lead is most frequently monitored using blood lead levels (Pb-B). Currently there is no minimum agreed Pb-B below which no toxic effect is seen. Otto (1983) reported effects on children at Pb-B of about 15 $\mu g\ dl^{-1}$. Symptoms of frank lead poisoning occur at Pb-B over 80 $\mu g\ dl^{-1}$. These symptoms include anaemia, constipation, colic, renal damage and neurological disturbance. The symptoms of lead poisoning are more variable in children, with irritability, loss of appetite, loss of muscle co-ordination and coma being seen (Batuman, Candy, Maesaka and Weeden, 1983). The current recommendation is that Pb-B over 25 $\mu g\ dl^{-1}$ be investigated (Lawther, 1980).

There have been several large surveys of blood lead levels. The U.S. National Health and Nutrition Examination Survey II (NHANES II) revealed that blood lead levels were associated with race (being higher in blacks than whites), sex (being higher in men than women) and income (being higher in the poor than the rich). NHANES II indicated a significant correlation between the fall in Pb-B in the U.S. population and the decrease in gasoline usage. However the data are not conclusive in this respect (Pirkle, 1985). Blood lead levels are commonly used to estimate body burdens of lead because they can be measured on a large scale and are fairly cheap (Harding, 1981), but they only indicate recent exposure (David, Wintrob and Arcoles, 1982).

After ingestion lead is absorbed from the small intestine. Once in the body it becomes widely distributed and exerts toxic effects. The principal target organs for lead toxicity are brain, haematopoietic system, liver and kidney. Lead

Figure 1.1



The heme synthetic pathway with inhibitory effects of lead shown (Petering, 1980).

also adversely effects the immune, cardiovascular and reproductive systems and the auditory and visual pathways.

Lead's effect on the brain has promoted study into possible intellectual deficit in children (Moore, 1980 and Needleman, 1982). The data presented suggest moderate blood levels (30 $\mu g\ dl^{-1}$) cause significant impairment in IQ, reading and mathematical ability. However it is important that the weighting effect of social class be taken into account since children from lower social classes have high lead exposure and greater intellectual impairment (Lansdown, 1983).

Lead affects the brain in several ways. At low Pb-B the primary effect is biochemical, with subtle changes in neurotransmitter synthesis. At higher Pb-B irreversible demyelination occurs causing neural damage (Feldman, Hayes, Younes and Aldrich, 1977). At Pb-B in excess of 80 $\mu g\ dl^{-1}$ encephalopathy occurs due to capillary damage; this may be fatal (Damastra, 1977). In the liver and kidney lead affects metabolism. The liver has impaired carbohydrate and protein metabolism (De Michele, 1984) with reduced reabsorption from the kidney tubule cells (Varvrossum, 1985).

Lead affects haem biosynthesis via inhibition of several of the enzymes involved in the synthetic pathway (Petering, 1980) see figure 1.1.

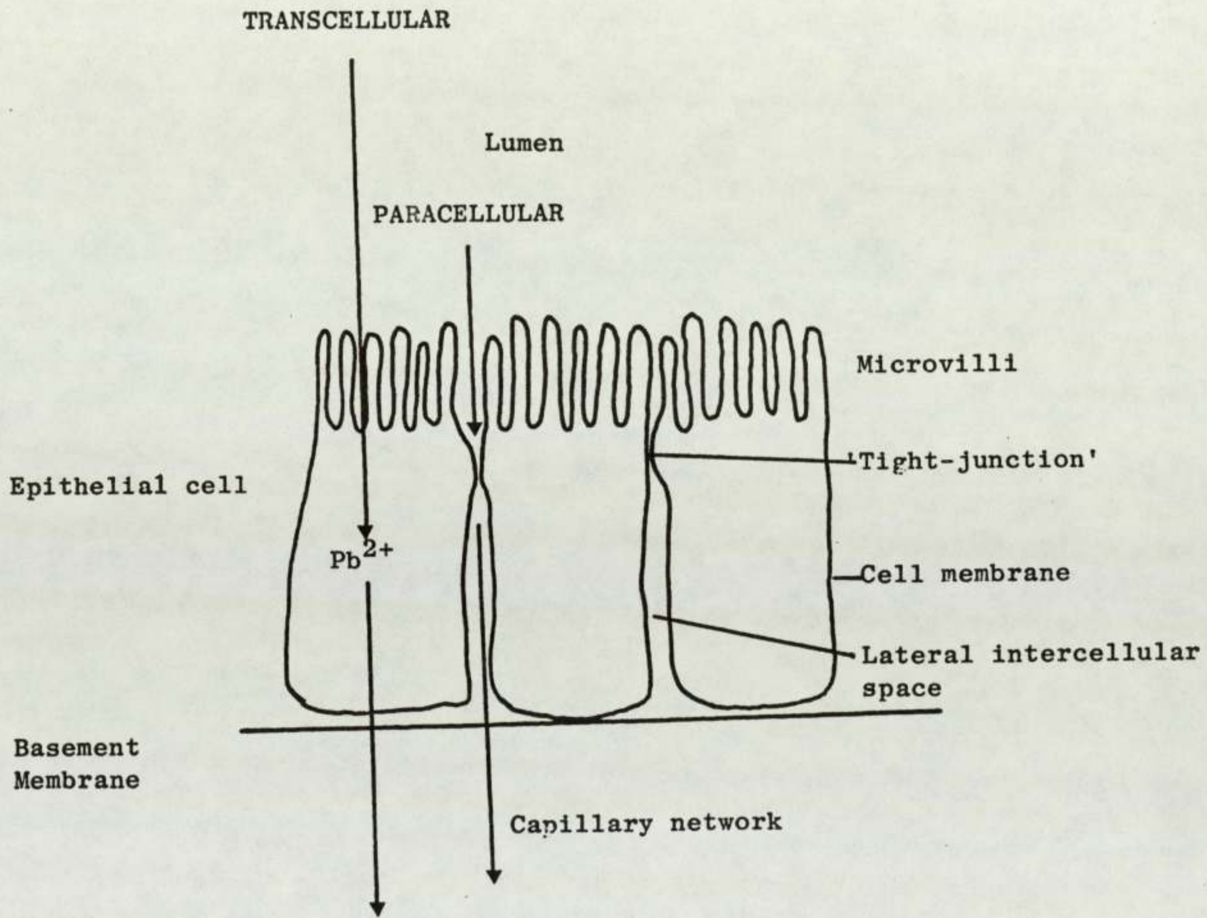
1.3 MORPHOLOGY OF THE SMALL INTESTINE

A knowledge of intestinal morphology is useful when attempting to analyse the transport of lead into the body. The small intestine is an organ of absorption and secretion. It is a long muscular tube which can be divided lengthwise into three regions, duodenum, jejunum and ileum.

The wall of the small intestine is essentially composed of three layers, the epithelium, the lamina propria and the muscularis. To maximise absorption the wall of the small intestine is specialised. These specialisations include the villi (finger like projections approximately 1mm high) which project into the lumen of the

Figure 1.2

Possible transport routes for lead across the Intestinal Epithelium



small intestine. The epithelium is composed of a single layer of cells. The columnar epithelial cells are mostly absorptive in function, although other cell types are present (Holt, Kotler and Pascal, 1983). These epithelial cells are produced at the crypts of Leiberkuhn and as they migrate from villus base to tip they mature, finally when senescent, being sloughed off into the gut lumen. The luminal surface of the absorptive cell is folded to form microvilli (1 µm long and 0.1 µm wide, see figure 1.2). This surface is known as the brush border membrane and is the primary site for absorption.

The surface of the small intestine is covered by a continuous layer of mucus. This viscous layer is protective in function. In the duodenum mucus protects the gut surface from the harmful effects of HCl and pepsin. Throughout the gut it protects against shearing forces (produced by food movement) and pathogenic organisms (Allen, 1978). Embedded in the mucus layer is a layer of glycoprotein called the glycocalyx. The glycocalyx serves as a support for the mucus layer and the enzymes responsible for digestion (Ugolev et al. 1979 and Quaterman, 1982). The role of the glycocalyx in metal absorption is obscure. Binding of metals to the glycocalyx has been reported (Quaterman, 1982) and mucin itself reduces permeability to small positively charged species (Kellaway and Marriot, 1975).

1.4 ABSORPTION OF LEAD BY THE SMALL INTESTINE

There are two possible routes by which lead can cross the epithelial layer of the small intestine, see figure 1.2. Paracellular transport involves passage of lead through the tight junctions and lateral intercellular spaces. Transcellular transport is more complex. Lead must be transported across the brush border membrane, cytoplasm and basement membrane of the absorptive cell. The transcellular route would be involved if a carrier were used to translocate lead from the gut lumen into the cell.

1.4.1 Possible Mechanisms for Lead Absorption

Three mechanisms have been proposed to describe the intestinal absorption of inorganic lead.

1.4.1.1 A carrier model

This model proposes the energy dependent translocation of lead from the gut lumen using metal binding proteins located in the brush border membrane as carriers. The metal binding proteins that have been proposed are iron binding protein (FeBp) (Flanagan, Hamilton, Haist and Valberg, 1979) and the calcium binding protein CaBp (Barton, Conrad, Harrison and Nuby, 1978). This process would be active and would exhibit saturation kinetics.

1.4.1.2 A passive model

This model is energy independent . Transport depends upon simple diffusion. This model includes binding of lead to the gut surface possibly by precipitation with phosphate (Blair, Coleman and Hilburn, 1979; Coleman, Blair and Hilburn, 1982; Coogan, 1982 and Heaven, 1985). This model would exhibit linear kinetics without saturation.

1.4.1.3 A combined model

This model was proposed by Aungst and Fung, (1981). The active component is a carrier which saturates at luminal lead concentrations of 1×10^{-5}M. The passive component occurs at higher luminal concentrations where absorption occurs by diffusion. This process would exhibit mixed kinetics.

1.4.2 Previous Studies of Lead Absorption

The intestinal absorption of lead is affected by numerous dietary, physiological and chemical factors (Moore, 1979; Coleman, 1979; Mahaffey, 1981; Coogan, 1982; Mahaffey, 1983 and DeMichele, 1984). A full discussion of all the

factors which are involved is beyond the scope of this thesis but some of the more important ones will be considered.

Deficiency of calcium has been shown to enhance the absorption of lead in the rat (Six and Goyer, 1970; Mahaffey, Goyer and Haseman, 1973; Barltrop and Khoo, 1976; Meredith, Moore and Goldberg, 1977; Barton et al., 1978 and Moore, 1979). The mechanism behind this effect is unknown but the transport of lead by a vitamin D dependent calcium binding protein (CaBp) has been suggested (Barton et al., 1978).

Iron deficiency, as seen in anaemia, has been shown to increase lead absorption in the rat (Mahaffey, Six and Goyer, 1971; Hamilton, 1978; Flanagan, Hamilton, Haust and Valberg, 1979). Increased lead transport seen is suggested to be due to the binding of lead to a mucosal carrier produced in the deficiency state. (Flanagan, Chamberlain and Valberg, 1982).

It is well known that children are more susceptible to lead poisoning than adults. A similar age dependent absorption is seen in the rat. Kostial, Siminovic and Pisonic (1971) reported that lead absorption was markedly enhanced in 5 - 7 day old rats when compared to adults. This correlates with the phagocytosis seen to occur in weanling animals (Keller and Doherty, 1980). In a long term experiment Forbes and Reina (1972) showed lead absorption in rats decreased with increasing age up to the threshold age of 29 days, after which the amount of lead absorbed becomes independent of age.

The presence of food in the gastrointestinal tract has been shown to decrease lead absorption in the rat when compared to the fasting state (Garber and Wei, 1974; Quaterman, Morrison and Humphries, 1976; Conrad and Barton, 1978 and Aungst and Fung, 1981). A similar effect has been seen in man (Rabinowitz, Kopple and Wetherill, 1980; Heard and Chamberlain, 1982; Blake, Barbezat and Mann, 1983 and James et al 1985).

The effect of chelating agents on intestinal absorption has been studied

and conflicting data has been published with regard to ethylenediamine tetra acetic acid (EDTA). In rat whole body studies EDTA had no effect on the intestinal absorption of lead (Garber and Wei, 1974). This data does not agree with the everted sac studies of Coogan, (1982), which showed increased rates of absorption with EDTA. Human studies have shown that EDTA reduces absorption (James, personal communication).

1.4.3 The Importance of Lead Speciation for Absorption

There have been few studies on the speciation of lead with relation to absorption. The studies published to date have investigated the absorption of different lead salts and ligands on absorption.

In the gut lead is likely to become speciated since it readily forms complexes with amino acids and food components, particularly dietary fibre.

Therefore the species formed in the gut may be crucial in an understanding of lead absorption since previous work has indicated that lead ligand complexes are transported in a different way to the lead cation (Coleman et al., 1982). Cirkt and Teschy (1975) reported the existence of lead bile complexes in the small intestine but the role of bile in lead absorption is complex, since as well as having an excretory function bile is also actively reabsorbed in the colon (Maenz, 1982).

1.5 AIMS OF THIS WORK

1. To determine the kinetics of lead absorption from solutions of orally dosed lead acetate using ^{203}Pb as radiotracer.

2. To investigate tissue distribution of orally dosed lead with increasing time after dosing.

3. To study the interaction of lead with the wall of the small intestine and to determine the effect of region of tissue, glucose, iron competition, EDTA and factors likely to vary with age on this interaction.

4. To investigate the effect of nutritional, chemical and physical factors on the intestinal absorption of lead in the rat.

5. To study the chemical interaction of orally dosed lead acetate with the contents of the gastrointestinal tract using gel permeation chromatography and a sequential leaching technique.

6. To determine the effect of the presence of food, bile duct ligation and a large dose of lead on the chemical speciation of lead within the small intestine.

7. To describe a model of lead absorption in the small intestine of the rat.

CHAPTER 2

MATERIALS AND METHODS

2.1 CHEMICALS

All chemicals used in these experiments were of Analar grade supplied by BDH Limited, Fisons Limited, Sigma Company Limited or Pharmacia Limited. All gases were of medical grade supplied by British Oxygen Company. Radioisotopes were supplied by Amersham International and the Medical Research Council Cyclotron Unit (Hammersmith Hospital, London).

2.2 ANIMALS

The Wistar rats used throughout these studies were supplied by Bantin and Kingman (Hull, U.K.). Adult rats (10 weeks old) 180-200g and young (3-4 weeks old) 50-80g were supplied and used in the suppliers batches. Animals were maintained on Rat and Mouse Breeding Diet (Pilsburys Limited) and tap water. Where appropriate, animals to be fasted were placed in a stainless steel fasting unit with grid floors (to prevent coprophagy) for the 18 hours prior to dosing, and had access to tap water alone. After dosing fasted animals were not allowed food prior to being killed unless otherwise stated.

2.3 DOSING REGIME

All animals were dosed under a light ether anaesthesia. Adult rats were dosed using an olive bulbed oropharyngeal needle attached to a sterile hypodermic syringe (care was taken to deliver to the stomach). For young rats the oropharyngeal needle was replaced with a polythene catheter (Portex Limited) attached to a hypodermic syringe needle. All animals were dosed between 9 and 10 am. After dosing, the animals were tail marked for identification purposes and returned to cages before sacrifice. In those experiments where the animals were to survive for more than four hours after being dosed, and in the balance studies, the animals were placed in individual glass metabolic cages (Jencons Limited) which permitted the separate collection of urine and faeces.

2.4 DISSECTION

Animals were sacrificed under light ether anaesthesia by cervical dislocation. The body was opened by a dorsal midline incision from a point below the base of the sternum to the lower abdomen. The small intestine was excised between the pyloric sphincter and a point 5 cm above the ileo-caecal junction. The small intestine was gently washed through with 20 cm^3 of ice cold isotonic saline (0.9%), the resultant fluid being designated as gut contents. The small intestine was divided into three regions: the first 16cm of gut distal to the pylorus being the duodenum, and the remaining intestine being divided into two equal lengths, the proximal portion designated as jejunum with the distal portion as ileum.

When tissue distribution of radioisotopes was determined the remaining gastrointestinal tract and organs of the body cavity, and brain were removed, weighed and placed into counting vials for assessment of radioactivity.

2.5 LIGATION OF THE BILE DUCT

Ligation of the bile duct was performed under direct supervision of Dr C Bailey (Department of Molecular Sciences, Aston University). Anaesthesia was induced by a mixture of 3% Fluothane in a stream of 80% N_2O and 20% O_2, and then maintained by 1.5% Fluothane in 80% N_2O and 20% O_2. The abdomen was surface sterilized by swabbing with a mixture of 5% Hibitane and 1% ethanol in aqueous solution. The abdomen was opened by midline incision and the bile duct located then ligated with a pair of 3/O silk ligatures at the point just proximal to the entry into the duodenum. The abdominal wall was closed by silk suture (3/O) and the skin closed by stainless steel suture clips. Sham operated animals were subjected to the same procedure without ligation of the bile duct.

Animals were fasted for 18 hours prior to surgery and then allowed to recover for 48 hours; access to normal diet was permitted. Before dosing with lead the

animals were fasted for 18 hours.

2.6 RADIOISOTOPES

The gamma emitting isotope ^{203}Pb was used as the radiotracer for lead throughout these studies. The isotope was supplied as $^{203}PbCl_2$ in isotonic saline by the Medical Research Council Cyclotron Unit (Hammersmith Hospital, London). The half life of ^{203}Pb is short (52.1 hours) and counts in the sample were corrected for decay using the automatic decay correction facility on the LKB Compugamma. The gamma emissions of ^{203}Pb and ^{59}Fe were counted over the range 110-550 KeV.

Other gamma emitters used in the studies were supplied by Amersham International. ^{59}Fe was supplied as $^{59}Fe\ Cl_2$ in 0.1M HCl.

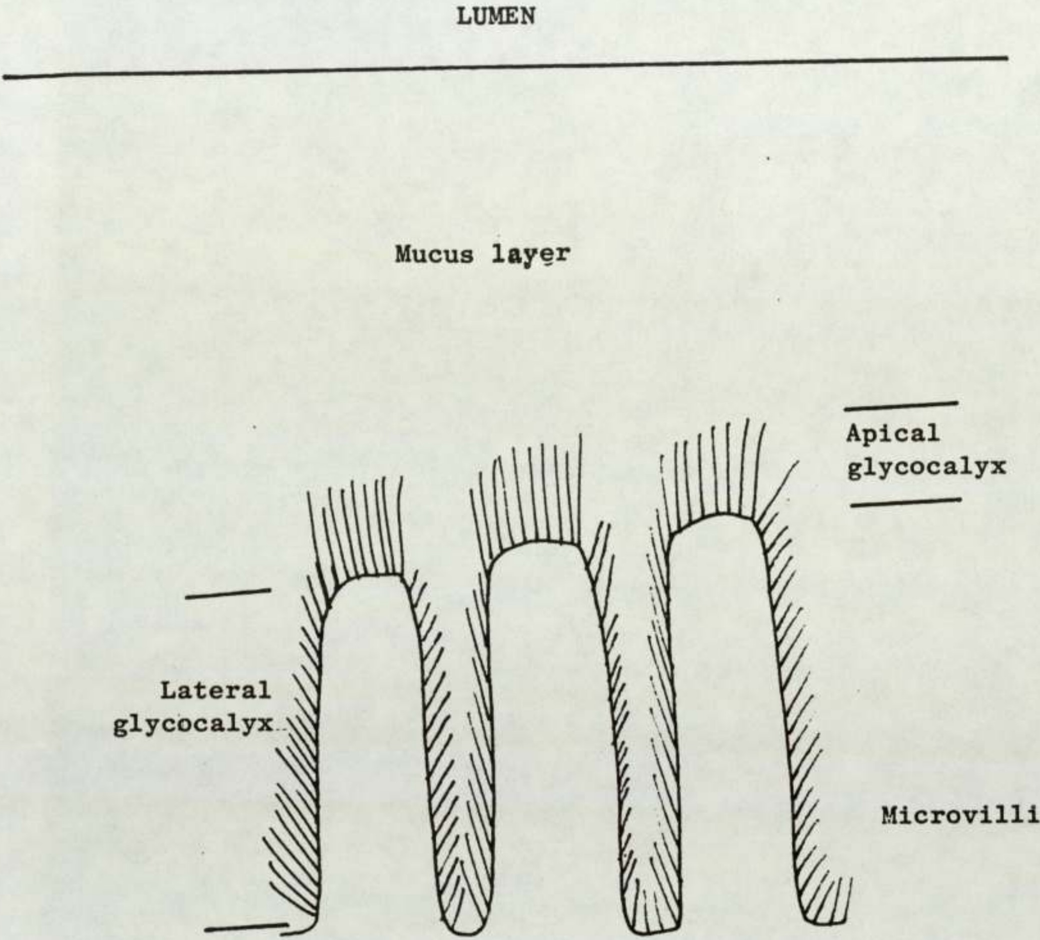
All samples containing gamma emitters were counted for 60 seconds or 200,000 counts in an LKB Compugamma. Background and decay were automatically corrected. Any sample not reaching twice the background count was rejected.

The beta emitters used, L-[U- ^{14}C] histidine and [carboxyl - ^{14}C] deoxycholic acid (sodium salt), were supplied by Amersham International. Samples were prepared for counting by the addition of $1cm^3$ of sample to $10cm^3$ of a premixed scintillation cocktail (LKB Optiphase Safe) then counted in a Beckman LS 7500 beta counter for 10 minutes or 10,000 counts. Quenching was corrected by comparison with a curve of differentially quenched standards of known activity.

2.7 THE AGAR REPLICA TECHNIQUE

First described by Ugolev et al., (1979) this technique used a solution of 3% agar at $40^{o}C$ to remove the apical portion of the glycocalyx (see figure 2.1). An 8cm long tube of intestine was tied off at one end using a cotton ligature, filled with $1cm^3$ of agar solution then tied off using cotton ligature. The agar filled sac was then

Figure 2.1



Schematic diagram of the microvilli and apical glycocalyx.

placed into 25cm^3 of ice cold oxygenated (95% O_2/5% CO_2) Krebs - Henseleit bicarbonate buffer (Krebs and Henseleit, 1932) until the agar had set. The sac was then removed, placed onto a cold glass plate, slit open lengthwise using a scalpel blade and the agar cylinder gently removed using forceps. The agar was then placed into a counting vial.

2.8 PREPARATION OF MUCOSAL SCRAPES

A mucosal scrape allows the epithelial cells and submucosa to be separated from the underlying circular and longitudinal muscle layers. After application of the agar replica technique (described in section 2.7) the intestinal tissue was laid onto a cold glass plate with the mucosal surface uppermost. The mucosa was then scraped off with an angled glass microscope slide. The mucosal scrape and muscle layers were put into separate counting vials.

2.9 PREPARATION OF INTESTINAL CONTENTS FOR ANALYSIS

The gut contents (see section 2.4) from adult rats were centrifuged at 100,000g for 1 hour in a MSE Superspeed 50 ultracentrifuge at 4°C. This centrifugation produced a supernatant phase which was analysed by gel permeation chromatography and a pellet which was investigated using a sequential leaching technique (see section 2.11).

2.10 ANALYSIS OF THE SUPERNATANT PHASE BY COLUMN CHROMATOGRAPHY

The lead species in the supernatant phase of the gut contents were separated using gel permeation chromatography. The columns were eluted with a 10^{-2}M lead acetate buffer pH 3.8. The chromatographic media used in these studies was Sephadex G-15 (Pharmacia Limited). Sephadex is a bead forming gel prepared

by cross linking dextran with epichlorohydrin.

The permeation columns were prepared as follows. Dry gel powder was allowed to swell in excess buffer for 24 hours. The slurry was then washed with 1000 cm^3 of buffer, degassed under vacuum and packed into a glass column (Amicon Wright Limited) using a peristaltic pump (Gilson Minipuls 2, Anachem Limited). To complete column preparation the gel bed was washed ascendingly with 800cm^3 of degassed buffer. Samples of the supernatant phase of the gut contents were loaded onto and eluted from the column ascendingly. Fractions were collected using an LKB Redirac 2112 fraction collector. Fractions were assayed by beta or gamma counting as required.

2.11 INVESTIGATION OF THE PELLET PHASE OF THE GUT WASHINGS BY A SEQUENTIAL LEACHING TECHNIQUE

This technique employs a series of chemical extraction procedures to isolate the metal species in a sample. The method used was that of Tessier et al., (1979). Sequential leaching procedure is shown in figure 2.2.

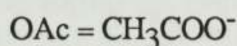
After each stage in the technique the soluble phase was separated by ultra centrifugation at 100,000g for 1 hour, the pellet being used for the next stage in the extraction technique.

2.12 STATISTICS

Unless stated otherwise the Students t-test was used to determine the significance of differences between two sample means drawn from a normal population. When appropriate the 'method of least squares' (see Holman, 1969, p68) was used to obtain the best fit of the data to a straight line. Probabilities were determined from tables (Murdoch and Barnes, 1979) and probabilities of 5% or less were taken as being significant. An Olivetti programma 101 computer was used for all statistical analysis.

Figure 2.2

Fraction extracted	Procedure for 1g pellet
Exchangeable pH = 7	1M NH_4OAc (10ml) pH = 7, 20°C, 1hr continuous agitation.
Carbonates pH = 5	1M NaOAc (20ml) pH = 5 (with HOAc) pH = 5, 5h continuous agitation
Oxides	0.04M NH_2OH HCl in 25% (v/v) HOAc (20ml) 96°C, occasional agitation.
Organic - Sulphides	0.02M HNO_3 (10ml) + 30% H_2O_2 (5ml), pH = 2, 2hr, 85°C, further 30% H_2O_2 (5ml), 3hr, 85°C, then 3.2M NH_4OAc in 20% v/v HNO_3(10ml) continuous agitation.
Residual	HNO_3 (conc) (20ml) 4hrs. 20°C.



The sequential leaching technique (Tessier et al., 1979).

CHAPTER 3

A KINETIC ANALYSIS OF LEAD ABSORPTION BY THE SMALL INTESTINE

3.1 INTRODUCTION

A kinetic analysis of lead absorption would provide an insight into the absorptive mechanism for lead in the small intestine. Three models have been proposed for lead absorption; the carrier model, the passive model and a combined model (as described in sections 1.4.1.1, 1.4.1.2, and 1.4.1.3).

These models have been proposed on the basis of data obtained using isolated tissue preparations. These experiments are not necessarily comparable to the situation seen in the whole animal, since they often involve alternative energy and oxygen supplies. Together with the lack of any hormonal and neurological influence this may result in patterns or mechanisms of absorption which differ from that seen in the intact animal.

The experiments detailed here were designed to perform an analysis of lead absorption in the intact animal. The uptake of lead by the rat small intestine and its subsequent distribution within the tissues of the animal was ascertained at (i) various times after oral dosing with 10^{-6}M lead acetate radiolabelled with ^{203}Pb, and (ii) four hours after oral dosing of various concentrations of radiolabelled lead acetate solutions.

3.2. TIME BASED STUDIES OF LEAD ABSORPTION

3.2.1 Methods

Adult male rats were fasted overnight for 18 hours and then orally dosed with 0.3cm^3 of 1 x 10^{-6}M lead acetate solution each 0.3cm^3 contained 1μCi of ^{203}Pb as radiotracer. 1, 2, 4, 24, 48 and 72 hours after dosing the animals were sacrificed and organs removed (as described in section 2.4). Those animals sacrificed 48 and 72 hours after dosing were allowed access to food 24 hours into the experiment to prevent any effects of prolonged fasting.

3.2.2 Results and Discussion

After 1 to 2 hours most of the ^{203}Pb administered is found in the stomach and small intestine with little radioactivity found in the caecum and colon (non absorptive region) table 3.1. Small but measurable quantities of lead were found in the liver, kidneys and spleen at one hour after dosing. Lead levels in these organs reach a peak at 4 hours and subsequently decline (table 3.1).

The amount of lead present in the intestinal tissue decreases with time after dosing. There is a parallel fall in the amount of lead in the gut contents; after 24 hours very little lead is associated with the tissue (table 3.1). At 24 and 48 hours very low amounts of lead are found in the organs examined. Faecal loss is approximately 67% of the initial dose, with about 5% of the dose being lost via the urine. After removal of the gastrointestinal tract and the organs of the abdominal cavity the remaining tissue (the carcass) was assayed for lead. The data reveal that the carcass contains approximately 17% of the initial dose at 4 hours and 21% at 24 hours.

The main excretory route for ingested lead is via faecal excretion. Faecal loss of approximately two thirds of the dose occurs within 48 hours of ingestion.

Using the data in balance studies; ie. (Uptake = amount ingested - amount excreted) and neglecting loss of absorbed lead through saliva, sweat, hair growth and biliary recirculation a retention of approximately 30% of the initial dose is seen in overnight fasted animals.

The interaction of lead with the small intestine may be crucial in determining the amount of lead taken up by the body. The interaction of lead with the small intestine is considerable at short time periods after dosing but this declines to approximately 3% of the initial dose after 24 hours.

The data are not in agreement with a prolonged retention of lead at the luminal surface as proposed by Coleman (1979). Nevertheless the binding of lead

to the gut surface may serve a protective function for short time periods after dosing.

3.3 LEAD ABSORPTION WITH VARYING CONCENTRATIONS OF LEAD

3.3.1 Methods

Adult rats were dosed by gastric intubation, after fasting overnight, with $0.3cm^3$ of lead acetate (10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} or 10^{-1} M) containing $1\mu Ci$ of ^{203}Pb. The animals were sacrificed four hours after dosing and the tissue distribution of lead was determined as described previously (section 2.4).

3.3.2 Results and Discussion

Lead was concentrated mainly in the kidneys and liver (table 3.2). This observation agrees with the previous studies of Hejmancik, Dawson and Williams (1982) and Heaven (1985). Measurable but lower quantities of lead were found in the spleen and brain (table 3.2). Less lead was present in the brain than in any other tissue examined, as has been found previously (Collins, Hrdina, Whittle and Singhal, 1982).

In all tissues examined there was a linear relationship between the concentration of lead dosed and uptake, with no evidence of saturation (figure 3.1-3.8). The data does not support the proposed models for lead uptake which involve a carrier for lead absorption. The data are indicative of absorption by a diffusion pathway as described by Coleman (1979); Coogan (1982) and Heaven (1985) who all found a lack of saturation in kinetic studies of the intestinal uptake of lead. These workers used the in vitro everted sac technique whilst this study involved intragastric dosing of radiolead.

Table 3.1 Distribution of Lead After Oral Dosing. Data Expressed as a Percentage of Total Dose Administered

	TIME AFTER DOSING (Hours)					
	1	2	4	24	48	72
Stomach	26.48 ± 22.01	26.05 ± 8.01	2.18 ± 0.60	0.17 ± 0.08	0.05 ± 0.03	0.03 ± 0.02
Absorptive	25.93 ± 20.39	23.17 ± 8.23	17.02 ± 7.96	3.24 ± 1.07	0.16 ± 0.07	0.11 ± 0.03
Gut Contents	17.79 ± 3.07	18.91 ± 0.55	10.67 ± 4.63	0.84 ± 0.59	0.09 ± 0.05	0.04 ± 0.03
Non-Absorptive (Caecum + Colon)	0.09 ± 0.07	2.29 ± 2.05	35.94 ± 7.45	26.40 ± 10.09	0.69 ± 0.12	0.16 ± 0.07
Liver	1.34 ± 1.32	3.17 ± 2.53	3.67 ± 1.52	1.49 ± 0.90	0.69 ± 0.12	0.48 ± 0.26
Kidney	0.70 ± 1.02	2.53 ± 1.22	2.89 ± 0.31	2.70 ± 1.88	1.59 ± 0.50	0.76 ± 0.49
Spleen	0.10 ± 0.08	0.30 ± 0.10	0.24 ± 0.31	0.20 ± 0.11	0.02 ± 0.01	0.02 ± 0.02
Faeces	0	0	0	38.23 ± 12.69	67.35 ± 13.80	65.28 ± 14.29
Urine	0	0.18 ± 0.16	0.30 ± 0.20	1.48 ± 0.80	2.24 ± 1.04	0.54 ± 0.28
Carcass	-	-	16.86 ± 4.19	20.65 ± 5.43	-	-
Recovery	68.55 ± 7.52	78.30 ± 5.30	72.50 ± 5.73*	73.97 ± 5.90*	71.06 ± 13.36	66.93 ± 15.16

Each Value is the mean of observations ± standard deviation

*Recovery does not include carcass radioactivity.

Table 3.2 Concentration of Lead (ng Pb/g wet wt. tissue) in Rat Tissues at four hours with Varying Concentrations of Lead Acetate

	LEAD CONCENTRATION (M)					
	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
Stomach	0.33 ± 0.21	2.05 ± 0.87	26.7 ± 29.1	130 ± 24.8	2603 ± 45.88	82666 ± 167400
Absorptive	1.57 ± 0.34	20.4 ± 4.03	117 ± 52.1	515 ± 273	5889 ± 4712	117552 ± 934960
Gut Contents	1.21 ± 0.67	8.68 ± 8.68	44.0 ± 34.1	1035 ± 682	6696 ± 50222	193158 ± 956660
Non Absorptive	2.52 ± 2.34	37.2 ± 27.2	608 ± 232	6712 ± 1754	10571 ± 1402	566680 ± 332182
Liver	0.42 ± 0.12	8.56 ± 1.24	52.7 ± 26.7	260 ± 43.4	868 ± 434	5137 ± 1505
Kidney	1.50 ± 0.38	20.58 ± 4.15	251 ± 110	918 ± 198	1674 ± 682	9610 ± 3720
Spleen	0.17 ± 0.14	1.67 ± 0.37	14.9 ± 13.6	62 ± 31	49.2 ± 55.8	12400 ± 1238
Brain	1.05 ± 0.12	8.07 ± 2.02	85.74 ± 39.6	698 ± 116	6200 ± 4659	10330 ± 2810

Each value is the mean of six observations ± standard deviation.

Figure 3.1 Uptake of lead into the stomach of fasted rats orally dosed with various concentrations of $^{203}PbAc$

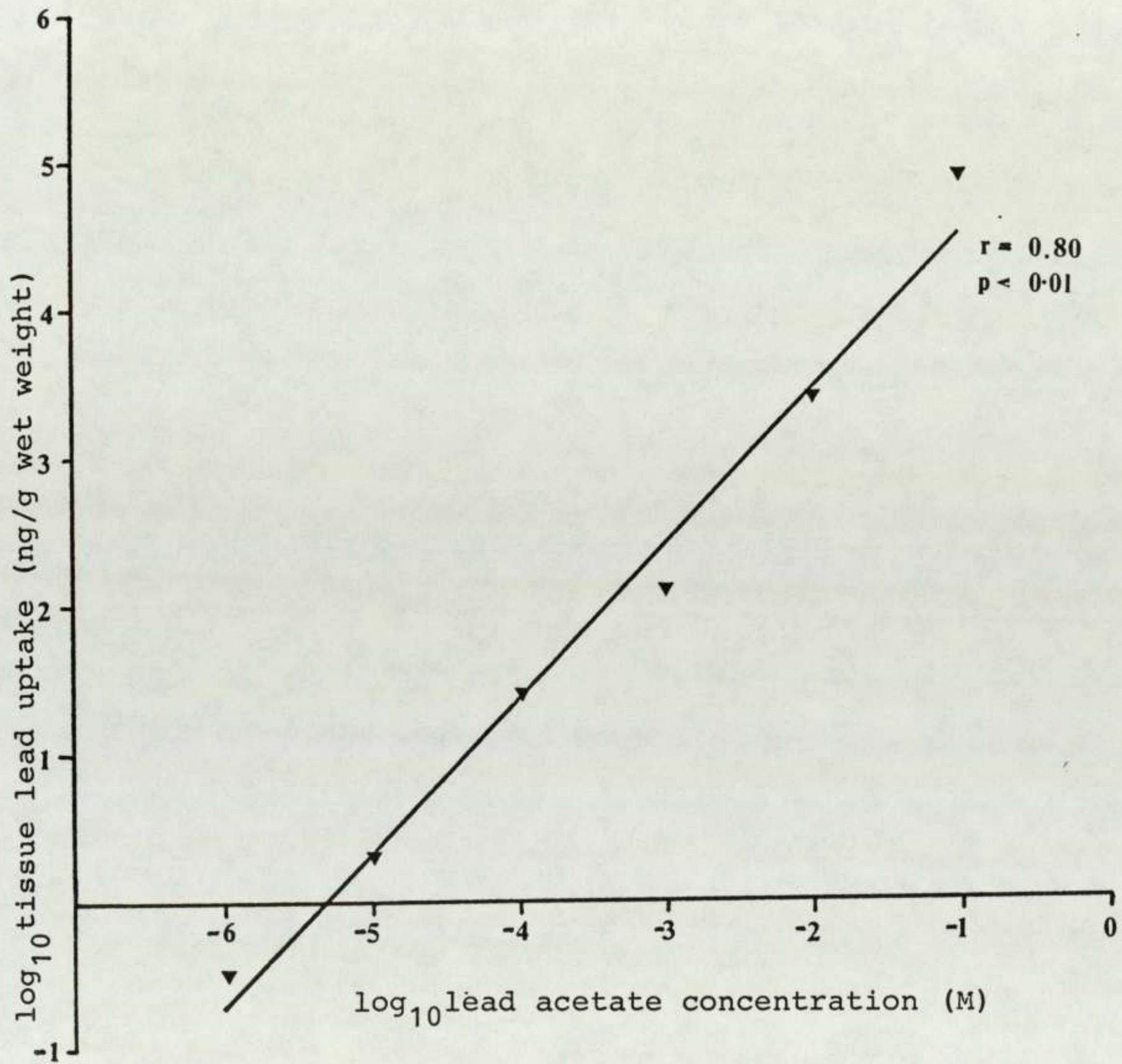


Figure 3.2 Uptake of lead into the absorptive region of rats orally dosed with various concentrations of $^{203}PbAc$.

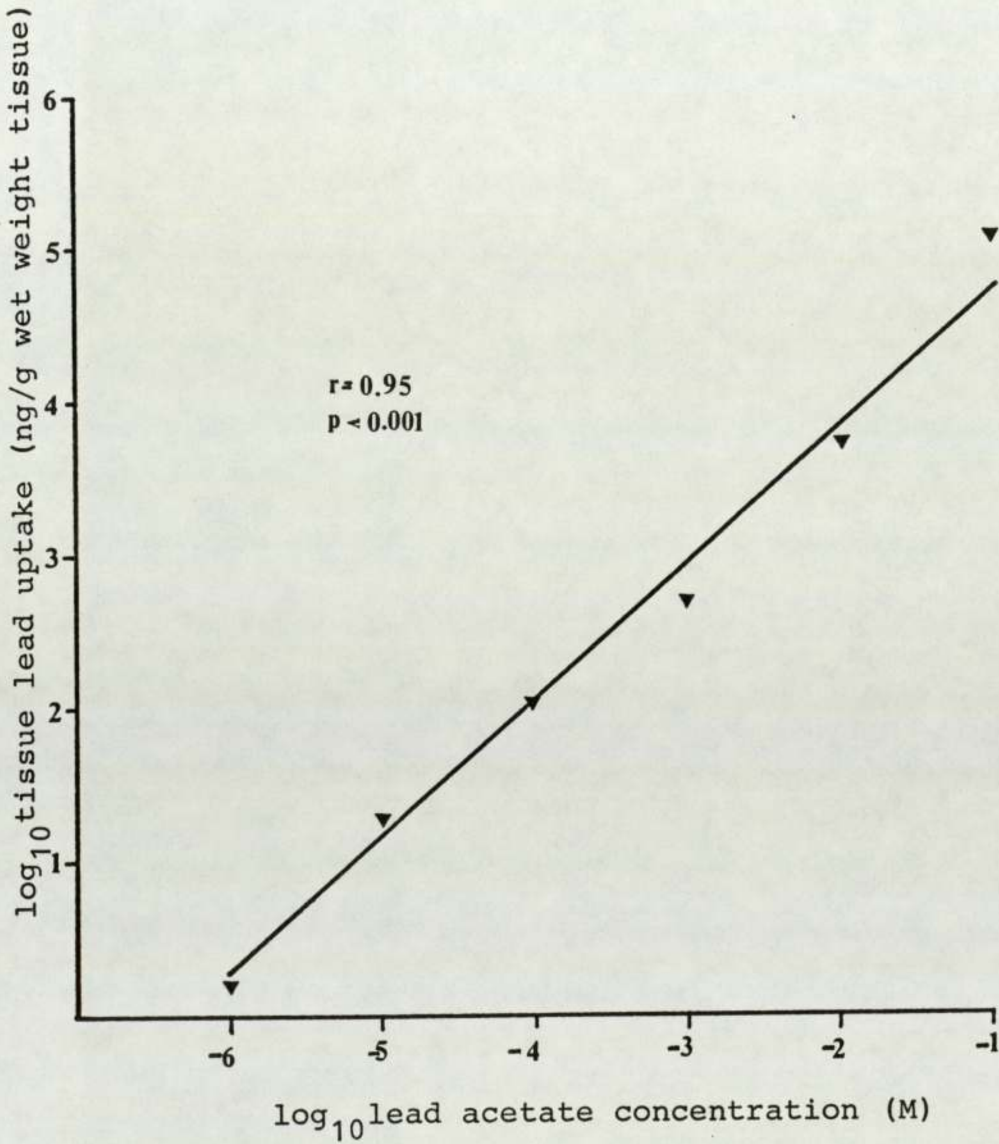


Figure 3.3 Amount of lead present in the small intestine contents of rats orally dosed with various concentrations of $^{203}PbAc$

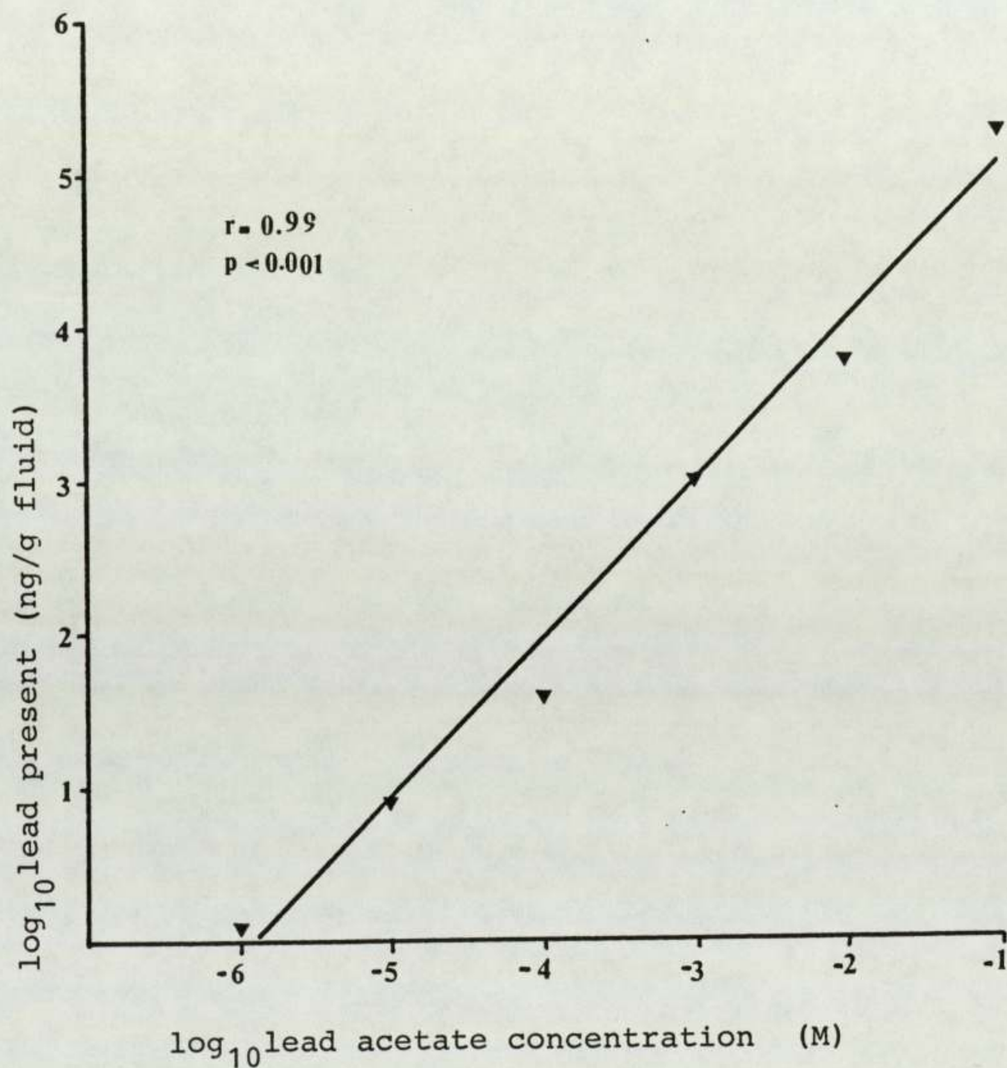


Figure 3.4 Uptake of lead into the spleen of rats orally dosed with various concentrations of ^{203}PbAc

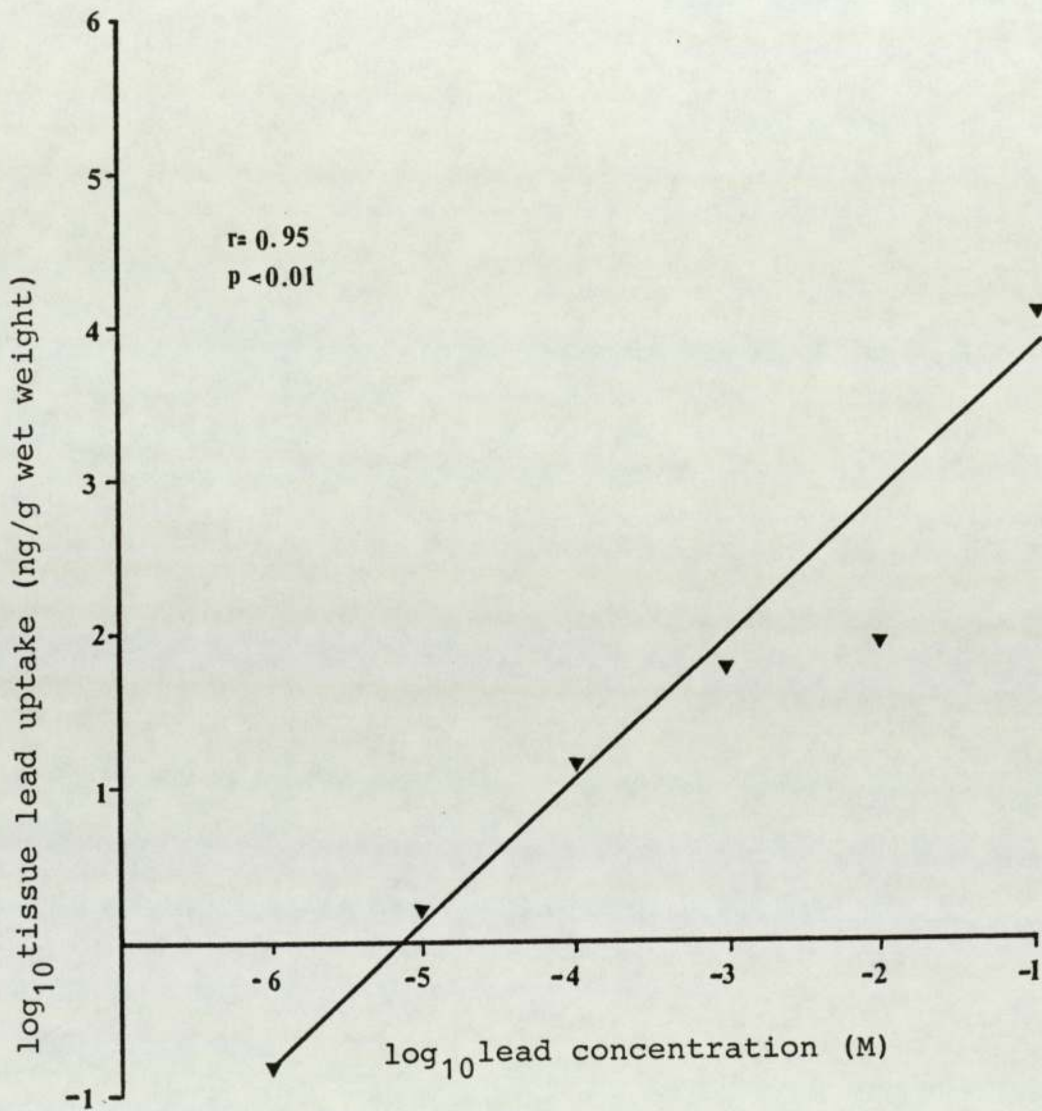


Figure 3.5 Movement of lead into the non absorptive region of fasted rats orally dosed with various concentrations of $^{203}PbAc$

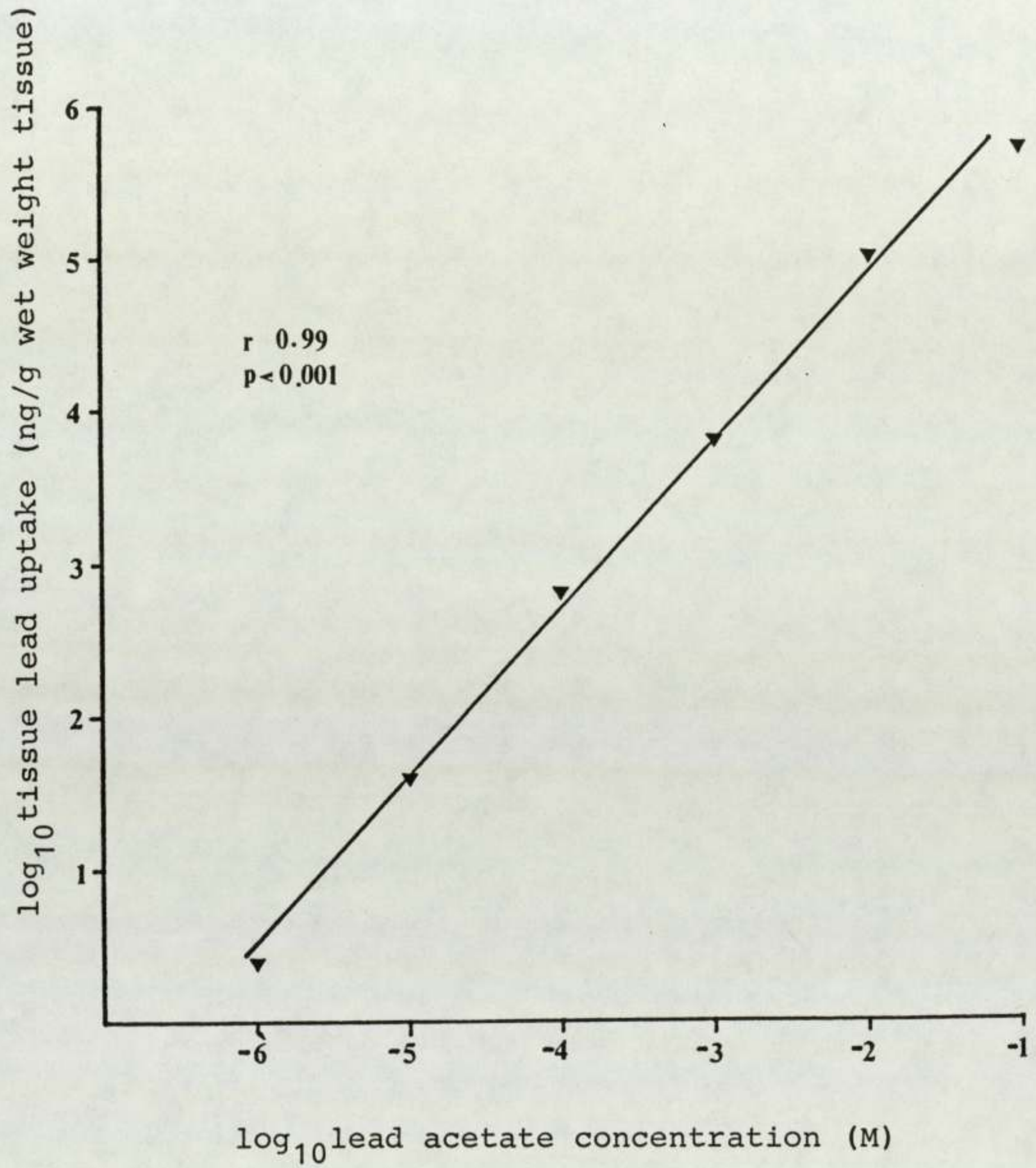


Figure 3.6 Uptake of lead into the liver of fasted rats orally dosed with various concentrations of $^{203}PbAc$

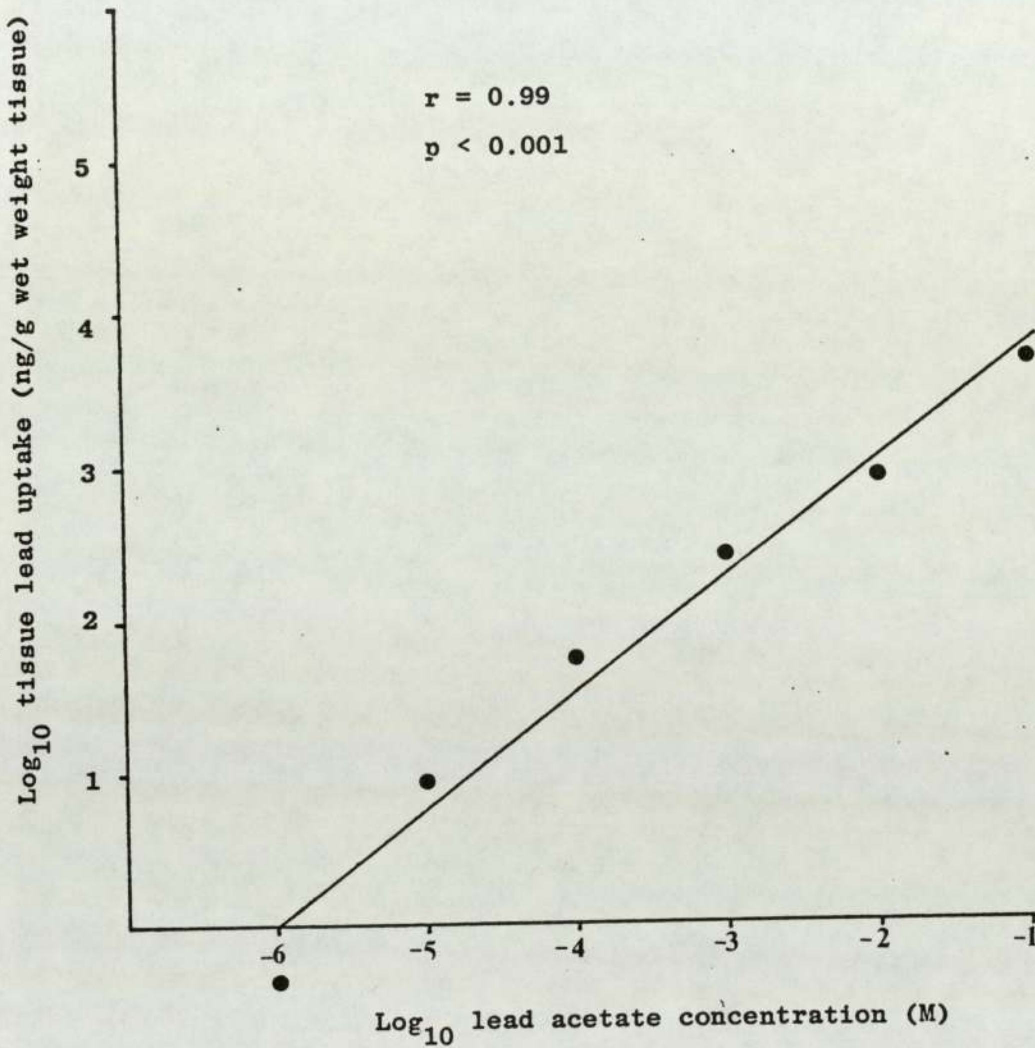


Figure 3.7 Uptake of lead into the kidney of fasted rats orally dosed with various concentrations of $^{203}PbAc$

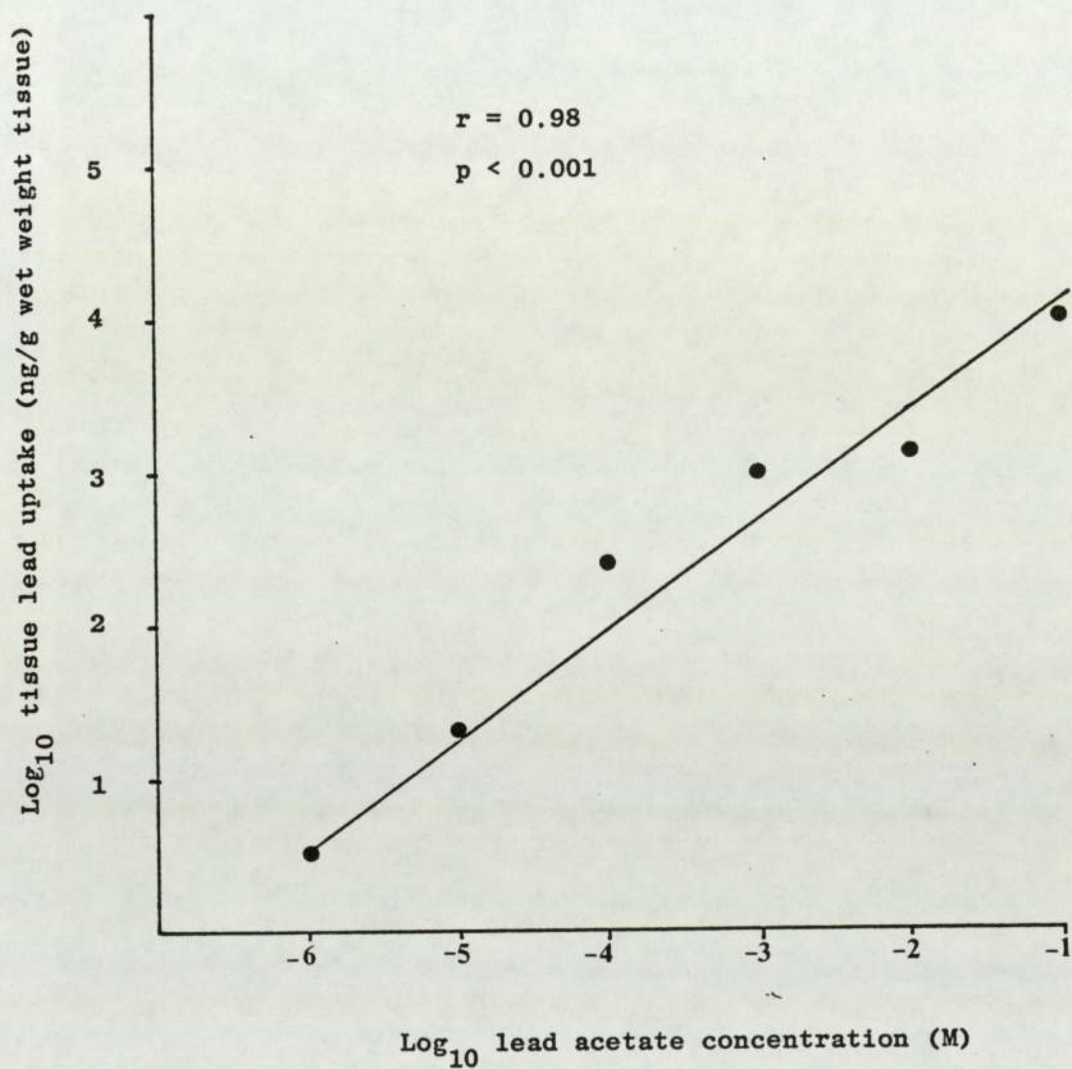
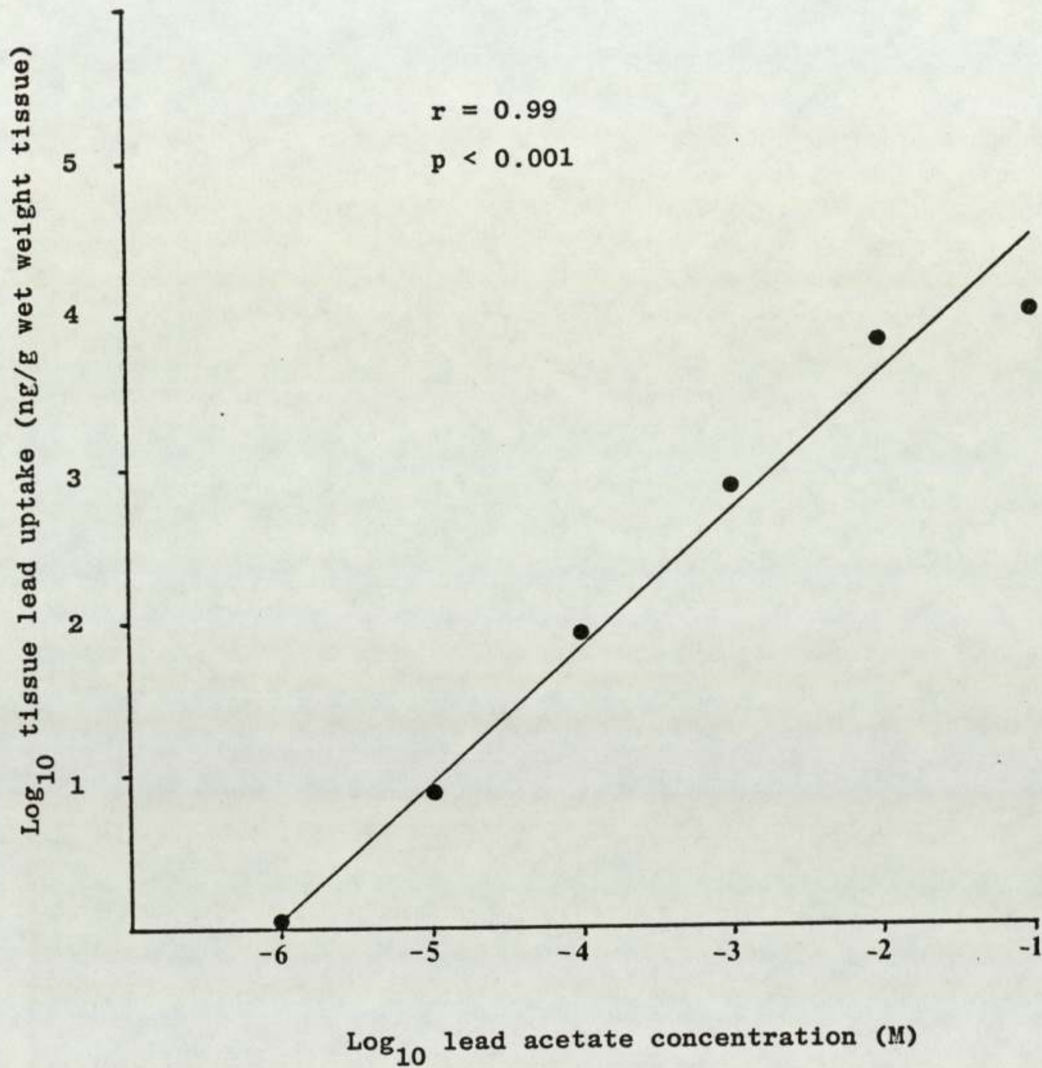


Figure 3.8 Uptake of lead in to the brain of fasted rats orally dosed with various concentrations of $^{203}PbAc$



CHAPTER 4

THE INTERACTION OF LEAD
WITH THE WALL OF THE SMALL INTESTINE

4.1 INTRODUCTION

Absorption of lead from the small intestine is the main transport route into the body (Mahaffey, 1981), and the initial interaction of lead with the small intestine may be involved in regulation of the amount of lead absorbed. Previous workers have reported a surface interaction of lead with the intestinal wall but the function of this remains unknown, although it may be protective in nature (Coleman, 1979 and Coogan, 1982).

There are many factors which could influence the interaction of lead with the small intestine. These include (i) the region of the small intestine (duodenum, jejunum or ileum) (ii) the availability of an energy source in the lumen (eg glucose), (iii) the possible competition of other metals (eg iron) for binding sites and transport pathways (iv) the physical form of the lead species (v) the amount of lead present or (vi) factors likely to vary with age.

These factors were investigated in this series of experiments.

4.2 METHODS

Unless stated otherwise only jejunal tissue was used. The wall of the small intestine was sub divided into three layers using the procedures detailed in sections 2.7 and 2.8. The procedures allowed the intestinal tissue to be divided into three components (i) the apical glycocalyx (ii) lateral glycocalyx, cells and sub mucosa and (iii) the muscle layers. The procedures are summarised in figure 4.1.

4.3 EXPERIMENTAL

4.3.1 The Effect of Time After Dosing on the Distribution of Lead in the Intestinal Wall

4.3.1.1 Methods

Adult rats were dosed intragastrically with $0.3cm^3$ of $10^{-6}M$ lead acetate each $0.3cm^3$ containing $1\mu Ci$ of ^{203}Pb as radiotracer. At 1, 2 and 4 hours

Figure 4.1

Schematic diagram to show the division of the rat small intestinal wall into three components.

after dosing the animals were sacrificed and the small intestinal wall divided into apical glycocalyx, mucosal scrape and muscle layers (as described in figure 4.1). In this experiment the distribution of lead in the wall of the duodenum, jejunum and ileum was investigated to give a comprehensive analysis of the way lead behaves with time after dosing.

4.3.1.2 Results and discussions

In this study the data were analysed using a 2 way ANOVA. This analysis was done on the MINITAB system.

No significant difference can be seen in the amount of lead present with any layer of the duodenal, or jejunal tissue at 1, 2 or 4 hours (table 4.1).

The data indicates that there is a rapid interaction of lead with the tissue in both duodenum and jejunum.

The ileal data are more complex. They indicate that even at 1 hour after dosing a significant amount of the lead dose is present in the ileum (table 4.1). The amounts of lead present in the apical glycocalyx, mucosal scrape and muscle layers rise significantly with time ($P<0.05$). This rise is probably due to the arrival of a greater proportion of the lead dose to the ileal tissue with increasing time after dosing.

The interactions seen in duodenum and jejunum are similar and it is likely that lead interacts with the intestinal wall and is absorbed from these areas in a similar manner. This supports the studies of Coleman (1979) who found no significant difference in lead absorption along the small intestine. However the observations in the ileum may reflect an alternative transport system for lead in this tissue, although it is likely they simply reflect the transit of lead along the small intestine. It is interesting to speculate however that these data may reflect alterations in the length of glycocalyx or in the mucus coating the absorptive surface (Ito,1964).

4.3.2. The Effect of Glucose on the Distribution of Lead in the Jejunal Wall of the Rat

4.3.2.1 Methods

Rats were dosed with $0.3cm^3$ of a solution of $10^{-6}M$ lead acetate containing glucose (500mM). The animals were sacrificed and the small intestine removed and divided as described previously, (figure 4.1).

4.3.2.2 Results and discussion

The concomittant administration of 500mM glucose with the dose of lead caused significantly more lead to become bound to the apical glycocalyx compared with the value obtained from animals dosed lead alone (table 4.2). There was no significant difference between lead dosed and lead plus glucose animals for the values for the mucosal scrape or muscle layers.

This suggests that glucose exerts an effect on the intestinal interaction of lead within the apical glycocalyx. This may be due to increased metabolic activity caused by the presence of glucose which possibly results in an increase in phosphate production and subsequent precipitation of lead (in the form of lead phosphate) at the apical glycocalyx (Blair et al., 1979). However the presence of glucose has no effect on the interaction of lead with the tissue or muscle layers; a general increase in metabolic activity would be expected to increase binding to the lateral glycocalyx which was not seen, suggesting that other factors may be involved.

4.3.3 The Effect of the Chelating Agent EDTA on the Distribution of Lead in the Jejunal Wall of the Rat

The chelating agent EDTA (ethylenediamine tetraacetic acid) binds lead and has been shown to alter its absorption (Coogan, 1982). The following experiments were performed to investigate the effect of EDTA on the binding of lead to the small intestinal wall.

Table 4.1 Distribution of Lead Present in Duodenum, Jejunum and Ileum of Fasted Adult Rats at 1, 2, and 4 hours. Data are Percentages of Total Dose Administered.

	TIME AFTER DOSING (HOURS)		
	1	2	4
DUODENUM			
Apical glycocalyx	0.76 ± 0.41	0.64 ± 0.32	0.51 ± 0.31
Mucosal scrape	0.91 ± 0.37	0.97 ± 0.25	0.85 ± 0.42
Muscle layers	0.71 ± 0.61	0.91 ± 0.67	0.99 ± 0.26
JEJUNUM			
Apical glycocalyx	0.69 ± 0.47	0.56 ± 0.14	0.31 ± 0.17
Mucosal scrape	0.64 ± 0.44	0.84 ± 0.26	0.47 ± 0.20
Muscle layers	0.51 ± 0.37	0.75 ± 0.25	0.79 ± 0.36
ILEUM			
Apical glycocalyx	0.06 ± 0.04	0.17 ± 0.12	0.42 ± 0.19
Mucosal scrape	0.04 ± 0.03	0.09 ± 0.06	0.67 ± 0.35
Muscle layers	0.04 ± 0.02	0.14 ± 0.09	0.92 ± 0.31

Each value is mean of six observations ± standard deviations.

Table 4.2 The Effect of 500mM glucose, 1 x 10^{-5}M EDTA and 1 x 10^{-4} $FeCl_3$ on ^{203}Pb Distribution in the Jejunum of Adult Rats at 4 Hours. Data are Lead Present in % Total Dose Administered

	10^{-6}M Lead Acetate (Control)	PbAc + 500mM Glucose	PbAc + 1×10^{-5}M EDTA	PbAc + 10^{-4}M $FeCl_3$
Apical Glycocalyx	0.13 ± 0.02	0.19 ± 0.03 **	0.51 ± 0.13 **	0.20 ± 0.24 NS
Mucosal Scrape	0.36 ± 0.10	0.37 ± 0.10 NS	0.72 ± 0.31 **	0.27 ± 0.26 NS
Muscle Layers	0.76 ± 0.31	0.72 ± 0.10 NS	0.46 ± 0.21 *	0.40 ± 0.29 *
n	6	14	6	12

Each value is the mean of n observations ± standard deviation.

* = $p<0.05$

** = $p<0.01$

NS = not significant

4.3.3.1 Methods

Rats were fasted overnight and dosed with each $0.3cm^3$ of $10^{-6}M$ lead acetate containing $10^{-5}M$ EDTA and 1µCi ^{203}Pb. Control animals were given lead acetate alone ($10^{-6}M$). All other conditions were as described previously (section 4.3.2.1.).

4.3.3.2 Results and Discussion

The presence of EDTA ($10^{-5}M$) causes a significant increase in the amount of lead associated with the apical glycocalyx and mucosal scrape in animals dosed with EDTA when compared with the values obtained for animals dosed with lead acetate alone (table 4.2). Rats dosed with EDTA had significantly less lead associated with the muscle layers of the small intestine than in animals dosed with lead alone (table 4.2).

The data suggest that EDTA alters the interaction of lead with the wall of the intestine; EDTA causes lead to be distributed in a manner which is different to that seen in animals dosed with lead acetate alone. The reason for this may lie in the ability of EDTA to form Pb-EDTA complexes which are more lipid soluble. Movement of a Pb-EDTA complex across the mucosal membrane would lead to the presence of lead within the epithelial cells, and this would explain the increased lead seen in the mucosal scrape. The reduction in the amount of lead present in the muscle layers could be because the Pb-EDTA complex is very rapidly transported into the blood supply (being more lipophilic than the Pb^{2+} species) and so is unable to penetrate and/or accumulate in the underlying muscle layers. The increase in the lead seen in the apical glycocalyx is less easy to explain; a neutral Pb-EDTA would not be expected to bind to the glycocalyx as readily as Pb^{2+}. The increase may represent better penetration of Pb-EDTA through the mucus layer at the surface of the small intestine than is seen with Pb^{2+}, but there is no available evidence to support this concept.

4.3.4. The Effect of Iron on the Interaction of Lead with the Jejunal Wall

4.3.4.1. Introduction

Previous workers have proposed that lead is transported across the gut by the iron binding protein (FeBp), (Flanagan et al., 1979, and Conrad and Barton, 1978).

If lead were transported by this system an excess of iron should reduce absorption, as competition for the carrier protein would decrease the amount of lead transported. Also the possibility of an insoluble iron-lead coprecipitate being produced may affect the interaction at the surface of the intestine. The interaction of lead and iron at the jejunal surface was investigated in these experiments.

4.3.4.2. Methods

Rats were dosed with (i) $0.3cm^3$ of 10^{-6}M lead acetate + 10^{-4}M iron chloride (ii) $0.3cm^3$ of 10^{-6}M lead acetate alone or (iii) $0.3cm^3$ of 10^{-6}M iron chloride alone. Solutions containing lead were labelled with 1µCi of ^{203}Pb in $0.3cm^3$. The solution of iron chloride was labelled with 1µCi of ^{59}Fe in $0.3cm^3$. All other conditions were as described previously.

4.3.4.3. Results and discussion

A hundred fold excess of iron over lead had no effect on the amount of lead bound to the surface of the preparation (ie the apical glycocalyx or the mucosal scrape), but it did significantly reduce the amount of lead associated with the muscle layers when compared to controls dosed with lead acetate alone (table 4.2). The distributions of a comparable dose of iron and lead in the jejunal wall indicates that the same amounts of lead or iron are associated with the apical glycocalyx (table 4.3) but that significantly less iron than lead is seen in the mucosal scrape and muscle layers (table 4.3). The lack of an effect of an excess of iron on the amount of lead

present in the mucosal scrape suggests that lead is not transported by the iron binding protein to any significant extent. The reduction in the amount of lead seen in the muscle layers when iron is present is difficult to explain. It suggests enhanced clearance or reduced accumulation of lead in the muscle layer when there is excess iron present. It is difficult to describe a mechanism for this effect and may simply reflect an artefactual result. The differences in the amount of iron or lead bound to the tissue after comparable doses (10^{-6}M) is likely to simply reflect the different clearance rates of iron and lead from the jejunal wall.

4.3.5 The Effect of a Large Dose of Lead on Jejunal Lead Distribution

4.3.5.1 Introduction

The binding of lead to the tissue may be dependent upon a specific chemical interaction with ligands on the intestinal wall or within the lumen. The possible saturation of these ligands was investigated in these experiments by comparing the interaction of lead within the intestinal wall of rats dosed with 10^{-6}M and 10^{-1}M lead acetate.

4.3.5.2 Methods

Adult fasted rats were dosed with either 10^{-6}M or 10^{-1}M lead acetate. Each animal received 0.3cm^3 of lead by gastric intubation. Lead acetate solutions were labelled with 1µCi of ^{203}Pb in 0.3cm^3. All other conditions were as described previously (see Figure 4.1).

4.3.5.3. Results and discussion

Following administration of a large dose of lead (10^{-1}M) a greater percentage of the initial dose is found associated with the apical glycocalyx and mucosal scrape when compared to values obtained in rats given a low (10^{-6}M) lead dose (table 4.4), whereas the amount of lead found in the muscle layers was not

significantly different (table 4.4).

Table 4.3 Comparison of the Distributions of Lead and Iron in the Jejunal Wall of the Rat at 4 hours. Data are Percentages of Total Dose Administered

	Lead (10^{-6}M)	Iron (10^{-6}M)
Apical Glycocalyx	0.13 ± 0.02	0.15 ± 0.14 NS
Mucosal Scrape	0.36 ± 0.10	0.12 ± 0.08**
Muscle Layers	0.76 ± 0.31	0.22 ± 0.18**
n	6	14

Each value is the mean of n observations ± standard deviation.

** = $p < 0.01$

NS = not significant

significantly different (table4.4).

This increased surface interaction seen in animals dosed with 10^{-1}M lead may indicate lead becomes precipitated onto the gut surface. This precipitation may arise from the decreased solubility of lead as it moves from the acid stomach to the near neutral intestine. The solubility coefficient for lead becomes exceeded and the lead precipitates out of solution onto the intestinal surface.

4.3.6 The Effect of Age and Vitamins A and D_3 on the Interaction of Lead with the Jejunal Wall

4.3.6.1 Introduction

Forbes and Reina (1972) showed that young animals absorbed more lead than adults. Whether this increased absorption results from changes in the intestinal surface (wider tight junctions and increased phagocytotic activity) or alterations in the diet remains unknown. These experiments were performed to investigate the effect of age and of two milk components, vitamins A and D_3 on the interaction of lead with the jejunal wall.

4.3.6.2 Methods

Young rats (3-4 weeks old) were dosed with (i) $0.3cm^3$ of lead acetate (10^{-6}M) alone (ii) lead acetate (10^{-6}M) + 16μg vitamin D_3 or (iii) lead acetate (10^{-6}M) + 50μg vitamin A. The doses of vitamin were equivalent to a fifty fold daily dietary excess and were given in $0.1cm^3$ of corn oil. All lead acetate solutions were labelled with 1μCi ^{203}Pb in $0.3cm^3$. All other conditions were as described previously.

Table 4.4 Comparison of the Distribution of Lead in the Jejunal Wall of the Rat after Oral Dosing with 10^{-6}M or 10^{-1}M Lead Acetate. Data Are % of Total Dose Administered.

	(10^{-6}M) Lead	(10^{-1}M) Lead
Apical Glycocalyx	0.13 ± 0.02	0.48 ± 0.07 **
Mucosal Scrape	0.36 ± 0.10	0.65 ± 0.19 **
Muscle Layers	0.76 ± 0.31	0.85 ± 0.42 NS

Each value is the mean of six observations ± standard deviation.

** = $p<0.01$

NS = not significant.

4.3.6.3 Results and discussion

In young animals the apical glycocalyx and mucosal scrape had significantly more lead associated with them than had adult animals, but there was no significant difference between the amount of lead associated with the muscle layers in these two groups (table 4.5).

Vitamins A and D_3 exerted different effects on the jejunal tissue interaction with lead. Vitamin A significantly increased the amount of lead bound to the apical glycocalyx and mucosal scrapes but had no effect on the amount present in the muscle layers (table 4.6).

The data suggest a possible change in the transport pathway for lead in young animals. The surface of the cell binds more lead then is seen in adults and this may be in part responsible for the increased absorption seen in these animals. However the possibility of a chelating species produced in young animals which allows the lead to be moved into the cell cannot be excluded.

Increased amounts of lead were associated with the apical glycocalyx and mucosal scrape of vitamin A dosed animals. No effect on the amount of lead present in the muscle layers was observed. The increased interaction of lead with the apical glycocalyx could reflect increased cellular metabolism as observed following acute dosage of Vitamin A (Flodin, 1979). The increase in the amount of lead associated with the mucosal scrape may reflect the previously reported toxic effect of vitamin A on cell membrane integrity which could allow lead to enter the cell (Frankel, Sejhadari, McDowell and Cornish, 1986).

Vitamin D_3 caused a decrease in the amount of lead present in the apical glycocalyx. No change in the amounts of lead associated with the mucosal scrape or muscle layers was observed. The decreased amount of lead present in the apical glycocalyx is difficult to explain. It is possible that vitamin D_3 causes a decrease in

the number of lead binding sites within the apical glycocalyx. In previous studies vitamin D_3 had no effect on the amount of lead bound to the chick intestine (Mykkanen and Wasserman, 1982). This observation was based upon the whole intestinal wall. This may be responsible for the differences with the data reported here although they may reflect the different species used.

Table 4.5 Distribution of Lead in the Jejunum of Fasted Adult (7-10 weeks Old) Fasted Young Rats (3-4 weeks old). Data Are % of Total Dose Administered

	Adult Rats	Young Rats
Apical Glycocalyx	0.13 ± 0.02	0.36 ± 0.16 *
Mucosal Scrape	0.36 ± 0.10	0.57 ± 0.21 *
Muscle Layers	0.76 ± 0.31	0.81 ± 0.61 NS

Each value is mean of six observations ± standard deviation.

* = $p < 0.02$

NS = non significant

Table 4.6 The Effects of Vitamins A and D_3 on Lead Distribution in Jejunum of Young Rats. Data Are % of Total Dose Administered.

	Control (carrier only)	Vitamin A (50μg)	Vitamin D_3 (16μg)
Apical Glycocalyx	0.40 ± 0.13	0.72 ± 0.24 **	0.27 ± 0.15 *
Mucosal Scrape	0.64 ± 0.24	1.26 ± 0.86 *	0.69 ± 0.29 NS
Muscle Layers	0.71 ± 0.38	0.97 ± 0.56 NS	0.80 ± 0.37 NS
n	13	9	15

Each value is mean of n observations ± standard deviations

* = $p < 0.02$, ** = $p < 0.001$, NS = non significant.

CHAPTER 5

FACTORS AFFECTING THE INTESTINAL ABSORPTION OF LEAD

5.1. INTRODUCTION

A number of factors such as fasting, the presence of food, the availability of bile, chelating agents and other metals are likely to change the chemical form of lead in the intestinal lumen and may influence the bioavailability of lead. The chemical form of the administered lead species might be expected to affect the amount of lead absorbed into the body because of differences in solubility and chemical behaviour of various inorganic lead salts. Experiments were performed to investigate the effect of some of these factors on lead absorption.

5.2 METHODS

Rats were dosed with lead acetate solutions radiolabelled with ^{203}Pb. All dosing was by gastric intubation. Tissue distribution of ^{203}Pb was determined (as described in section 2.4). The liver, kidney and spleen were used as indicator organs for lead uptake as it had been previously established that these are the main target organs for lead (see section 3).

5.3 THE EFFECT OF FOOD ON LEAD ABSORPTION

The presence of food in the gastrointestinal tract may be expected to alter the absorption of lead; ligands present in food may act to bind lead prior to digestion and absorption. Lead may enter the body as a lead ligand complex, possibly by other routes of uptake. Food may also precipitate lead and make it unavailable for absorption.

Studies of lead absorption in man have shown that fasted volunteers absorb about 60% of the lead dose (Flanagan et al., 1982; Blake et al., 1983; Blake and Mann, 1983 and James et al., 1985). In the fed state lead absorption is decreased to about 4% (Chamberlain et al., 1978 and James et al., 1985). It has been suggested that this reduction may be due to a co-precipitation of lead with calcium phosphate (James et al.; 1985). The experiment described here aimed to

investigate the effect of dietary status on lead absorption in the rat.

5.3.2 Methods

Adult male Wistar rats were dosed by gastric intubation with ^{203}Pb labelled lead acetate solutions (as described in section 2.3). The distribution of lead in various tissues was determined 4 hours after dosing as described previously (see section 2.4).

5.3.3 Results and Discussion

Significantly more lead remains in the stomach and intestinal washings of fed compared to fasted animals (table 5.1). The fed animals had significantly more lead in the caecum and colon than the fasted rats (table 5.1). This presumably reflects the transport of lead down the intestine in a non absorbable form. Uptake into the body of the rat was reduced by the presence of food; this was indicated by significantly lower levels of lead in the organs and a significantly higher recovery of radioactivity in the fed rat compared to the fasted (table 5.1).

The presence of food in the lumen of the intestine decreases lead absorption and increases the amount of lead moving into the large intestine for subsequent excretion. This action is presumably due to the formation of insoluble lead salts which are not absorbed (Barltrop & Meek, 1975) or by the interaction of lead with non absorbed species such as components of dietary fibre. It is also possible that lead becomes trapped in the food matrix and passes through the small intestine into the large intestine.

The interaction of lead with the wall of the small intestine is reduced in fed animals when compared to fasted (table 5.1). Although the amount of lead in the intestinal washings is significantly increased in fed animals it is likely that most of this lead is in an insoluble form and as such is not available for absorption into

Table 5.1 The effect of food on the tissue distribution of lead in the adult rat 4 hours after dosing with $0.3cm^3$ of 10^{-6}M PbAc

	Lead uptake % of total dose administered Fed	Fasted
Stomach	17.85 ± 4.92**	2.18 ± 0-.60
Absorptive	2.28 ± 1.54**	17.02 ± 7.96
Gut washings	17.36 ± 3.50*	10.67 ± 4.43
Non absorptive	57.16 ± 7.27**	35.94 ± 7.45
Liver	0.07 ± 0.04**	3.67 ± 1.52
Kidney	0.05 ± 0.01**	2.89 ± 1.09
Spleen	0	N.D.
Recovery	93.27 ± 6.17**	72.50 ± 5.73

Each value is the mean of five observations ± standard deviation.

** = $p < 0.01$, * = $p < 0.05$, N.D. = not determined.

the body. The reduction of the soluble lead concentration within the lumen may in part be responsible for the diminished interaction of lead with the tissue. However it is possible that food or the presence of food in the intestine alters the affinity of the intestinal tissue for lead. Such alterations in the interaction of lead with the tissue could be due to changes in the surface environment, possibly as a result of increased mucus secretions or pH changes following the digestion of food at the luminal surface.

Using this data, calculation of the amount of lead taken up into the body (ie., Uptake = amount ingested - amount excreted) indicates that in fed rats only 7-8% of the initial dose is taken up by the body, whereas a much greater uptake of lead (approximately 30% of the initial dose) is seen in fasted animals.

A similar decrease in lead absorption in the presence of food is also observed in man, although, in man the differences are more marked (James et al., 1985).

5.4 THE EFFECT OF EDTA ON LEAD ABSORPTION

5.4.1 Introduction

Ethylenediaminetetraacetic acid (EDTA) is a chelating agent used in the treatment of lead toxicity. It enables a rapid excretion of lead by mobilizing lead from the body stores. EDTA therapy causes a fall in blood lead level to within acceptable levels in most cases (Piomelli et al., 1985).

The reported effects of EDTA on the intestinal uptake of lead are conflicting. EDTA has been shown to increase the rate of lead absorption in everted gut sacs of rat small intestine (Coogan, 1982), whereas in rats orally dosed with lead there was no apparent effect of EDTA on lead absorption (Garber and Wei, 1974). In humans EDTA has been shown to reduce lead absorption (James et al., 1985). In an attempt to rationalise these conflicting ideas the effect of EDTA on the uptake and subsequent tissue distribution of lead was determined after oral dosing.

5.4.2 Methods

Fasted animals were dosed with either 0.3 cm^3 of 10^{-6}M lead acetate or 0.3 cm^3 of 10^{-6}M lead acetate containing 10^{-5}M EDTA (disodium calcium salt). Dosing was by gastric intubation. All other conditions were as described previously (see section 2). Each 0.3cm^3 dose contained 1μCi ^{203}Pb.

5.4.3 Results and Discussion

The simultaneous dosing of EDTA with lead had no significant effect on lead uptake into any of the tissues examined (table 5.2). This observation indicates that in rat, EDTA has no effect on lead absorption as previously suggested by Garber and Wei (1974). Possible explanations for this effect are that (a) chelating agents are poorly absorbed from the G.I. tract (Foreman and Trijillo, 1954) and (b) the Pb-EDTA complex dissociates in dilute solution (Catsch, 1979).

An apparently increased rate of lead absorption in the presence of EDTA in the everted sac of the rat (Coogan, 1982) may simply reflect the different techniques used. In vivo the administered Pb-EDTA dose passes through the stomach and it is possible that the form in which lead is presented is altered in a way that affects its subsequent absorption. In humans concommitant administration of EDTA decreased absorption (James et al., 1985). These differences may be due to the fact that in the rat studies the EDTA does not have to pass through the stomach as occurs in the human experiments.

5.5 THE EFFECT OF GLUCOSE ON LEAD ABSORPTION

5.5.1. Introduction

It is not possible to show whether energy is necessary for the intestinal absorption of lead in vivo without producing deleterious effects in other systems. The in vitro everted sac studies of Coleman (1979) and Coogan (1982) showed lead absorption to be unaffected by a lack of an exogenous energy substrate. Whilst this

Table 5.2 The effect of 1×10^{-5}M EDTA on lead absorption in fasted rats at 4 hours

	Lead uptake % of total dose administered	
	Pb + EDTA	Pb Only
Stomach	1.29 ± 0.96	1.34 ± 0.53
Absorptive	22.22 ± 7.02	20.07 ± 3.66
Gut washings	13.11 ± 6.13	6.92 ± 3.22
Non absorptive	29.76 ± 18.74	27.08 ± 18.13
Liver	3.69 ± 1.35	6.04 ± 3.09
Kidney	4.92 ± 1.71	5.00 ± 2.49
Recovery	75.76 ± 11.31	71.99 ± 4.06

Each value is mean of five observations ± standard deviations. There are no significant differences in the data as determined by Student's t-test.

experiment is extremely difficult to repeat in the whole animal the possible effect of an additional energy source on lead absorption can be studied. A large dose of glucose was given concommitantly with lead to rats to determine the effect of the presence of an energy source in the lumen on absorption.

5.5.2 Methods

Each $0.3cm^3$ of 10^{-6}M PbAc contained 1μCi ^{203}Pb and 500 mM glucose was administered by gastric intubation to fasted animals. All other conditions were as described previously (section 5.4.2).

5.5.3 Results and discussion

Glucose caused a significant decrease in the amount of lead present in the stomach compared to controls (table 5.3.). There was no change in any of the other tissues examined (table 5.3). This effect may be due to glucose causing an increase in the rate of gastric emptying or may simply be an artefactual result. Clearly, glucose does not significantly increase lead absorption in vivo. It is unlikely that there is any interaction between glucose and lead which may influence lead absorption.

5.6 THE EFFECT OF IRON COMPETITION ON LEAD ABSORPTION

5.6.1 Introduction

It has been proposed that lead is transported from the intestinal lumen into the body by the iron transporting protein (FeBp) (Flanagan et al., 1979). Iron and lead may compete both for the protein carrier and paracellular route of transport (see section 1). The effect of excess iron on lead absorption was investigated.

5.6.2 Methods

Adult male rats were fasted overnight and then dosed by gastric

Table 5.3 <u>The effect of 500mM glucose on lead absorption in fasted adult rats</u>

	Lead uptake % of total dose administered	
	Pb + Glucose	Pb Only
Stomach	0.88 ± 0.43***	4.55 ± 1.33
Absorptive	8.56 ± 4.08	7.24 ± 4.38
Gut washings	7.49 ± 6.81	2.40 ± 3.00
Non absorptive	57.46 ± 21.22	46.29 ± 21.64
Liver	5.70 ± 1.63	2.65 ± 3.08
Kidney	4.71 ± 3.08	2.28 ± 2.82
Recovery	86.14 ± 14.39	79.20 ± 12.56

Each value is the mean of six observations ± standard deviations. Significances of differences between means was determined by Students-t-test.

*** = $p < 0.005$.

intubation with each 0.3 cm^3 of 10^{-6}M PbAc containing 1μCi of ^{203}Pb and 10^{-4}M $FeCl_3$. Control animals were given lead acetate alone. Four hours post dosing the animals were sacrificed and the distribution of lead was determined as described previously (see section 2.4).

5.6.3 Results and discussion

In the presence of a hundred fold excess of iron there was no significant effect on the uptake of lead into any of the tissues examined (table 5.4). The observations show that tissue distributions of lead in the rat are unaffected by iron as reported by Flanagan et al., (1979) and Barton et al., (1981). From this data it is unlikely that lead is absorbed to any significant extent by the iron transporting system.

5.7 THE EFFECT OF LIGATION OF THE BILE DUCT ON LEAD ABSORPTION

5.7.1 Introduction

Lead absorption was previously shown to be stimulated by the dietary addition of bile salts (Quaterman et al., 1977). This may be due to the formation of a more readily transported lead-bile salt complex. The aim of this experiment was to assess the effect of bile duct ligation on lead absorption.

5.7.2 Methods

The bile duct was located and ligated (as described in section 2.5). Control animals were sham operated (as described in section 2.5). Following a 48 hour recovery from surgery the animals were fasted overnight then dosed with $0.3cm^3$ of 10^{-6}M lead acetate by gastric intubation. Two hours after dosing the animals were sacrificed and lead distributions in tissue were determined (as

Table 5.4 The effect of 10^{-4}M iron on lead distribution in fasted male rats

	Lead uptake % of total dose administered	
	Pb + Fe	Pb Only
Stomach	5.81 ± 10.48	1.34 ± 0.53
Absorptive	13.30 ± 8.88	20.07 ± 3.66
Gut washings	10.68 ± 4.11	6.92 ± 3.22
Non absorptive	35.34 ± 20.43	27.08 ± 18.13
Liver	2.38 ± 1.43	6.04 ± 3.09*
Kidney	2.53 ± 2.04	5.00 ± 2.49
Recovery	75.75 ± 21.49	71.99 ± 4.06

Each value is the mean of five observations ± standard deviations.

* = < 0.05

described in section 2.4).

5.7.3 Results and discussion

Less lead was absorbed into the body of rats in which the bile duct had been ligated. Uptake into the liver and kidney was significantly reduced in animals with the bile duct ligated compared to controls (table 5.5). More lead remains in the stomach of the ligated animals than is seen in the controls (table 5.5). This may indicate a delay in gastric emptying which causes an increase in intestinal transit time in ligated animals compared to controls. The data is in agreement with that of Cirkt et al, (1975).

The decreased absorption of lead in bile duct ligated rats indicates that bile is involved in lead absorption. This data suggests the absorption of lead bile complexes may occur. However the effect may represent the detergent effect of bile on the small intestine (Heaton, 1972) or the involvement of bile as a lead ionophore in a manner similar to the effect of bile on calcium absorption (Maenz, 1982).

5.8 THE EFFECT OF DIFFERENT LEAD SALTS ON LEAD ABSORPTION

5.8.1 Introduction

Data in the literature proposes that the chemical form of the ingested lead species is important in determining the uptake of lead (Kehoe, 1976). However the literature available is conflicting (Fairhall and Sayers, 1940 and Kaurhausen, 1972).

Lead salts would be expected to be absorbed differently depending on the nature of their chemical properties (particularly solubility in acid and in the intestinal contents). These experiments were performed to investigate the effect of differing lead salts on lead absorption in the rat.

Table 5.5 Distribution of lead 2 hours after oral dosing in animals with bile duct ligation and in sham operated controls

	Lead uptake % of total dose administered	
	Ligated	Control
Stomach	27.26 ± 21.88*	0.94 ± 0.29
Duodenum	4.54 ± 3.30	3.14 ± 0.86
Jejunum	6.51 ± 3.49*	10.45 ± 5.79
Ileum	8.90 ± 9.22	11.16 ± 6.61
Caecum	4.64 ± 6.64*	20.28 ± 15.45
Colon	9.69 ± 32.50	0.45 ± 0.42
Liver	1.08 ± 0.95**	5.55 ± 1.90
Kidney	0.55 ± 0.63**	2.04 ± 0.61
Spleen	0.03 ± 0.03	0.07 ± 0.07
Gut supernatant	4.00 ± 3.02	3.02 ± 0.95
Gut pellet	24.4 ± 9.19	27.28 ± 12.81
Recovery	85.72 ± 9.58	80.42 ± 8.18
n	12	6

Each value is the mean of n observations ± standard deviations. Significance of difference of means were determined by Student's-t-test.

** = $p < 0.001$ * = $p < 0.01$.

5.8.2 Methods

Adult male rats were fasted overnight and dosed with $0.3cm^3$ of either lead acetate (control), lead nitrate, lead chloride or lead sulphate. Each $0.3cm^3$ of solution contained 1μCi of ^{203}Pb. Solutions were mixed for two hours prior to dosing. Four hours after dosing the animals were sacrificed and distribution of ^{203}Pb determined as described previously (see section 2.4).

5.8.3 Results and Discussion

The inorganic salts (acetate, chloride, sulphate and nitrate) were absorbed to differing extents by fasted rats following oral dosing. The data shows that less lead sulphate is absorbed compared to lead acetate (table 5.6), this may simply reflect the relative insolubility of lead sulphate. However the data does show ^{203}Pb to be present in the organs of the lead sulphate dosed animals. This observation is probably an artefactual result due to the presence of ^{203}Pb in the dosing solution which is not in the $^{203}Pb\ SO_4$ form.

Whilst significantly more lead remains in the stomach of lead nitrate dosed animals there was no significant difference in any of the other tissues examined, (table 5.6). Significantly more lead was associated with the intestinal tissue, liver and spleen of lead chloride dosed animals than controls (table 5.6). The reason for this is unknown, but it has been reported that lead chloride is relatively toxic (Barltrop and Meek, 1975).

Kaurhausen (1972) proposed that the lead compounds which were most soluble or were able to be converted to soluble forms within the gastrointestinal tract are most readily absorbed. The data presented here do not support this hypothesis since lead acetate is the most soluble of the lead salts administered. It may be that the absorption of different lead salts may reflect the variation in intraluminal composition particularly of anions such as phosphate, sulphate and carbonate which by reaction with the ionic lead species alter its absorption (Kehoe, 1976).

Table 5.6 Tissue distributions of lead 4 hours after oral dosing of $10^{-6}M$ solutions of PbAc (control) $PbCl_2$, $Pb(NO_3)_2$ and $PbSO_4$ in fasted rats

	Lead uptake % of total dose administered			
	PbAc(control)	$PbCl_2$	$Pb(NO_3)_2$	$PbSO_4$
Stomach	0.22 ± 0.05	0.71 ± 0.20***	0.41 ± 0.17*	1.12 ± 0.94
Absorptive	9.61 ± 4.80	21.15 ± 2.39***	13.99 ± 7.67	9.34 ± 3.41
Gut washings	5.57 ± 3.75	2.91 ± 0.31	5.00 ± 4.31	1.96 ± 0.94*
Non absorptive	50.13 ± 19.55	47.37 ± 5.42	45.49 ± 22.33	75.80 ± 12.53*
Liver	3.48 ± 1.20	6.18 ± 1.42*	4.87 ± 3.24	2.17 ± 1.28
Kidney	2.38 ± 1.20	3.65 ± 0.29	3.56 ± 2.61	1.99 ± 1.00
Spleen	0.08 ± 0.03	0.16 ± 0.005*	0.10 ± 0.07	0.04 ± 0.04
Recovery	69.42 ± 10.23	82.15 ± 6.45	72.88 ± 10.84	92.44 ± 0.73***

Each value is the mean of six observations ± standard deviations. Significances of differences of means between test and control means was determined by Students-t-test.

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

CHAPTER 6

SPECIATION OF LEAD IN THE INTESTINAL TRACT

6.1 INTRODUCTION

Lead is a soft Lewis acid and is likely to form covalent bonds with soft bases. Lead binds with sulphur donors, and thiolate ligands are by far the most avid monodentate ligand for lead. Phosphate, carboxyl and amino groups on organic molecules will also bind lead (Sillen and Martell, 1971) as will free phosphate, carbonate and hydroxide ions. The potential for the formation of lead ligand complexes is considerable in the lumen of the intestinal tract where food and intestinal secretions present many ligands which may bind lead. It is currently accepted that the amount of lead available for transport is dependent on the soluble lead concentration (Kehoe, 1976). The experiments described in chapter 5 indicate that many factors influence lead absorption and the formation of lead ligand complexes may be important in determining the extent of absorption.

However, characterisation of the types of lead species formed within the small intestine has not been a subject of intensive research. The studies presented here involve the use of techniques to identify the types of lead complexes found within the lumen of the small intestine.

6.2 GENERAL METHOD

The following methodology was used for all experiments detailed in this chapter.

Each animal was dosed by gastric intubation with $0.3cm^3$ of lead acetate containing 2μCi of ^{203}Pb as radiotracer. The animals were sacrificed two hours after dosing, this time period being optimum for retaining lead in the small intestine (see section 3). The small intestine was excised between the pyloric sphincter and a point 5cm above the ileo-caecal junction. The luminal contents were flushed from the small intestine using 20 cm^3 of ice cold isotonic saline solution. The intestinal contents were centrifuged at 100,000g for 1 hour in an MSE 50 Ultracentrifuge. This produced a pellet, containing insoluble lead species and

particulate matter and a supernatant phase containing soluble lead species. The pellet produced was subjected to a sequential leaching technique using the method of Tessier et al., (1979), which is outlined in figure 6.1. After each step in the extraction process, centrifugation (100,000g for 1 hr at $4^{o}C$) was used to separate the supernatant (which contained the released radioactivity) from the pellet. The amount of lead in both the supernatant and pellet was determined. The pellet was then used in the next stage of the extraction process. This process yields five categories of lead species:

(i) exchangeable cations

(ii) carbonates

(iii) oxides

(iv) organic and sulphides

(v) residual fraction.

The supernatant from the centrifuged intestinal contents was analysed by gel filtration chromatography using Sephadex G-15 (Pharmacia Ltd.), as described in section 2.10. The performance characteristics of the column were determined using Dextran blue 2000 for the void volume and $^{3}H_2O$ for the exclusion volume. Chromatograms were also prepared for a number of standard solutions.

6.3 RESULTS AND DISCUSSION

6.3.1 Analysis of the Pellet Phase of the Intestinal Contents using the Sequential Leaching Technique

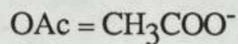
Four groups of animals were used in these experiments. The animals were:

(a) Overnight fasted then dosed with 10^{-6}M PbAc

(b) Dosed with 10^{-6}M PbAc without fasting.

Figure 6.1

Fraction extracted	Procedure for 1g pellet
Exchangeable pH = 7	1M NH_4OAc (10ml) pH = 7, 20°C, 1hr continuous agitation.
Carbonates pH = 5	1M NaOAc (20ml) pH = 5 (with HOAc) pH = 5, 5h continuous agitation
Oxides	0.04M NH_2OH HCl in 25% (v/v) HOAc (20ml) 96°C, occasional agitation.
Organic - Sulphides	0.02M HNO_3 (10ml) + 30% H_2O_2 (5ml), pH = 2, 2hr, 85°C, further 30% H_2O_2 (5ml), 3hr, 85°C, then 3.2M NH_4OAc in 20% v/v HNO_3(10ml) continuous agitation.
Residual	HNO_3 (conc) (20ml) 4hrs. 20°C.


$OAc = CH_3COO^-$

The sequential leaching technique (Tessier et al., 1979).

Table 6.1 Distribution of lead between the supernatant and pellet phases of intestinal contents and fractionation of lead in the pellet phase in fasted high (10^{-1}M), low (10^{-6}M) fed and ligated animals two hours after dosing

	% of lead present in pellet			
	FASTED		FED	LIGATED
Fraction	10^{-6}M PbAc	10^{-1}M PbAc	10^{-6}M PbAc	10^{-6}M PbAc
Exchangeable Cations	0.001	2.6	2.2	4.2
Carbonates	71	25.4	67.0	87
Oxides	16.9	44	33.1	9.6
Organic and Sulphides	6.3	10.6	7.6	1.9
Residual	1.7	13.1	1.8	0.95
	^{203}Pb % of total dose administered.			
Supernatant	3.9	0.36	2.1	2.6
Pellet	41	36	48.3	21

(c) Overnight fasted and dosed with 10^{-1}M PbAc.

(d) Bile duct ligated then overnight fasted and dosed with 10^{-6}M PbAc.

The distribution of lead in the gut contents of fed, fasted, bile duct ligated and high lead (10^{-1}M) dosed rats was studied. Lead was shown to be preferentially associated with the pellet phase of the intestinal contents with only 0.3 - 4% of the lead dose remaining in the supernatant (soluble) phase (Table 6.1). Fasted animals dosed with a large dose of lead (10^{-1}M) had less lead in the intestinal supernatant than was observed in any other group of animals.

The pellet phase of the intestine was studied using a sequential leaching technique (Tessier et al., 1979). In all animals dosed with 10^{-6}M lead the majority of the lead dose is found in the carbonate fraction, the remaining lead being distributed throughout oxide, organic-sulphide and residual fractions (Table 6.1). The data suggest that when given a large dose of lead the speciation within the lumen is altered. In the 10^{-1}M lead dosed rats most lead is found in the oxide fraction, however a large proportion of the dose is still present as carbonate (Table 6.1). Both the organic and residual fractions contain increased amounts of lead in these animals (Table 6.1). The reason for these alterations in chemical form remain unknown but it is likely that they result from the large dose of lead binding all available anions particularly if insufficient carbonate is present. It is also possible this reflects a toxic effect of lead on the absorptive surface.

Bile duct ligation was shown to alter lead speciation within the pellet phase. Increased amounts of exchangeable cation and lead carbonate were seen in these animals (Table 6.1) with decreased amounts of lead present as oxide and residual fractions (Table 6.1). This data suggests that the formation of lead carbonate is independent of the availability of bile. Bile, however, is possibly involved in formation of the other lead species observed particularly phosphates

which would be seen in oxide and residual fractions.

The slight shift away from the carbonate fraction seen in fed animals may be due to the effect of competing food ligands or differences in the intraluminal pH between fed and fasted animals which may alter the solubility of lead complexes in the small intestine.

6.3.2 Gel Permeation Chromatography of the Supernatant Phase of the Intestinal Contents

Chromatograms of the supernatant phase of the intestinal contents were prepared from fasted, fed and bile duct ligated animals (figure 6.2, 6.3 and 6.4). These were compared to chromatograms of PbAc alone (figures 6.2 and 6.3) and lead-histidine and lead-bile salt complexes (figures 6.5 and 6.6). The associated ligands were labelled with ^{3}H or ^{14}C and were detected by beta counting (as described in section 2.6).

Chromatograms of ^{203}Pb labelled PbAc produced a single peak at fraction 21. The void volume of the column was at fraction 7 and the exclusion volume at fraction 23. Chromatograms of ^{14}C deoxycholic acid gave good recovery of ^{203}Pb (89%) when a mixture of ^{14}C deoxycholate and ^{203}Pb were eluted from the column; this is probably due to the acidic nature of the elution media (pH 3.8) Lead deoxycholic complexes were seen at fraction 21 (figure 6.6). Lead histidine complexes were seen at fraction 20 (figure 6.5). Histidine alone eluted at fraction 13.

The supernatant fraction of intestinal contents were eluted using the G-15 system. The supernatant fraction of intestinal contents of fed rats gave chromatograms with a single peak at fraction 22. By comparison fasted (16 hours) animals produced chromatograms with two peaks one at fraction 22 the other a minor peak being at fraction 10 (figure 6.2). This peak eluting at fraction 10 contained upto 30% of the recovered radioactivity in the supernatant and was

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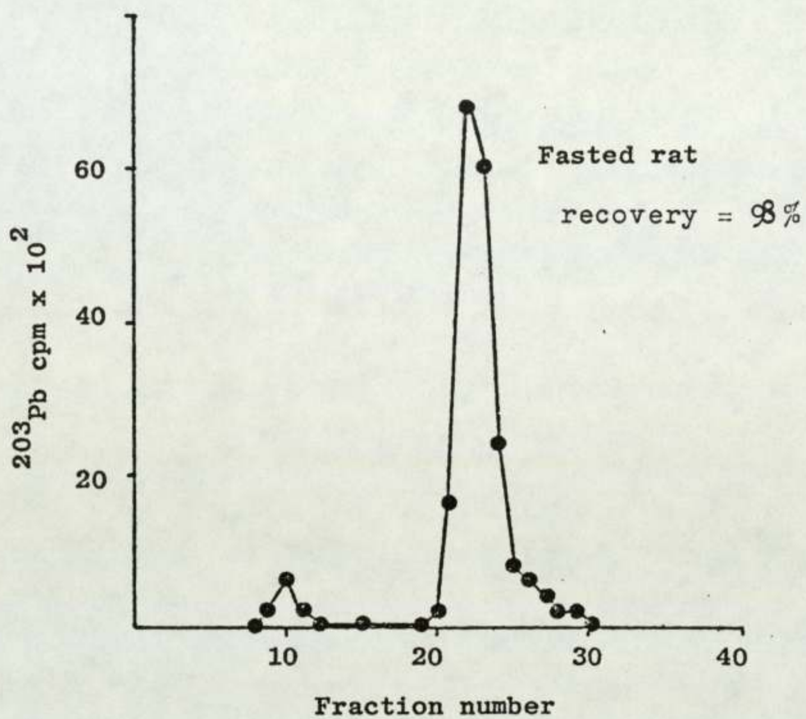
Figure 6.2 Comparison of ^{203}Pb elution profiles from a fasted male rat and a $^{203}PbAc$ standard on Sephadex G-15



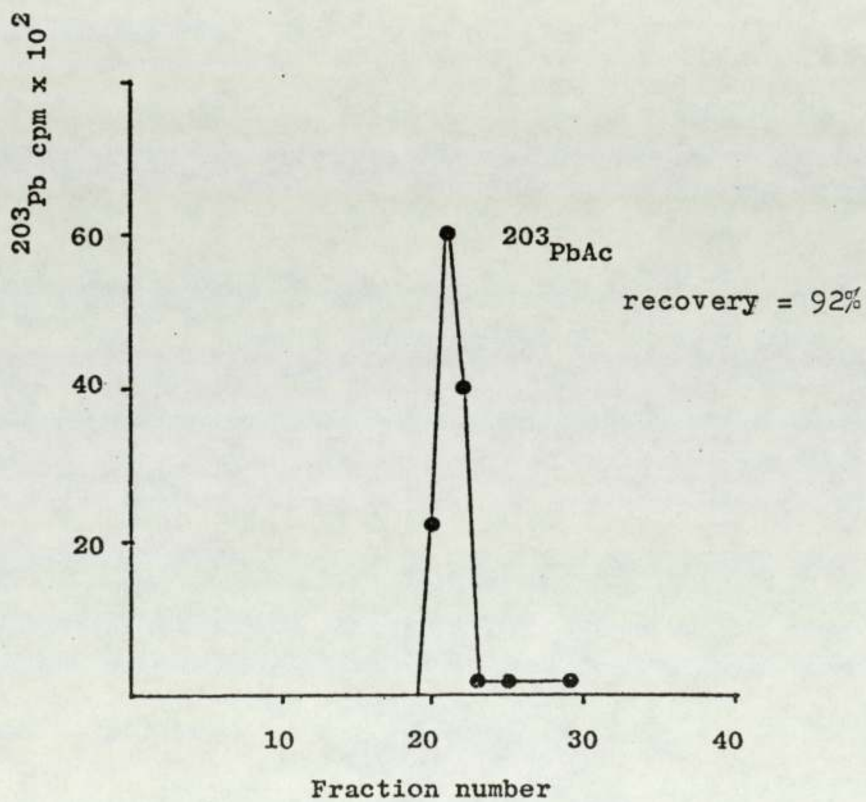
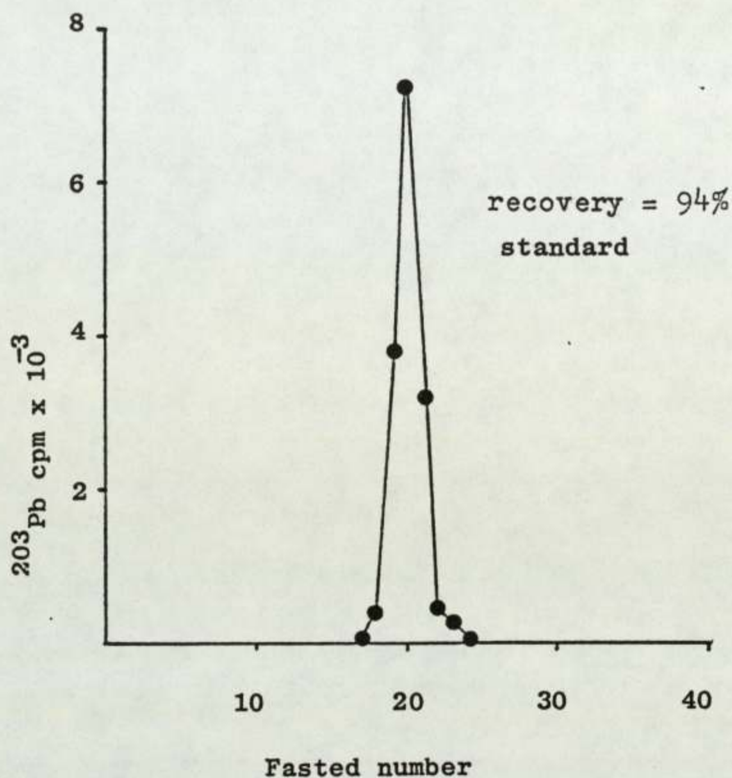


Figure 6.3 Comparison of elution of ^{203}PbAc (standard) with intestinal contents from fed wistar rats



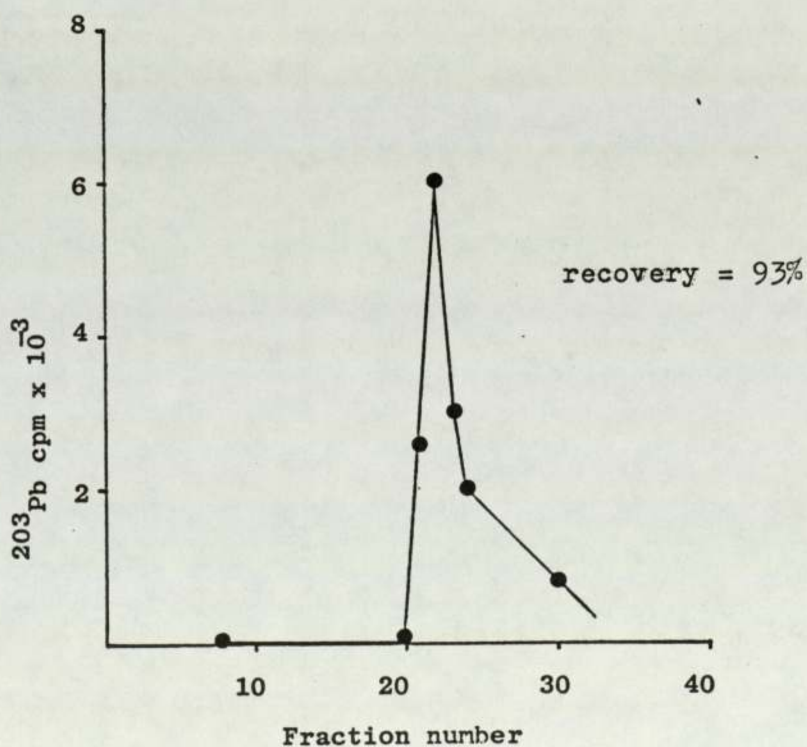


Figure 6.4 Elution profile of intestinal contents from fasted, bile duct ligated animals dosed with ^{203}PbAc on Sephadex G-15

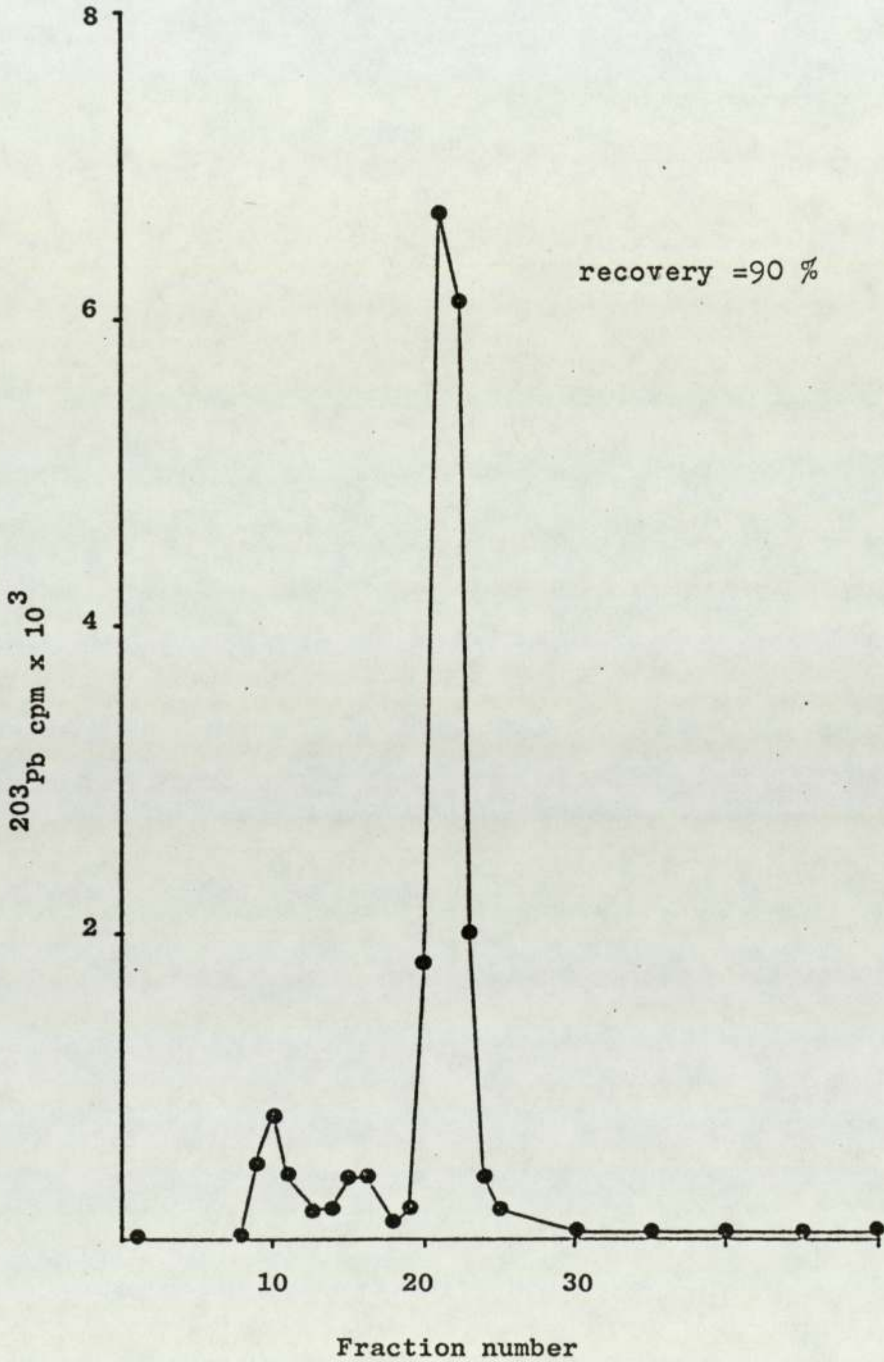


Figure 6.5 Elution of a mixture of 10^{-4}M ^{14}C histidine + 10^{-6}M ^{203}PbAc on G-15.

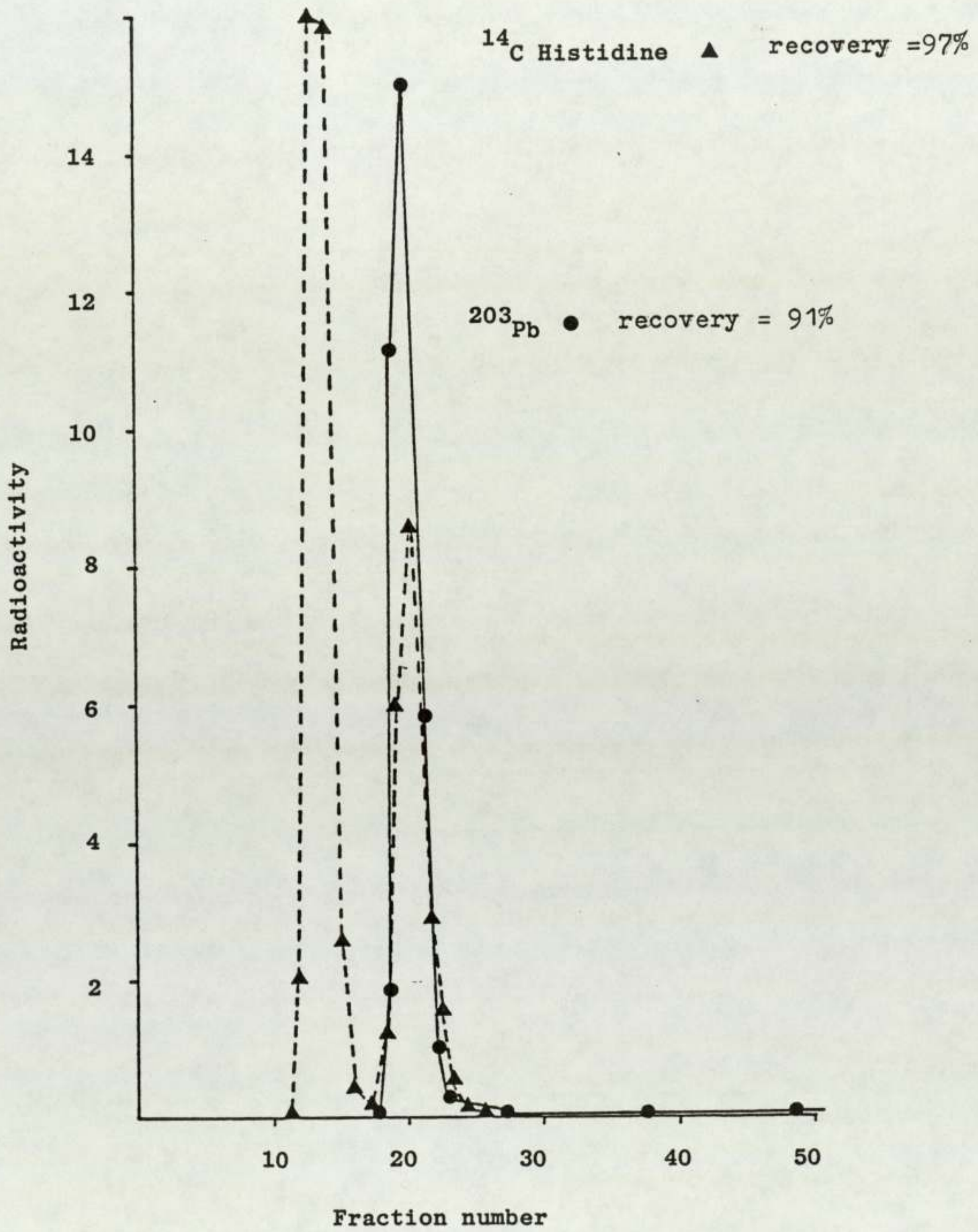
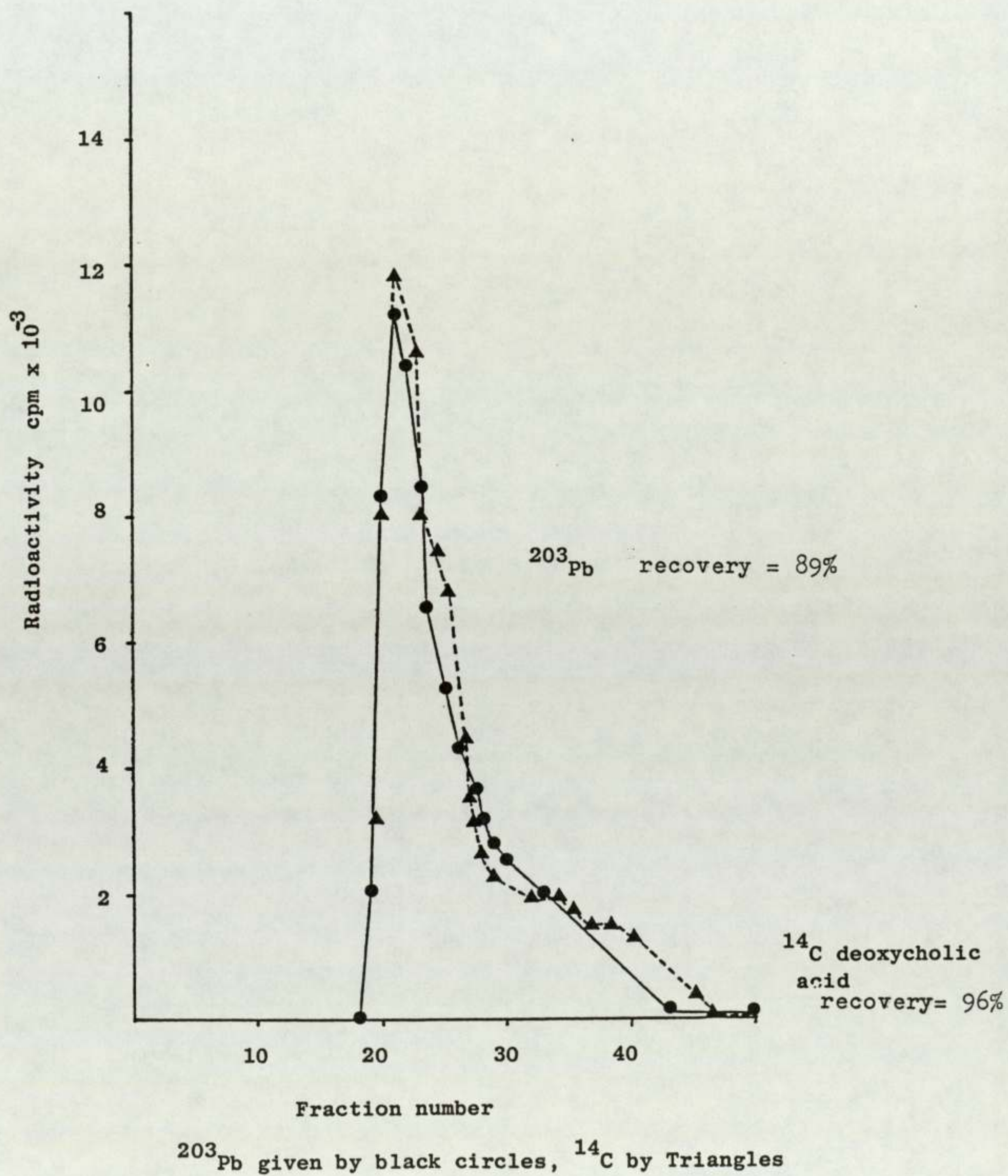


Figure 6.6 Chromatogram of a mixture of 10^{-6}M ^{203}PbAc and 5% ^{14}C deoxycholic acid on G-15



^{203}Pb given by black circles, ^{14}C by Triangles

labelled Pb-X. The chromatograms produced from bile duct ligated animals indicated that three peaks were produced at fractions 10, 15 and 22, (figure 6.4).

Identification of these peaks is presently incomplete although it is likely that the broad peak produced at fractions 21-30 is probably a mixture of lead and lead ligand complexes. Possible elution of lead acetate, lead-bile and lead-histidine complexes within these fractions may account for the broad peak observed. This data suggests that the resolution of this chromatographic system is not capable of completely separating these compounds. This may be due to their elution being close to the separation limit of the gel. The absorption properties of Pb-X were investigated in the next series of experiments.

6.4 THE ABSORPTION OF REDOSED (SUPERNATANT DERIVED) LEAD SPECIES

In these studies the absorption of unfractionated supernatant and Pb-X was investigated. The experiments were to investigate whether Pb-X was absorbed differently from the whole supernatant or lead acetate alone.

6.4.1 Methods

A group of animals were dosed with $0.3cm^3$ of 10^{-6}M PbAc containing 10μCi of ^{203}Pb as radiotracer. Two hours after dosing the animals were sacrificed and the intestinal washings removed. The supernatant phase was prepared (as described in section 6.2), and chromatographed on G-15 (as described in section 6.2). The chromatograms produced were very similar to that seen previously in fasted animals (figure 6.2). The fraction containing Pb-X or supernatant were redosed into further groups of animals by gastric intubation. These groups of animals were sacrificed two hours after dosing and lead distributions were determined (as described in section 2.4).

6.4.2 Results and Discussion

The soluble phase of the fasted rat intestinal contents contains at least two lead species. In the Pb-X dosed animals significantly more lead is seen in the stomach and intestinal washings (table 6.2). An increased recovery of lead was also observed (table 6.2). This data combined with significantly decreased tissue lead levels in the liver, kidney and spleen of Pb-X dosed animals compared to controls suggested that Pb-X is not absorbed to any significant extent in the rat (table 6.2). In animals dosed with whole supernatant no significant difference was seen in the amount of lead taken up into the liver, kidney or spleen (table 6.2) compared to control (PbAc) dosed animals. However the whole supernatant interacted in a significantly different way with the intestinal tissue when compared to PbAc dosed animals (table 6.2). In the redosed animals both jejunum and ileum had significantly increased lead levels compared to control (table 6.2). The duodenum was not significantly different (table 6.2).

Both species formed in the intestinal lumen are absorbed differently. Pb-X is not absorbed to any significant extent in the rat and may reflect the formation of a protective lead-ligand complex, as suggested by Coleman (1979). The uptake parameters of the whole supernatant showed similar characteristics to lead acetate. However, a significantly increased tissue interaction is seen in the jejunum and ileum. This may be due to the lead being chelated or may reflect the presence of a compound in the lumen which alters tissue permeability to lead

Table 6.2 Tissue distribution of 203PbAc (control), whole gut supernatant and Pb-X in fasted Wister rats, 2 hours after dosing

	^{203}Pb % total dose administered		
	Control	Whole supernatant	Pb-X
Stomach	2.76 ± 5.67	1.21 ± 1.59	14.31 ± 3.62**
Duodenum	1.67 ± 1.04	3.83 ± 2.22	1.16 ± 0.67
Jejunum	3.85 ± 2.53	10.65 ± 4.38**	2.19 ± 0.81
Ileum	4.44 ± 14.95	14.23 ± 9.69*	0.76 ± 0.58
Gut washings	44.99 ± 14.95	27.7 ± 18.43	76.40 ± 6.28**
Caecum	15.11± 17.41	26.85 ± 23.45	0.25 ± 0.44
Colon	0.11 ± 0.13	5.17 ± 8.65	0.007 ± 0.002
Liver	2.10 ± 1.47	3.26 ± 1.03	0.03 ± 0.04**
Kidney	1.70 ± 1.24	2.11 ± 1.02	0.033 ± 0.034**
Spleen	0.03 ± 0.01	0.026 ± 0.005	0.0025 ± 0.005***
Recovery	75.06 ± 7.81	93.35 ± 6.11*	96.50 ± 6.53***
n	6	6	4

Each value is the mean of (n) observations ± standard deviations. Significances of differences between test and control were determined by Students t-test.

* = $p < 0.05$, *** = < 0.01, *** = $p < .001$.

CHAPTER 7

DISCUSSION

7. DISCUSSION

Three models have been proposed to account for the mechanism whereby lead is transported from the intestinal lumen into the body.

1. The diffusion model (Blair et al., 1979 and Hilburn, 1979).

Lead is absorbed into the body by diffusion via the tight junctions and lateral intercellular spaces between the epithelial cells. This process is energy independent and exhibits no saturation phenomena.

2. The carrier model (Conrad and Barton, 1978 and Flanagan et al., 1979).

A protein carrier located in the brush border membrane binds lead from the lumen and translocates it into the cell from where it moves through the cytoplasm and across the basolateral membrane of the cell into the blood stream. Kinetic data show evidence of saturation at high lead concentrations.

3. The carrier-diffusion model (Aungst and Fung, 1981).

Up to luminal lead concentration of 5×10^{-5}M lead is absorbed by a carrier. At higher concentrations absorption occurs by diffusion. The kinetic data are complex and composed of saturatable and linear components.

The observations presented in this thesis support the diffusion model proposed by Blair et al., (1979) and Hilburn, (1979). The linearity of absorption with the absence of saturation even at high concentrations of lead (10^{-1}M) makes it possible to dismiss both the carrier model (Conrad and Barton, 1978 and Flanagan et al., 1979) and the carrier diffusion model (Aungst and Fung, 1981) on the basis that both these models involve carrier mediated lead transport with kinetics described by saturation at high concentrations.

In previous studies of lead absorption using everted sacs of rat small intestine Coleman, (1979) and Coogan, (1982) both reported lead absorption kinetics without saturation and concluded that lead was absorbed by a diffusion process. Using a whole animal technique with dosing directly into the intestinal lumen, Gerber and Deroo, (1975) reported the amount of lead absorbed was independent of the

amount given. The data presented in this thesis support the previous in vitro studies of Coleman, (1979) and Coogan, (1982).

Conflicting roles for the intestinal wall in the absorption of lead have been reported. Coleman, (1979) proposed that lead interacted with the intestinal wall as part of a protective effect against a large lead insult, whilst Heaven, (1985) suggested the interaction of lead with the intestinal wall was part of the absorptive process. In an attempt to resolve this conflict the interaction of lead with the intestinal wall was studied.

In rats orally dosed with lead the small intestine wall was divided into apical glycocalyx, mucosal scrape and muscle layers. The interaction of lead with the surface glycocalyx was investigated using an agar replica technique (Ugolev et al., 1979), a process which removes the apical glycocalyx from the luminal surface of the intestine. Any lead associated with the apical glycocalyx is removed by this technique. The data reveal the presence of large amounts of lead within the apical glycocalyx. This suggests a reasonably loose association of lead within the tissue. The nature of the lead species within the glycocalyx remains unknown. However it is likely that lead interacts with mucus itself (Kellaway and Marriot, 1975) and other biomolecules present in the mucus layer particularly fragments of exfoliated cells (Bandurko, Brodskii, Galperin and Lazarev, 1984). Bandurko et al., (1984) have speculated that enzymes present in the mucus layer act as anion sources and these may convert soluble lead into insoluble species and prevent its absorption.

The effect of glucose on the interaction of lead with the intestinal wall was studied. Concommitant oral dosing of glucose and lead caused a significant increase in the amount of lead present in the apical glycocalyx. No effect on the amount of lead associated with the mucosal scrape or muscle layers was observed. This data suggests that glucose causes an increase in the lead binding capacity of the apical glycocalyx.

The data in this thesis is in agreement with previous studies of the effect of glucose on lead absorption. Coleman, (1979) Coogan, (1982) and Heaven (1985) all demonstrated significant uptake of lead into the wall of the small intestine when using glucose containing buffer and the everted sac preparation. These workers did not show any influence of glucose on lead absorption which agrees with the studies presented here. It is likely that glucose causes an increase in the amount of lead bound to the apical glycocalyx by stimulating metabolism with a resultant increase in phosphate levels which precipitate lead in the apical glycocalyx. However no data are currently available to support this concept. The independence of lead transport and glucose availability suggests that lead absorption is independent of metabolic energy as proposed by Blair et al., (1979), and this further supports a diffusive model for lead transport.

The role of the chelating agent EDTA (ethylenediamine tetraacetic acid) on lead absorption has been studied and conflicting data reported. Using everted sac studies Coogan (1982) showed increased rates of absorption with EDTA. In rat whole body studies Garber and Wei, (1974) showed EDTA had no effect on the intestinal absorption of lead. Human studies have shown that EDTA reduces lead absorption (James, personal communication).

In this study the influence of the chelating agent EDTA on the interaction of lead with the intestinal wall, absorption and tissue distribution of lead in the rat was investigated. EDTA was seen to increase the amount of lead associated with the apical glycocalyx and mucosal scrapes but not the muscle layers. This observation may simply reflect the previously reported effect of EDTA on the intestine whereby it generally increases intestinal permeability (Aronson and Rogerson, 1972). The mechanism of this effect remains unknown but it may be that EDTA by chelation of other divalent metals in the intestinal wall causes an increase in the number of lead binding sites within the tissue. However, it is likely that the lipid soluble EDTA - lead

complex crosses the cell membrane and enters the absorptive cell.

When lead was dosed with EDTA there was no increase in the amount of lead absorbed as measured by organ lead levels. This observation agrees with those of Garber and Wei, (1974) and Jugo, Maljkovic and Kostial, (1975) who found no effect of EDTA on intestinal lead absorption in orally dosed rats. The increased rate of absorption of lead with EDTA reported by Coogan, (1982) is not supported by the observations in this thesis. However it may be that this is a reflection of different techniques used. The whole animal studies presented here are more complex than the previous everted sac studies since in the whole animal dissociation of the lead-EDTA complex in the stomach may occur. It is also possible that the rate of lead absorption is faster in lead - EDTA dosed rats and that this observation was not observed due to increased urinary excretion of Pb-EDTA within the dosing period. The reduced absorption reported by James (personal communication) may reflect species differences in gastric pH between man and rat.

The concept of lead absorption by iron binding proteins has been reported previously (Conrad and Barton, 1978 and Flanagan et al., 1979). To investigate this proposal the distribution of ^{59}Fe was compared to that of lead within the intestinal wall and the effect of excess iron on lead absorption was also studied.

If lead were transported by the iron binding proteins to any significant extent iron and lead would be expected to have similar tissue distributions within the intestinal wall. Excess iron, by competing for binding sites and transport pathways should also reduce lead absorption.

Hundred fold excess of iron over lead had no effect on the interaction of lead with the intestinal surface (i.e. the apical glycocalyx and mucosal scrape). Iron however did reduce the amount of lead present in the muscle layers. The reason for this remains unknown. The distribution of ^{59}Fe and ^{203}Pb in the intestinal wall showed that similar amounts of lead and iron were seen in the apical glycocalyx but

that iron and lead were distributed differently in both mucosal scrape and muscle layers. Excess iron did not reduce lead absorption into the body as measured by organ lead levels. From these observations it may be concluded that lead is not transported by the iron binding protein to any significant extent.

The reports of Conrad and Barton, (1978) and Flanagan et al., (1979) showed an influence of iron absorbing systems on lead absorption. This may simply reflect the iron deficiency of the animals used and as such may differ from the normal iron replete state. The observations recorded in this thesis agree with the previous studies of Coogan (1982) and Heaven (1985) who showed lead absorption to be independent of iron in the everted sac preparation.

The effect of age on the intestinal absorption of lead was studied. The role of phagocytosis in the increased absorption of lead was suggested by Kostial et al., (1971) and Keller and Doherty (1980). Significantly increased amounts of lead were associated with the apical glycocalyx and mucosal scrape of the young rat compared to the adult. This indicates that in young animals more lead becomes associated with the intestinal tissue. This may be due to the phagocytosis reported in these animals (Keller and Doherty, 1980). However it could reflect the presence in the lumen of a chelating species produced in young animals which allows lead to enter the cell although identity of this compound remains unknown.

The presence of food in the lumen of the intestine reduces lead absorption significantly in the rat (Garber and Wei, 1974, Quaterman et al., 1976 and Conrad and Barton, 1978) and in man (Rabinowitz et al., 1980, Heard and Chamberlain, 1982, Blake et al., 1983 and James et al., 1985).

In these studies lead absorption in the rat falls from approximately 30% of the dose in the fasted state to about 5% in the fed animal. A similar trend is revealed in human studies but the reduction is much greater, from 60% in the fasted to about 3-4%

in the fed state (James et al., 1985). The variation in data from man and rat may simply reflect nutritional differences since feeding rats with diets similar to humans causes similar absorption values to be seen in both species (Kostial and Kello, 1979).

It is possible the simultaneous presence of lead and food in the intestine causes lead to become bound to food components particularly dietary fibre or phosphates (Wise and Gilbert, 1981 and Rose and Quaterman, 1984). The presence of food additives may also be important (Furia, 1968). Thus the conversion of lead into a non-bioavailable form may be responsible for the reduced absorption seen in the fasted rat. This process was suggested previously (Kehoe, 1976). However physiological changes in the intestine (pH and bile availability) may reduce lead solubility and prevent absorption.

Previous theoretical studies have shown that lead may form complexes with several anionic groups particularly carbonate, phosphate, hydroxyl and sulphide (Rickard and Nriagu, 1978). The formation of lead carbonate species has been described as crucial to lead solubility in aquatic systems (Jackson and Sheilham, 1980).

To investigate the chemical forms of lead within the intestinal lumen speciation studies were performed on the small intestine luminal contents. The contents were subjected to a single centrifugation step (100,000, 1hr) which produced supernatant and pellet phases. Speciation studies were performed on both phases. The supernatant was investigated by gel permeation chromatography on columns of Sephadex G-15, whilst the pellet was studied using a sequential leaching technique (Tessier et al., 1979).

Speciation of the pellet phase reveals that fed, overnight fasted and fasted bile duct ligated animals the main constituent of the pellet phase is lead carbonate although significant amounts of oxide and phosphate are present. Animals dosed with a large dose of lead had lead present mainly in oxide species with a significant portion

of the dose also being found as carbonates and phosphates.

The observations reported in this thesis show that the formation of lead carbonate is an important part of the lead speciation process within the intestinal lumen. Since bile and pancreatic secretions are rich in bicarbonate the effect of bile duct ligation on the speciation of lead in the lumen was studied. Bile duct ligation caused no significant effect on the amount of lead carbonate produced. From this data it seems likely that the carbonate involved in the formation of lead carbonate is either produced at the surface of the intestine by enzymic activity (carbonic anhydrase) or simply diffuses from plasma to lumen.

The soluble phase of the luminal contents was studied using gel permeation chromatography on Sephadex G-15. This approach is of interest since following chelation the absorption properties of the metal ligand complex may be altered (as a result of changes in lipid solubility and chemical charge of the chelating ligand).

In fasted rats the elution profile contains two peaks the first (minor) peak, arbitrarily labelled Pb-X at fraction 9 and the second (major) peak at fraction 21. In fed rats only the second peak at fraction 21 was observed, Pb-X being absent. Bile duct ligation gave a similar chromatographic profile to fasted animals with an intermediate peak at fraction 15.

The broad peak at fraction 21 is likely to be a mixture of the lead cation and lead bile complexes, since lead bile complexes and lead alone standards elute at this position. The intermediate peak produced in bile duct ligated animals was dissimilar in chromatographic properties to any standard and remains unidentified. The larger molecular weight species Pb-X also remains unidentified although it is unlikely that Pb-X is a lead-bile complex since Pb-X is produced in the bile duct ligated animal. The absorption properties of Pb-X were studied by redosing animals with chromatographically produced Pb-X. The data indicate Pb-X is not absorbed. It is possible that Pb-X is a protective compound produced in the fasted animal only.

Another possibility is that Pb-X is similar to the low molecular weight zinc binding ligand which has been shown to be involved in zinc absorption (Song, Adham and Ament, 1984).

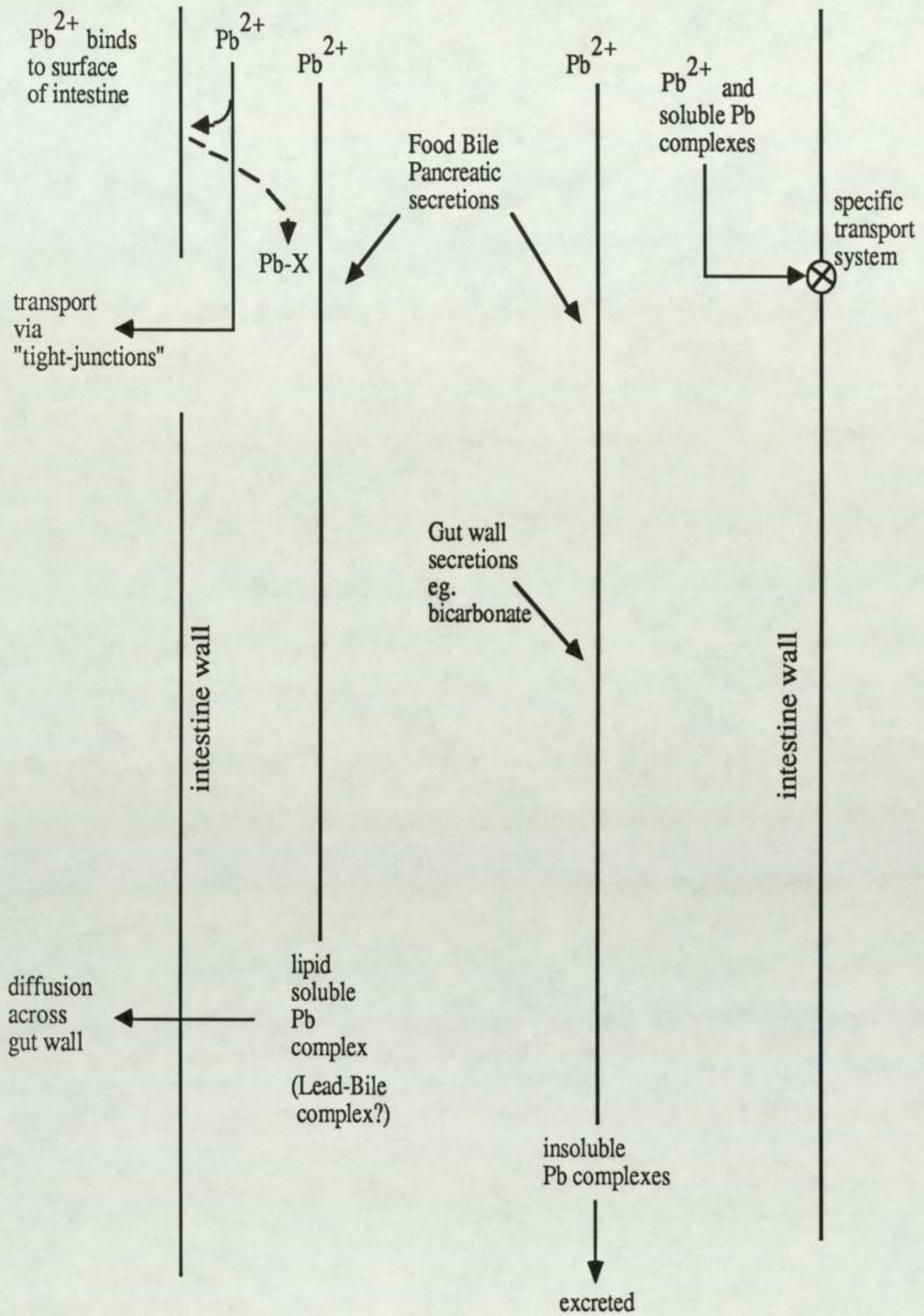
The chromatographic data, however must be treated with caution since problems with the interaction of lead with the Sephadex matrix made it essential that a low pH and high lead concentration (10^{-2}M) buffer be used to suppress the binding of lead with ionised carboxyl groups within the gel. However this buffer system gave good recoveries of lead (90%). This is in agreement with a similar system used to speciate lead in soils (Gregson and Alloway, 1984). The resolution of the system was not adequate to fully separate the low molecular weight lead species and consequently there can be no definitive identification of these compounds.

A proposed mechanism for lead absorption

Figure 7.1 shows an expanded model to explain lead absorption in the rat. The concept of lead absorption, being a simple diffusion process of the lead cation, remains unaltered by these studies. The data shows no active transport of lead in the intestine of the intact rat and hence it is unlikely that lead has a specific transport system in the intestinal wall. In all cases, except in young rats, no correlation between the interaction of lead with the intestinal tissue and absorption into the organs of the body cavity was seen. This suggests that the intestinal wall does not have a role in controlling lead absorption.

Speciation data show that the formation of lead carbonate is important in reducing the soluble lead concentration and hence the amount of lead absorbed. However other species are involved. Whilst the nature of the absorbed lead species remains unknown it is likely that it is simply the lead cation or lead ion pairs and lead-bile complexes.

Figure 7.1



Possible fates of lead upon entering the small intestine.

CONCLUSIONS

1. Lead is absorbed by an non saturating mechanism with kinetics indicative of a diffusion process.
2. After absorption into the animal lead becomes widely distributed in both soft and hard tissues.
3. Factors which influence the interaction of lead with the intestinal tissue do not necessarily influence the way lead is absorbed in the fasted rat.
4. Lead interacts with food components and may influence the way in which they are absorbed. This requires further study since micro nutrients are possibly lost to the body because of their interaction with lead.
5. In the gut lead is converted into several chemical forms. Lead carbonate is the most abundant species, but in conditions where the dose of lead is very large or in situations where bile is unavailable other forms particularly lead phosphate and lead oxides are produced in vivo.
6. The soluble phase of the gut contents has been shown to contain at least two lead species. One is likely to be the lead cation or a lead bile complex, the other as yet unidentified may be a protective compound.

7.1 FURTHER WORK

1. Further studies are necessary to determine the role of the small intestine wall in absorption in young animals. Such studies could investigate the increased interaction of lead observed in these animals using membrane vesicles produced from the microvilli of young rats.

2. The possibility that lead may form complexes with dietary components (especially micronutrients) and thus make them unavailable for absorption is an area requiring further studies.

3. An alternative approach to studying kinetics of lead absorption in the whole animal would be useful to support this data. Such experiments would use intestinal in vivo perfusion and cannulation of the mesenteric blood supply to study transport.

4. The sites of formation of the lead species observed remains unknown. The possible role of carbonic anhydrase on the formation of lead carbonate should be studied using the inhibitor Diamox.

5. An increased resolution system is needed to identify the lead ligand complexes formed in the lumen of the small intestine. This increased resolution could be obtained using an alternative chromatographic medium (e.g. Sephadex G-10) or by the use of two permeation columns in series.

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