THE ABSORPTION OF THE LEAD CATION FROM THE INTESTINE OF THE RAT

A Thesis submitted for the degree of Doctor of Philosophy

by

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SUMMARY

The gastrointestinal absorption of lead was studied using two techniques, one *in vitro*, the other *in vivo*. The *in vitro* technique used was that of everted intestinal sacs. The *in vivo* method was a whole body study where rats were dosed intragastrically with a known dose of radio-labelled lead acetate solution and sacrificed at time intervals after administration.

The distribution of lead within the tissue was investigated by the application of agar to remove the apical glycocalyx or by scraping the incubated tissue to remove the mucosal layer. The interaction of lead with the tissue was altered by the use of a number of agents *in vitro*. The effect of these agents on the tissue-binding of a chelated lead complex was also studied.

The *in vivo* absorption of lead was compared to that of iron and sodium cations. The effect on lead absorption of food was examined, both in the form of the normal rat diet and as individual components ie. an energy source, a chelating agent and fibre. Some comparison was made with the effects of these factors on the absorption of iron. The absorption of lead by weanling rats was examined.

From the experimental data obtained in these studies it was concluded that lead transport by the intestine is a passive process. The interaction of lead cations with the tissue is a mainly surface phenomenon. Lead absorption is reduced by the presence of food in the intestine, the main effect being that of fibre. The age of the rat also affected the extent of absorption.

KEY WORDS

intestinal absorption

tissue interaction

glycocalyx

DECLARATION

This thesis is an account of original work carried out in the Department of Chemistry of the University of Aston in Birmingham between July 1980 and March 1983. This work has been carried out independently and has not been submitted for any other degree.

Jennifer Ann Heaven.

Jennifer Ann Heaven

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I dedicate this thesis to my husband, Walter, and to my parents, with many thanks for their unfailing help and support.

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CHAPTER ONE

INTRODUCTION

1.1. THE MORPHOLOGY OF THE GASTROINTESTINAL TRACT.

In order to study the absorption of lead from the small intestine it is necessary to have a clear understanding of the morphology and function of the gastrointestinal tract.

The gastrointestinal tract is a long fibromuscular tube, stretching from the mouth to the anus. It is lined with an epithelium which, in places, is specialised for secretion and for absorption. The wall is composed of four concentric layers; mucosa, submucosa, muscularis externa and serosa.

There are four main sections of the gastrointestinal tract; (i) the oesophagus, a tube for conducting food to the stomach; (ii) the stomach, a food store, though some digestion of proteins is started, and water and alcohol is absorbed; (iii) the small intestine, comprising the duodenum, jejunum and ileum, which is the main area for absorption of digested matter; and (iv) the large intestine, where matter is prepared for elimination.

The structure of the small intestine is related to its absorptive function. The epithelium is one cell thick, covering the mucosa which contains blood and lymph vessels to carry away absorbed matter. The surface area is increased by thin protrusions known as villi. The cells covering the villi have their own small villi-like projections, known as

microvilli, to increase the surface area further. The epithelial cells are formed at the base of the villi, in the crypts of Lieberkühn, and migrate to the tips as they age. The cells at the tips of the villi are sloughed off continuously by the action of the food in the lumen. Maximum absorption of actively absorbed nutrients occurs midway up the villus, while the cells at the tip are senescent and have a low absorptive capacity. Lead is absorbed along the whole length of the small intestine, maximum absorption taking place in the duodenum and jejunum, (Coleman,1979).

The luminal face of the epithelial cells is covered by a mucous layer, secreted by the goblet cells. This layer may affect diffusion of substances from the lumen to the apical cell membrane, and therefore be a barrier to absorption.

The apical membrane is a typical cell membrane consisting of two layers of lipid with protein molecules randomly embedded in it. These molecules may act as transport routes for substances which are poorly lipid soluble, and would have difficulty crossing the lipid bilayer. The microvilli on the apical membrane are covered with a 'fuzzy coat' of glycoprotein, known as the glycocalyx. This coat maintains an acid atmosphere near the membrane, known as the acid microclimate, thought to be caused by the production of hydrogen ions during the breakdown of ATP at the surface of the membrane (Blair and Matty,1974). The acidity may facilitate the absorption of large charged molecules such as

folates, and may indeed have some effect on smaller charged metal ions.

1.2. LEAD IN THE ENVIRONMENT.

Lead has been used by man for many thousands of years. The Egyptians are known to have used lead compounds in pigments, both for cosmetics and in glazing pottery vessels. Cooking pots were often made from the metal itself. The Romans used lead for piping water, and slaves working in the lead mines are known to have suffered from lead poisoning (Hilburn,1979).

Lead consumption in historical times was very small compared to the amount used since the Industrial Revolution. Before 1850 an estimated 55 million tonnes of lead were used, whereas the consumption in the decade 1971 - 1980 was approximately 38 million tonnes (Table 1.1.).

Although the worldwide consumption of lead has fallen slightly in the last few years (Table 1.2.), due partly to the economic recession and partly to the replacement of lead by plastics, etc., a large amount (over 5 million tonnes) is used annually. In the United Kingdom lead is used principally in the manufacture of batteries, tetraethyl lead, cables, sheeting and piping (Table 1.3.).

Unchecked airborne emissions and uncontrolled dispersal by industry have resulted in widespread contamination, leading to levels in the environment which are far in excess

Table 1.1.

Historical worldwide consumption of lead.

Time Period	Lead Consumption (thousand tonnes)
Pre-1850	55,000
1850-1900	25,000
1901-1910	10,700
1911-1920	11,200
1921-1930	14,200
1931-1940	14,600
1941-1950	14,900
1951-1960	24,000
1961-1970	33,000
1971-1980	38,000
TOTAL	241,000

from: Nraigu,J.O.,(1979)

Table 1.2.

Consumption of Refined Lead.

Year	World Consumption (thousand tonnes)	UK Consumption (thousand tonnes)
1977	5,407	317.7
1978	5,464	336.5
1979	5,556	333.2
1980	5,285	295.5
1981	5,224	265.8
1982	no figures available	271.9

Accounting based on consumption of refined pig lead and the lead content of antimonial lead.
Remelted pig lead and remelted antimonial lead are excluded.

from: World Metal Statistics (April 1983).

Table 1.3.

Consumption of Lead by End Use - UK 1982.

	thousand tonnes
Cables	20.9
Batteries (as metal)	43.7
Battery oxides	45.0
Lead Alkyl anti-knock compounds	54.4
Sheet and Pipe	53.6
Other oxides and compounds	19.5
White Lead	0.5
Solder	9.0
Alloys	7.8
Shot (incl. bullet rod)	5.4
Collapsible tubes	1.4
Other rolled and extruded	0.4
Miscellaneous	20.4
TOTAL	281.8
(of which Refined Lead)	(271.9)

from: World Metal Statistics (April 1983).

of those found naturally. Emission and dispersal of lead are now under the control of the Industrial Air Pollution Inspectorate (formerly the Alkali and Clean Air Inspectorate) and the Factory Inspectorate in England and Wales, and with the Industrial Pollution Inspectorate in Scotland.

The lead in the atmosphere is largely due to man-made contamination, 90% of it being due to emission of exhaust fumes from motor vehicles (D.H.S.S. Working Party, 1980), the rest from industrial sources. As a result, airborne lead concentrations vary considerably, from below 0.1$\mu g/m^3$ in remote rural areas to between 0.2 and 2$\mu g/m^3$ in towns and cities. There are also 'hotspots' where the concentration of lead can reach 6$\mu g/m^3$ or higher, for example along busy roads flanked by high-sided buildings.

Motorway traffic is one of the main sources of airborne lead in rural areas, although the concentration of approximately 6$\mu g/m^3$ in the immediate vicinity falls to 10% of that figure by a distance of 150m from the roadway (D.H.S.S. Working Party,1980).

Lead occurs naturally in the soil due to erosion of the earth's crust, which contains large amounts of the metal ore. The levels are low, averaging 16ppm (Stubbs,1972) with a variation between 5 and 50$\mu g/g$ (Royal Commission Report,1983). However, industrial contamination has resulted in high localized lead concentrations, for example Brunekreef et al (1981) found levels as high as 1,126$\mu g/g$

in the soil of gardens near a lead smelter, and a survey in Walsall found levels exceeding 1,000µg/g in 1.5% of sites studied (Joint Unit for Research on the Urban Environment, 1982).

The average body burden of lead is approximately 120µg/70kg man (Schroeder and Tipton,1968). There is no direct measure of the total amount of lead in living persons, therefore the blood lead level has been suggested as a reasonable indicator of the body burden of lead. On average, the level of lead in the blood is approximately 11µg/100ml in urban dwellers, reaching 12.8µg/100ml in inner city dwellers, and 7µg/100ml in people who live in rural areas (Royal Commission Report,1983).

Frank lead poisoning usually occurs at blood lead levels over approximately 80µg/100ml. Symptoms include anaemia, constipation, colic and renal damage. In children there is loss of appetite, irritability, abdominal pain, loss of muscle co-ordination and coma. Lower blood lead levels than those which cause overt symptoms may cause damage to the nervous system (Lee,1981).

Although there is no controversy about high blood lead levels having adverse effects, there is some debate on the occurrence of effects associated with a blood lead concentration lower than about 35µg/100ml. It has been suggested that even slightly elevated blood lead levels may cause hyperactivity (David et al,1977) and mental

retardation in children (David et al,1976). Yule et al, (1981), reported a detrimental effect on IQ of blood lead levels above 12µg/100ml, and Needleman et al,(1979),warned of possible deficits in classroom performance of children with elevated blood lead levels.

1.3. DIETARY SOURCES OF LEAD.

1.3.1. Lead in Water.

The natural lead content of water is derived from leaching from rocks and soil. Man-made contributions include effluent from smelters and factories, and also as a result of precipitation of airborne lead by rain. Much of the lead becomes bound to particles in the water, and is deposited in the sediment.

The main source of contamination of domestic water supplies is as a result of the use of lead piping. Houses without lead piping have water with a low lead content. However, although the use of leaded water supply pipes was stopped after the Second World War, a great many dwellings and mains systems still exist which date before this period.

A survey carried out in 1975-76 (Department of the Environment,1977) found that the water supply of 10.3% of houses in the United Kingdom had a lead content above 50µg/l, the limit set by an EEC directive (1979) as the maximum permitted lead content of household water. In Scotland, where approximately 60% of housing has leaded pipes, and

40% of mains water has a pH of less than 6.8 (Pocock,1980), 34.4% of households could not meet the demands of the directive. Hence, the presence of lead piping plays an important role in determining the intake of lead from water.

Moore et al,(1979), found that food took up lead from cooking water, especially foods which were partially dehydrated, eg. macaroni. These workers suggested that the sulphydryl groups of denatured protein bound the lead. Smart et al,(1981), also found this phenomenon during cooking and suggested that lead in cooking water could contribute up to 10% of the normal dietary intake of lead, at cooking water concentrations of 20µg/l. This figure may be high for the majority of households in the United Kingdom, but the lead content of water is an important contributor to the amount of lead ingested by man.

1.3.2. Lead in Food.

The average intake of lead from food sources has been estimated to be 100µg/day (Ministry of Agriculture, Food and Fisheries,1982). The estimated lead concentration in some common components of the diet are laid out in Table 1.4.

Lead intake varies with the calorific intake; for example an adult male who consumed 2,850Kcal/day would have a lead intake of approximately 140µg/day. On the other hand, a two-year-old child who ate 1,350 Kcal/day would ingest approximately 70µg/day (D.H.S.S. Working party,1980). This

Table 1.4.

Lead concentration in components of the average diet.

Food Group	Max. Lead Concentration (mg/kg)
Bread and cereals	0.050
Meat and poultry	0.050
Offal	0.130
Fish	0.080
Sugar and preserves	0.055
Green vegetables	0.065
Potatoes	0.050
Other vegetables	0.050
Canned vegetables	0.135
Fruit	0.050
Fruit products	0.155
Beverages	0.014
Milk	0.020

from: M.A.F.F. Total Diet Study (1981).

is a proportionally greater amount, reflecting a child's greater calorific intake per body weight. The average intake of lead in the United Kingdom is well within the limit suggested by the WHO/FAO of 430µg/day. However, total intakes could rise far above these limits due to the ingestion of adventitious lead, ie. items which do not make up part of the normal diet but nevertheless are ingested.

1.3.3. Adventitious Sources of Lead.

Lead contained in dust, soil and paint are examples of adventitious sources of lead that may contribute significantly to the total amount of lead ingested. Children may be particularly at risk, because of the large number of hand-to-mouth actions they perform. Similarly, Tola and Nordman, (1977), reported a higher blood lead concentration in lead workers who smoked compared with those who did not. They attributed this to an increase in hand-to-mouth actions, together with contamination of the fingers while working.

A study carried out in households in the vicinity of a lead smelter (Brunekreef et al,1981) showed that the blood lead concentration of children aged between one- and three-years-old were elevated, probably due to the increased lead content of the soil and dust. Walter et al,(1980), reported that blood lead levels were dependent on a number of factors, eg. lead in household dust and garden soil, the occupation of the father and the incidence of pica (the persistent ingestion of non-dietary items eg. soil and paint flakes).

1.4. THE BALANCE BETWEEN INGESTED AND INHALED LEAD.

Until fairly recently, it has been generally accepted that approximately 10% of the lead ingested by man is absorbed (Thompson,1971; Blake,1976). However, more recent studies have questioned these results. For example, Rabinowitz et al,(1980), found that although 6-10% was absorbed by subjects who had recently taken a meal, in those who had fasted for 16 hours the percentage of lead absorbed fron a single dose rose to 30-37%. Chamberlain et al,(1978), reported the same effect in that 13-18% of the lead dose was absorbed in well-fed subjects, while an average of 45% was absorbed after a 12-hour fast. Few researchers have studied the absorption of lead from the lungs. Chamberlain et al,(1978), reported that approximately 35% of inhaled lead is absorbed.

Table 1.5. shows the estimated uptake of lead from inhaled and ingested sources, using absorption figures of 10% and 35% for ingested and inhaled lead respectively. The ratio of ingested to inhaled lead is 2:1 in urban regions and as much as 20:1 in rural areas. The ratio would increase if the percentage of lead absorbed was higher than 10%, as has been suggested. Furthermore, no account has been taken of adventitious sources of lead, which may far exceed the amount of lead ingested in food and water. Therefore, it is clear that ingestion is the more important route of entry

Table 1.5.

Estimated daily uptake of lead from ingested and inhaled sources.

Source	Amount (per day)	Approx. Lead Conc.	Approx. Absorption (%)	Estimated Uptake (μg)
Food	1.5 kg	0.067 μg/g	10	10
Water	1.5 l	10 μg/l	10	1.5
Air (urban)	15-20 m^3	1 μg/m^3	35	6
Air (rural)	15-20 m^3	0.1 μg/m^3	35	0.6

Ingested : Inhaled

Urban 2:1
Rural 20:1

ADVENTITIOUS SOURCES ?

of lead into the body.

Recent work has shown that body burdens of lead which were once thought safe may have harmful effects, especially in children. Therefore, it is important to gain a greater understanding of the mechanism of intestinal lead transport, and find factors which can reduce absorption as far as possible, in order to minimise the potential risk from lead.

1.5. FACTORS WHICH AFFECT THE INTESTINAL ABSORPTION OF LEAD.

The reason why a particular factor affects intestinal lead absorption must ultimately be explained in terms of a model which describes all the features of the absorption process. A considerable number of studies have been carried out, in different laboratories, in recent years, which have identified a large number of factors affecting lead absorption from the gastrointestinal tract. However, many of the studies suffer from the defect that no satisfactory explanation of the data, in terms of a mechanistic model, is put forward. The main points from some of the studies are summarised in the following section. The experiments (unless otherwise stated) have been carried out either on rodents or humans. Rodent studies used various techniques, eg. everted sac, isolated loops, whole body studies, while the human experiments were all whole body studies.

1.5.1. The Effect of Dietary Calcium and Iron on the Absorption of Lead.

a) Calcium.

A shared transport route for lead and calcium has been put forward by Barton et al,(1978), who found that calcium-deficient rats have an increased lead retention. These findings are supported by other studies on rats (Six and Goyer,1970; Mahaffey et al,1973; Barltrop and Khoo,1975); and Johnson and Tenuta,(1979), have linked low calcium diets and high blood lead levels in children. Meredith et al,(1977), found that high dietary calcium reduced lead absorption. Barton et al,(1978), reported that lead bound to calcium-binding protein, and proposed that in calcium deficiency, lead cations would be transported via this carrier mechanism. However, if a shared carrier-mediated mechanism was involved one may expect to find competition between lead and calcium for the carrier, and none has been demonstrated, (Mykkänen and Wasserman,1981). Demonstration of saturation of a carrier may also be expected, but this has not been found (Coogan,1982).

b) Iron.

Flanagan et al,(1979), suggested that the absorption of iron and lead were linked. They reported that the two metal ions competed for absorption from the small intestine. It has also been noted that a dietary iron deficiency results in an increase in lead absorption in mice,

(Flanagan et al,1979; Weimer and Kies,1981; Hamilton,1978), rats,(Barton et al,1981), and humans,(Watson et al,1980; Flanagan et al,1982). Anaemia caused by bleeding had no effect, however (Flanagan et al,1979; Barton et al,1981; Flanagan et al,1980).

No explanation has been put forward for the link between iron and lead transport. The effect may be solely due to competion for binding sites, as the physical absence of iron is required for the enhancement of lead absorption.

1.5.2. The Effect of Other Dietary Components on the Absorption of Lead.

A number of dietary components have been reported to affect lead absorption and retention. However, much of the data is conflicting and insufficient explanations for the mode of action have been put forward.

a) Milk.

Experiments carried out on weanling and adolescent rats have shown that both the absorption and retention of lead are enhanced by a diet rich in milk, (Kello and Kostial, 1973; Stephens and Waldron,1975; Kostial and Kello,1979). The lactose content of milk is reported to be the significant factor (Bell and Spickett,1981), as lactose has been found to increase lead absorption, (Bushnell and DeLuca,1981).

b) Protein.

Data concerning the effect of protein on lead

absorption is contradictory. Diets low in protein have been reported to either increase, or have no effect on, lead absorption, (Barltrop and Khoo,1975; Quarterman et al,1978; Conrad and Barton,1978). When specific amino acids were studied it was discovered that some caused an increase in absorption in weanling rats, eg. lysine, methionine, cystine, and tyrosine, while having the opposite effect in adult rats. Other amino acids had no significant effect at either age, (Quarterman et al,1980).

No full explanation has been advanced, but Moore et al,(1979), suggested that lead uptake by foodstuffs could be due to its chelation by sulphydryl groups of denatured protein.

c) Lipids.

The absorption of lead is reported to be enhanced both by a long-term diet high in fat (Barltrop and Khoo,1975), and by the simultaneous administration of fat with a dose of lead (Flanagan et al,1982). Quarterman et al,(1977), using individual fatty acids, found that most of those studied increased lead absorption, though to differing degrees.

d) Vitamin D.

Mykkänen and Wasserman,(1982), reported an increase in lead absorption when vitamin D was given to rachitic chicks. Barton et al,(1980), found that although the manipulation of dietary vitamin D had no effect on lead

absorption, both deficiency and repletion of vitamin D resulted in an increased absorption of lead in the intact rat. The authors suggested that the increase in transit time caused by vitamin D allowed the lead to be available for absorption for a longer period.

e) Miscellaneous Minerals.

Uptake of lead from the diet has been found to decrease with a high phosphate diet, (Quarterman et al,1978), while diets low in phosphate have been reported to increase lead absorption, (Barltrop and Khoo,1975; Quarterman and Morrison,1975; Barton and Conrad,1981). Lead toxicity was reduced by dietary zinc, (Cerklewski and Forbes,1976), and magnesium deficiency resulted in increased lead absorption, (Fine et al,1976).

These data point to some common transport mechanism for a large number of ions. However, no adequate explanation has been suggested for the observed experimental data.

f) Other Dietary Factors.

The dietary fibre pectate reduces lead absorption by up to 87%, (Paskins-Hurlburt et al,1977), though hemicellulose, another dietary polysaccharide, has been reported to enhance lead absorption, (Weimer and Kies,1981).

Chronic and acute exposure to ethanol has been reported to reduce lead absorption,(Barton and Conrad,1978), as has the presence of tannic acid in the intestine, (Peaslee and Einhellig,1977).

1.5.3. The Effect of Non-Dietary Factors on the Absorption of Lead.

a) Chelating Agents.

Lead absorption has been found to be enhanced by a number of chelating agents. These include ethylenediaminetetraacetic acid, (Jugo et al,1975; Coleman et al,1978; Coogan,1982), nitroloacetic acid, (Garber and Wei,1974; Coogan,1982), d-penicillamine, (Garber and Wei,1974), diethylenetriaminepentaacetate, (Blair et al,1979), dimercaptopropanol, (Jugo et al,1975), and sodium citrate, (Garber and Wei,1974; Jugo et al,1975).

Coleman et al,(1978), suggested that the chelated species are absorbed more readily than ionic lead due to their greater lipid solubility. The chelated species would have a greater molecular weight than the ion, and may use a different transport route. It would probably not be transported by a carrier system which some investigators believe operates for the ionic species, though the increased lipid solubility may result in a greater total absorption.

b) Bile.

Cikrt and Tichy,(1975), were the first to report that the absence of bile in the gastrointestinal tract reduces the absorption of lead. Quarterman et al,(1977), and Conrad and Barton,(1978), agreed with this finding, and Conrad and Barton went on to say that bile is also an important route of excretion of lead.

The action of bile may be due to the formation of a complex between the lead cation and one or more components of the bile, (Cikrt and Tichy,1975). It is known that bile salts are absorbed both passively and actively from the small intestine, and the lead-bile complexes may enter the body in this way.

c) Age.

The effect of the age of the experimental animal on the proportion of lead absorbed has been commented upon by several authors. Young rats, aged about three weeks, absorb a greater amount of lead than adults. This has been found in in vitro preparations of gut tissue, (Gerber and Deroo,1975), isolated intestinal loops of rats,(Conrad and Barton,1978), and chicks, (Mykkänen and Wasserman,1981), and in whole body studies, (Kostial et al,1971; Quarterman and Morrison,1978).

It has been suggested that absorption of all nutrients is enhanced in young rats due to the demands made by rapid growth, (Conrad and Barton,1978), but this may not be the whole answer. It is possible that pinocytosis may play a part, as this has been shown to occur in weanling mice, (Keller and Doherty,1980).

1.6. POSTULATED MECHANISMS FOR THE TRANSPORT OF LEAD BY THE SMALL INTESTINE.

At present there are two main models to explain the transport of lead by the small intestine, each derived from work carried out in different laboratories, using different techniques and experimental animals.

One theory is that lead transport is a carrier-mediated mechanism. The carrier is thought to be related either to calcium-binding protein, (Barton et al,1978), since some investigators believe that lead and calcium compete for binding sites; or to the carrier mechanism for iron, as competition for absorption has been reported between lead and iron, (Flanagan et al,1979). The proposed transport mechanism would be an active process.

In contrast, Blair et al,(1979), and Coleman,(1979), have described an energy independent, biphasic model :- a rapid binding of the lead cation onto the surface of the intestinal wall, followed by a slow, passive movement of lead into the body via a paracellular route. The binding is thought to act as a protective mechanism, to minimize the lead available for transport. No saturation of the absorptive mechanism has been shown by these authors.

A third proposal, by Aungst and Fung,(1981), combines features of the two theories already presented. These authors postulate a carrier as the main route of lead

transport, and report that the mechanism is sensitive to anoxia and is partially saturable, implying an active transport process. They further report that the carrier system becomes saturated at luminal lead concentrations of approximately 10^{-5}M, and any further increase in the amount of lead absorbed is accounted for by passive diffusion.

CHAPTER TWO

MATERIALS AND METHODS

2.1. ANIMALS.

Male Wistar rats supplied by Bantin and Kingman Ltd. were used throughout these studies. The majority of the rats used were aged approximately 10 weeks and weighed 170 - 200g. The younger rats used were aged 23 - 28 days and weighed 50 - 60g.

All the rats were kept at 23°C under a 12 hour light/dark cycle, and maintained on Heygates 41B diet and tap water *ad libitum*. The diet has been shown to contain 0.1 ppm lead (Coleman,1979). The cages in which the rats were housed had stainless steel grid floors to prevent copraphagy. 18 hours before experimentation, food was taken away from the rats, although they were allowed free access to water.

Treatment of the rats complied with the Cruelty to Animals Act, 1876, as laid down in the Home Office Licence Regulations, and qualified by Certificate A.

2.2. PHYSIOLOGICAL BUFFER SOLUTIONS.

Krebs Hensleit bicarbonate buffer was the principal buffer used in the incubation of everted sacs. The buffer contained the following final concentrations of reagents; sodium chloride (112mM), potassium chloride (4.5mM), calcium chloride (2.4mM), potassium hydrogen phosphate (1.12mM), magnesium sulphate (1.12mM), sodium bicarbonate (2.4mM), and glucose (20mM).

The buffer was corrected to pH 7.4 by the addition of 1M HCl added dropwise. The solution was gassed with a mixture of oxygen and carbon dioxide (95% : 5%) to prevent formation of the carbonate ion, which may cause precipitation of lead carbonate and therefore render the lead cation unavailable for the transport studies.

Where high concentrations of lead were required, Tris(hydroxymethane)-aminomethane buffer was used to maintain the lead in solution. The composition of this buffer was: Tris(hydroxymethane)-aminomethane (6 mM), sodium chloride (112 mM), potassium chloride (4.5 mM), calcium chloride (2.4mM), magnesium sulphate (1.12 mM) and glucose (20 mM). The solution was corrected to pH 7.4 with 1M HCl added dropwise. The buffer was gassed with oxygen (100%).

2.3. METHOD OF SACRIFICE.

The rats were anaesthetized with Inactin (5-sec butyl-5-ethyl-2-thiobarbituric acid, Promonta) in 0.9% saline at a dose of 100mg/kg body weight. The dose was administered intraperitoneally. When the rat was unconscious, a mid-line incision from the pelvis to the neck was made. The diaphragm was cut, to prevent breathing, and the rat died without regaining consciousness.

Anaesthetization, followed by incision, was preferred to cervical dislocation, as the former method insured the

maintenance of blood flow to and from the intestine up to the moment of removal of the intestine.

2.4. RADIOISOTOPES.

The gamma emitting isotope ^{203}Pb was used as a tracer in all the studies of lead transport presented in this thesis. The isotope was obtained from the M.R.C. Cyclotron Unit, Hammersmith Hospital, London. ^{203}Pb decays rapidly, with a half-life of 52.1 hours. For this reason, counts were corrected for decay to the nearest hour. The equation used was:

$$A = A_o e^{-\lambda t}$$

where A = counts at time t
A_o = counts at time t_o
λ = decay constant = 3.695×10^{-7} sec^{-1}

The isotopes ^{22}Na and ^{59}Fe, (obtained from Amersham International, Bucks.), were used for studies of sodium and iron absorption. Both are gamma emitting isotopes with half-lives of 2.6 years and 45.1 days respectively, and so no correction for decay during the experimental and counting periods was necessary.

Radiation was detected by an N.E.8312 gamma counter (Nuclear Enterprises). Samples were counted for 600 seconds or up to a maximum of 200,000 counts.

The use of radioisotopes to study the behaviour of

ions depends on the fact that when the appropriate radioactive tracer is thoroughly mixed with an unlabelled compound, the radioisotope will behave in exactly the same fashion as the unlabelled substance. If the factor of dilution of the radioactive tracer is known, the amount of the stable isotope in the samples can be calculated from a calibration curve. This curve consists of standard volume samples taken from the original radioactive solution before the commencement of each experiment.

2.5. PREPARATION AND INCUBATION OF EVERTED SACS.

Rats were anaesthetized and a mid-line incision made as described in section 2.3. The intestine was cut at the ligature of Trietz and the ileo-caecal junction, and the whole of the small intestine was removed.

The intestine was cut in half, and the proximal half was flushed through with Krebs Hensleit bicarbonate (KHB) buffer. The piece of intestine was then divided into six sections, each approximately 8 cm long, and the third, fourth and fifth from the ligature of Trietz were transferred into ice-cold KHB buffer gassed with 95%O_2:5%CO_2. No more than three sacs were prepared from any one rat. Using the method of Wilson and Wiseman (1954), each section was everted over a glass rod 3 mm in diameter and tied at one end. The section was then weighed on a torsion balance (White Elec. Inst. Co. Ltd.), (W1). Approximately 0.5 ml oxygenated buffer was introduced into the open sac using a

hypodermic syringe with a blunt needle. The filled sac was tied at the other end, and weighed again, (W2).

The sac was placed in a 25 ml conical flask containing 10 ml KHB buffer at 37°C. The buffer contained the required concentration of lead acetate labelled with ^{203}Pb at an activity of 3.7 kBq per ml. The sacs were incubated for the required period of time, in a shaking water-bath (Techne Ltd.), oscillating 80 times per minute. The time taken to prepare each sac was approximately 1.5 minutes. The period without oxygenation was minimised to reduce, as far as possible, any damage to the preparation due to oxygen starvation.

After incubation the sacs were removed from the media, blotted on damp filter paper (Whatman No.1) to remove any excess fluid, and reweighed,(W3). The sacs were cut at the bottom to drain the serosal fluid, which was collected in a small sample bottle. The empty sac was blotted again, and weighed a fourth time,(W4). The tissue and aliquots of serosal fluid were placed in vials, and the radiation assessed in the N.E.8312.

Fluid movement during the period of incubation was assessed from the following calculation:

W3 - W2 = total fluid uptake

W4 - W1 = tissue fluid uptake

(W3 - W2) - (W4 - W1) = fluid transferred into serosal compartment

The weight of the threads were found to be negligible (less than 1% of the sac weight) and were ignored in the calculation of fluid movement.

2.6. MUCOSAL SCRAPES.

Everted sacs prepared and incubated in lead acetate and ^{203}Pb, as described in section 2.5., were drained and laid flat on a piece of dry filter paper. The edge of a glass microscope slide was drawn along the length of the sac, leaving only the muscle layer. The sac was turned over and the process repeated. The muscle layer and the cells and filter paper were assessed for radioactivity separately to determine the distribution of lead between the two fractions.

The muscle layer of sacs incubated in solutions containing no radioactive isotope was examined histologically.

2.7. THE MEASUREMENT OF TRANSMURAL POTENTIAL DIFFERENCE.

The potential difference across the intestinal wall was measured by a modification of the method of Barry et al (1964). A section of everted intestine was tied at one end with a cotton thread attached to a glass weight. The other end was eased over a fluted glass cannula and tied securely in place. The sac was filled,via the cannula, with oxygenated Krebs Hensleit bicarbonate (KHB) buffer, taking care that no air bubbles remained. The filled sac

was lowered carefully into 30 ml KHB buffer maintained at 37°C and continuously gassed with 95%O_2:5%CO_2.

Two salt bridges were introduced, one into the mucosal solution, the other into the serosal solution. The ends of the salt bridges were kept level, about half-way down the sac. The other ends of the salt bridges were in separate test-tubes containing saturated potassium chloride and calomel half cells. The half cells were connected to a digital millivoltmeter (Pye Unicam, P.W.9409) and the potential difference across the gut wall could be measured. Readings were taken every five minutes over a one hour period.

The salt bridges were made by warming agar-agar in 3M potassium chloride over a boiling water bath, to make a 5% solution. While the agar solution was still warm it was sucked up into polyethylene tubes 2 mm in diameter. After cooling, the salt bridges were checked to ensure no air bubbles were present, and the potential difference along their length measured. Only salt bridges with a potential difference of 0 $\pm$ 0.1 mV were used.

2.8. THE MEASUREMENT OF GLUCOSE TRANSPORT.

Everted sacs prepared as described in section 2.5. were incubated for either 30 or 60 minutes at 37°C in KHB buffer containing 20 mM glucose. After incubation, 400µl aliquots were taken of the serosal and mucosal fluids.

The concentration of glucose in the serosal and mucosal fluids was measured using a Technicon autoanalyser (Ames Co., Slough), at the Birmingham General Hospital. The autoanalyser uses a colorimetric technique based on the action of the enzyme glucose oxidase.

2.9. HISTOLOGICAL PREPARATIONS.

Everted sacs prepared and incubated for 30 or 60 minutes as described in section 2.5. were removed from the incubation media and emptied. The empty sacs were cut to lie flat, and placed on sheets of stiff absorbent paper. The preparations were then put into buffered 10% formalin. Everted sacs which had been scraped were preserved in the same way.

The preparations were mounted and stained by the Histology Department in Birmingham General Hospital. Two types of stain were used, Periodic Acid Schiff (P.A.S.), and Haematoxylin and Eosin (H & E). P.A.S. is used to stain carbohydrates, polysaccharides and muco substances. H & E stains nuclei, cartilage, basophilic cytoplasm, muscle fibres and collagen.

2.10. WHOLE ANIMAL STUDIES.

The absorption of lead, iron and sodium from the intestine of the intact rat was examined. Ether anaesthetised rats were dosed with the metal ion solution containing

the appropriate radioactive label. Adult rats were given a dose volume of 0.3 ml from a hypodermic syringe, introduced into the stomach via a curved, wide-bore needle with a blunt end. Younger rats, aged 23 - 28 days, were given 0.2 ml of the radioactive dose, using a narrow polyethylene tube (Portex), attached to a hypodermic syringe needle.

After dosing the rats were left in gridded cages, without access to food but with water ad libitum, for a period of 2, 4 or 6 hours. For experiments lasting 24 hours the rats were housed individually in glass metabowls (Jencons Ltd.), with access to water but not food. The metabowls allowed the separate collection of urine and faeces.

At the end of the experimental period the rats were anaesthetized with inactin as previously described in section 2.3. A mid-line incision was made, and the stomach, small intestine, caecum and large intestine were removed. The small intestine was flushed through with 0.9% saline solution, to remove any loosely adhering material. This fluid was reserved.

Each of the sections of intestine, plus the washing fluid, were placed in vials and their radioactivity measured in the N.E.8312. A calibration curve was calculated from standard volumes (5 - 20 μl) of the administered dose, and the total radioactivity given to each animal determined. The amount of metal ion present in each section of intestine

and in the washing fluid was calculated as a percentage of the initial dose by extrapolation from the calibration curve. Recovery of the dose was calculated by the equation:

$$\text{Recovery} = \frac{\text{Total found in intestine and fluid}}{\text{Total dose}} \times 100$$

Absorption into the rat could then be calculated as follows:

$$\text{Percentage absorption} = 100 - \text{Recovery}$$

The liver, kidneys, spleen and heart were removed from some experimental animals. The counts from these organs gave an indication of the amount of metal ion absorbed, although the total absorbed could not be determined by this method.

2.11. PREPARATION OF AGAR REPLICAS.

Agar replicas were prepared according to the method of Ugolev *et al*, (1979). Agar-agar was heated in distilled water, over a boiling water bath, to make a 3% solution, and then stored at 40°C. Sections of small intestine, removed from rats previously dosed with a radiolabelled solution, were flushed with 0.9% saline solution. The sections were tied at one end with a cotton ligature, and agar solution introduced from a hypodermic syringe via a blunt, wide-bore needle. The filled sacs were fastened with cotton, and

placed in ice-cold saline for 5 minutes, in order for the agar to set.

The sac was removed from the saline and slit longitudinally with a scalpel blade. The agar replica was carefully removed and the radioactivity associated with the empty sac and the replica was assessed separately.

2.12. CHEMICALS.

All chemicals used in the experiments presented in this thesis were of Analar grade. Chemicals were obtained from Sigma Co. Ltd., B.D.H. Ltd., Fisons Ltd. and Ciba-Geigy. Radioisotopes were supplied by the M.R.C. Cyclotron Unit, Hammersmith Hospital and Amersham International, Bucks.

2.13. STATISTICAL METHODS.

All results presented in this thesis, unless otherwise stated, are the mean of six experimental observations $\pm$ standard errors of the mean.

The Student's 't'-test was used to determine the significance of difference between two groups of results. Regression lines through a series of points were determined by the method of least squares.

All statistical calculations were carried out using an Olivetti Programma 101 desk computer. Probability values of less than 5% were considered significant.

CHAPTER THREE

THE ASSESSMENT OF THE VIABILITY OF THE EVERTED SAC PREPARATION

AND

AN INVESTIGATION INTO THE CHARACTERISTICS OF LEAD TRANSPORT AND TISSUE UPTAKE.

3.1. THE ASSESSMENT OF THE VIABILITY OF EVERTED SAC PREPARATIONS.

This thesis presents results from two different methods of investigation of gastrointestinal transport processes : an *in vitro* technique, using the everted intestinal sac; and an *in vivo* technique studying the movement of lead in the intact rat. There can be no dispute as to the viability of the tissues in the intact animal, as the nutrient supply and clearance of toxins occurs continuously throughout life.

The viability of the tissue of the everted sac preparation is, however, sometimes questioned. The tissue has been removed from its site in the body, and its supplies of blood and lymph have been discontinued. As a result, the tissue will have lost its normal source of energy. There is also no nervous or hormonal control over the tissue. Clearance of toxins will not take place, and substances absorbed from the incubation media will not be removed. This will result in their accumulation in the tissue, with possible effects on further absorption.

Isolated preparations can be advantageous by their very simplicity, in that the experimenter is able to rigidly control the conditions of incubation while changing one or more parameters. Easy access to sampling is available, meaning that the transport process may be assessed very accurately. Another advantage of isolated tissue

preparations is that they permit the use of substances and conditions which could not be achieved in the intact animal. For example, chemicals which would either cause the animal discomfort, eg. metabolic inhibitors, or which would be denatured or metabolised before reaching the site under investigation, eg. enzymes.

Everted sacs have been used successfully for many years in the study of the absorption of a wide range of nutrients and non-nutrients. If proper care is taken to ensure control over temperature, pH and the adequate supply of energy and oxygen, they can have a useful, if restricted, life.

The viability of the preparations can be determined by studying a number of normal tissue functions. These include transmural potential difference and the transport of glucose and water. The intestinal tissue may also be examined histologically, to find the extent of damage which occurs after periods of incubation under experimental conditions.

3.1.1. The Measurement of Transmural Potential Difference, the Transport of Glucose and Water, and the Histological Examination of Everted Sac Preparations.

Sections of the mid-jejunum were everted, eased over glass cannulae, filled with Krebs-Hensleit bicarbonate buffer containing 20mM glucose, and suspended in the same buffer

at 37°C, and gassed with 95%O_2:5%CO_2. The transmural potential difference was measured by the method described in section 2.7., both in the presence and absence of 10^{-6}M lead acetate.

Everted sacs prepared as described in section 2.5. were incubated in Krebs-Hensleit bicarbonate buffer containing 20mM glucose, at 37°C and gassed with 95%O_2:5%CO_2 for, (i) either 30 or 60 minutes, and the concentration of glucose in the serosal and mucosal media measured as described in section 2.8., or, (ii) for time periods up to one hour, and the movement of water measured as described in section 2.5., or, (iii) for 30 minutes then removed, preserved in formalin and stained, as described in section 2.9., by the Histology Department of the Birmingham General Hospital. Everted sacs which had not been incubated were also prepared for histological examination.

The mean transmural potential difference over the one hour period of experimentation was 7.49 $\pm$ 0.08 mV in the absence of 10^{-6}M lead acetate and 9.26 $\pm$ 0.09 mV in its presence. In neither case did it fall more than one millivolt over the period of measurement, (Fig. 3.1., Table 3.1.).

Glucose was transported by the everted sac during the experimental period. The ratio of the glucose concentration on the serosal side to that on the mucosal side was 1.10 $\pm$ 0.03 (n=18) after 30 minutes of incubation, and 1.39 $\pm$ 0.03

Table 3.1.

Transmural potential difference in the absence and presence of 10^{-6}M lead acetate, (mV).

	Time (minutes) 5	10	15	20	25	30
No PbAc	7.6 ± 0.5	8.1 ± 0.4	7.7 ± 0.4	7.7 ± 0.4	7.6 ± 0.4	7.5 ± 0.4
10^{-6}M PbAc	9.6 ± 0.8	9.8 ± 0.7	9.7 ± 0.7	9.4 ± 0.7	9.3 ± 0.8	9.3 ± 0.8

	Time (minutes) 35	40	45	50	55	60
No PbAc	7.4 ± 0.4	7.4 ± 0.4	7.3 ± 0.3	7.3 ± 0.4	7.2 ± 0.4	7.1 ± 0.4
10^{-6}M PbAc	9.1 ± 0.8	9.0 ± 0.8	9.0 ± 0.8	9.0 ± 0.8	9.0 ± 0.8	8.9 ± 0.8

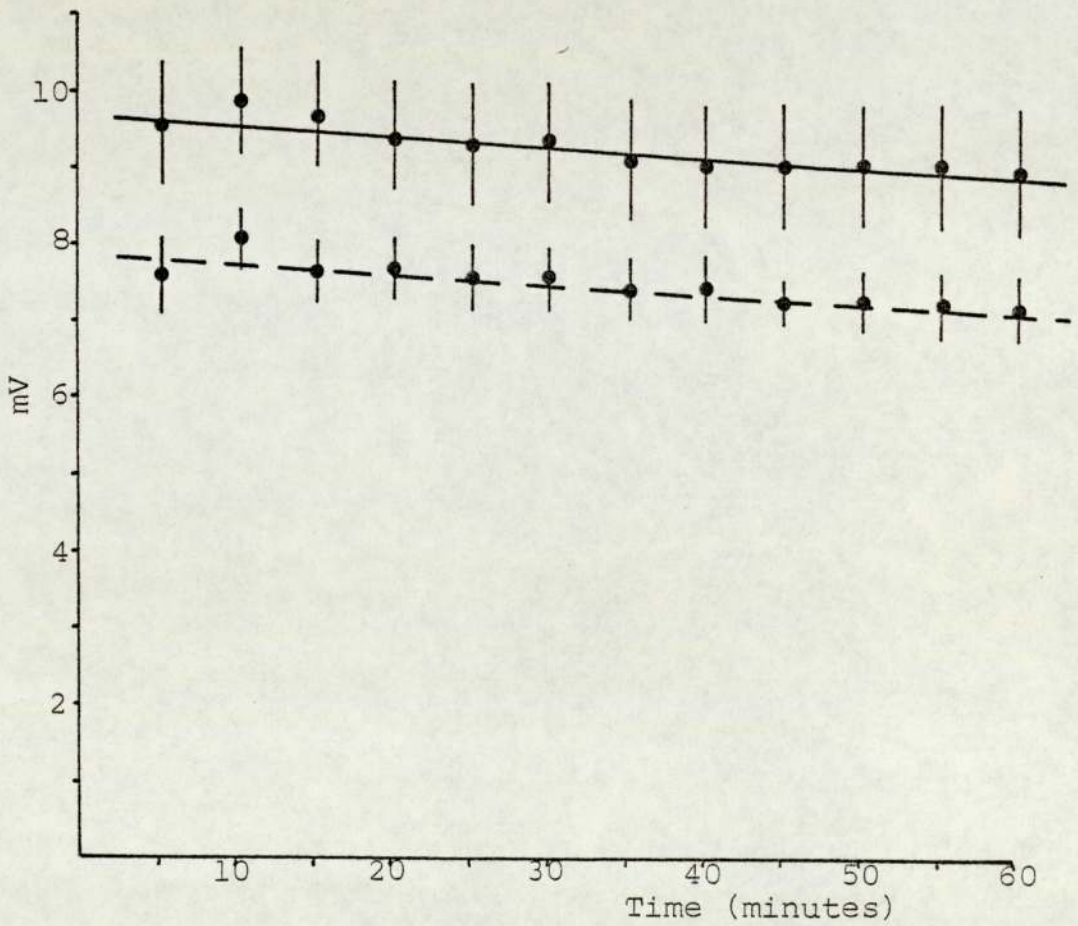
Each value is the mean ± S.E.M. of six experimental observations.

Fig. 3.1.

Transmural potential difference measured in the absence and presence of 10^{-6}M lead acetate, (mV).

- - - - No lead acetate in buffer.

——— 10^{-6}M lead acetate in buffer.



Each value represents the mean ± S.E.M. of six experimental observations.

(n=18) after 60 minutes.

Water entered the serosal space in a linear fashion with time at an overall rate of approximately 13.8 µl/g wet wt/min, after an initial short time lag, (Table 3.2., Fig 3.2.).

Plates 1(a) and 1(b) show the everted sac preparation before incubation, stained with P.A.S. and H & E respectively. The villi are intact, the mucous-producing goblet cells shown in dark pink in plate 1(a). Plates 2(a) and 2(b) show the intestinal preparation after incubation for 30 minutes in Krebs-Hensleit bicarbonate buffer. The villi are wider, and loosely associated mucous can be seen in plate 2(a). The vast majority of the villi remained intact after a 30 minute incubation and the subsequent histological procedure.

3.1.2. Discussion.

The potential difference across the intestinal wall is caused by an imbalance of ionic concentration between the two sides of the intestinal wall. This imbalance is the result of the active pumping of ions from the lumen into the body, or in the case of everted sacs, from the mucosal fluid to the serosal cavity. Transmural potential difference could, therefore, be regarded as an indirect measure of active ionic flow through the gut wall.

The existence of a transmural potential difference

Plate 1(a).

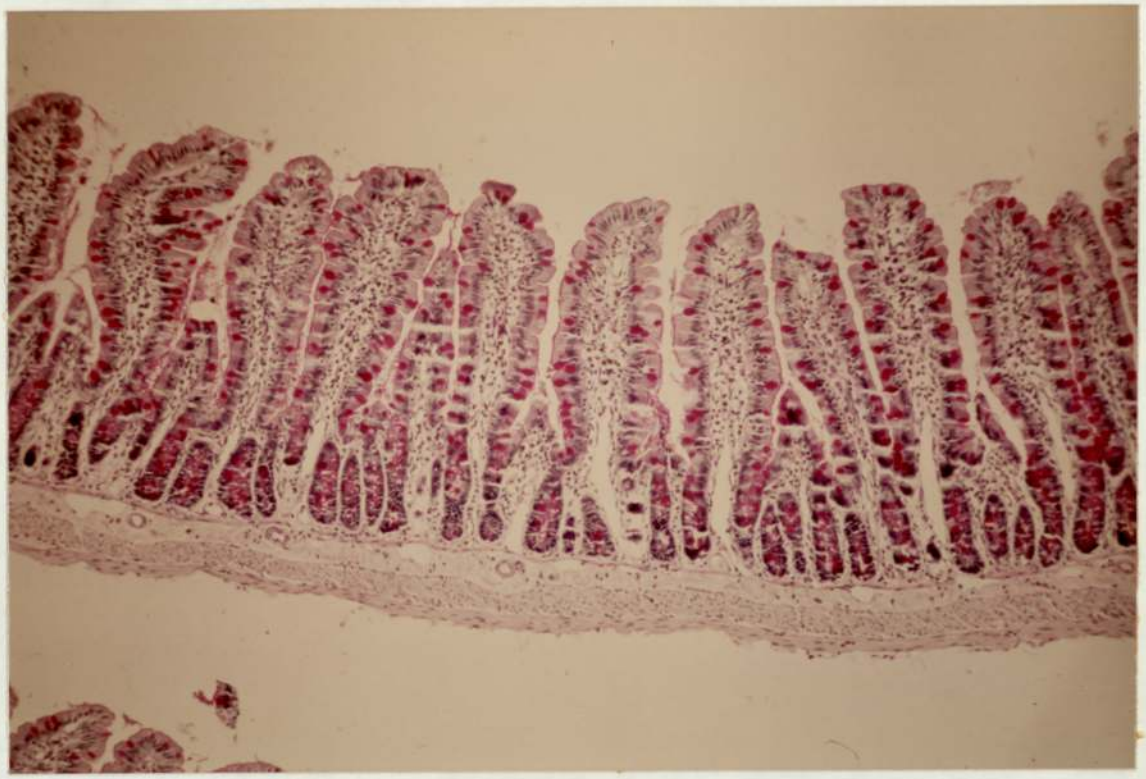
T.S. of mid-jejunal tissue after eversion.
Stained with P.A.S.

Magnification x 25.

Plate 1 (b).

T.S. of mid-jejunal tissue after eversion.
Stained with H & E.

Magnification x 25.



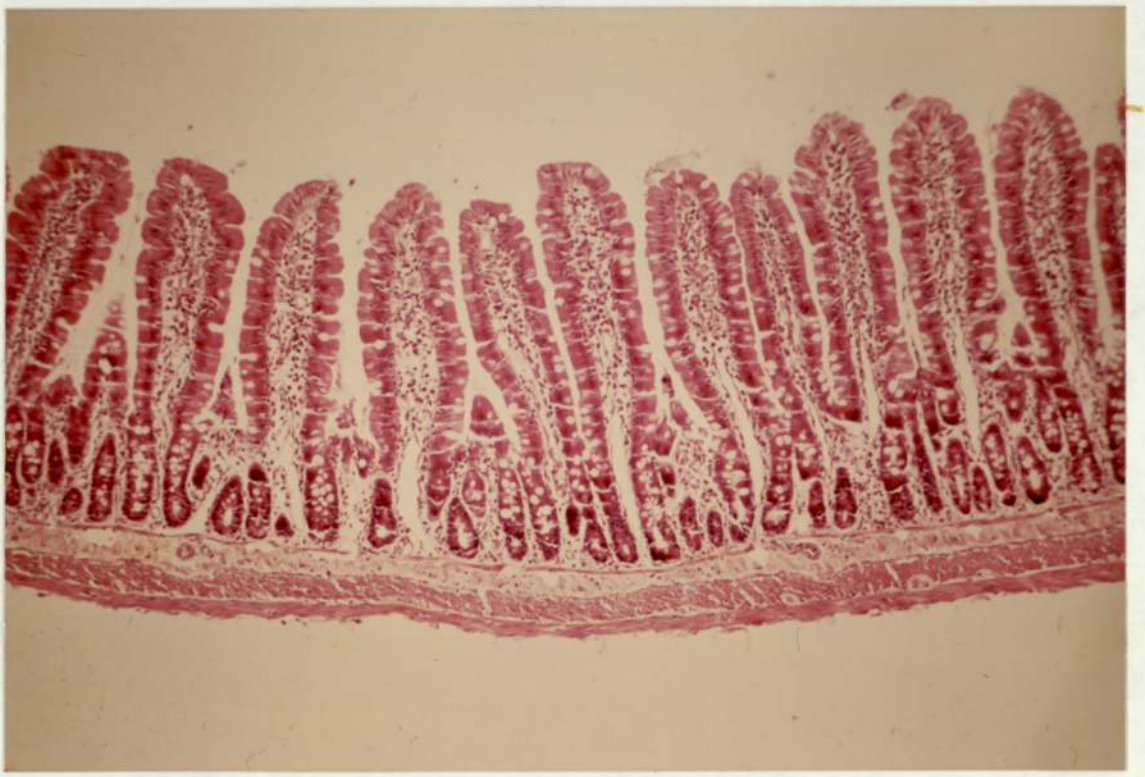


Plate 2(a).

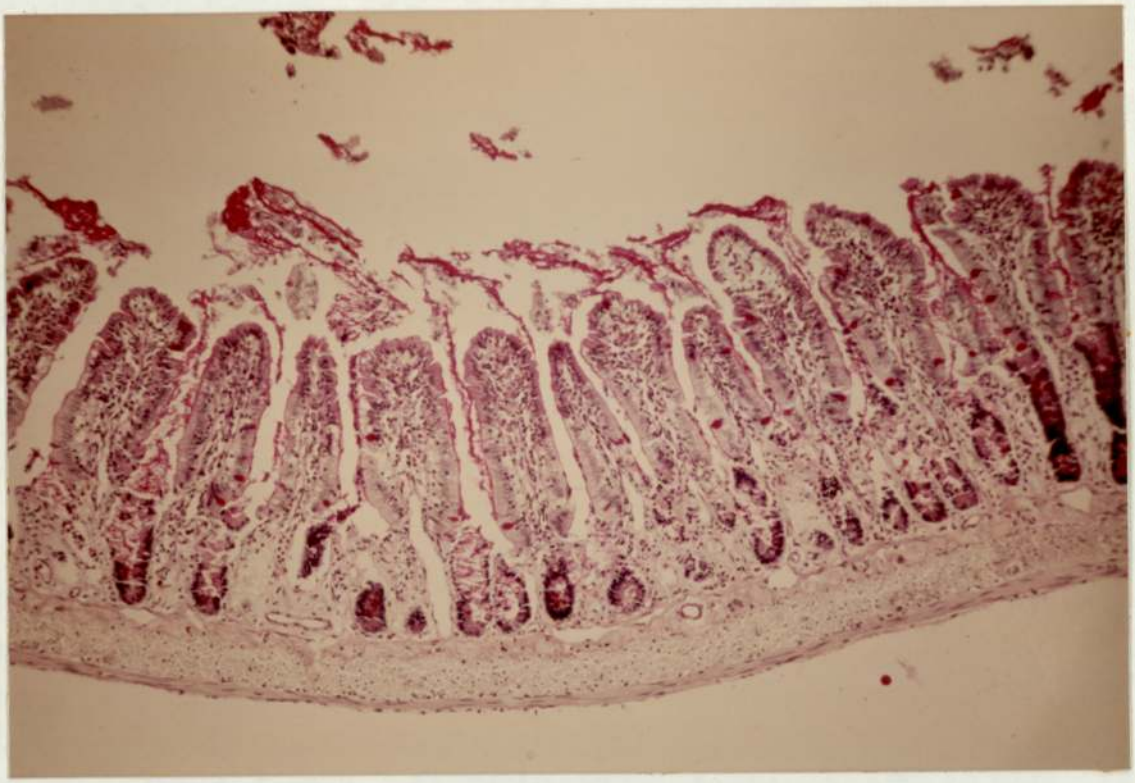
T.S. of mid-jejunal tissue after incubation for thirty minutes in Krebs-Hensleit bicarbonate buffer at 37°C. Stained with P.A.S.

Magnification x 25.

Plate 2(b).

T.S. of mid-jejunal tissue after incubation for thirty minutes in Krebs-Hensleit bicarbonate buffer at 37°C. Stained with H & E.

Magnification x 25.



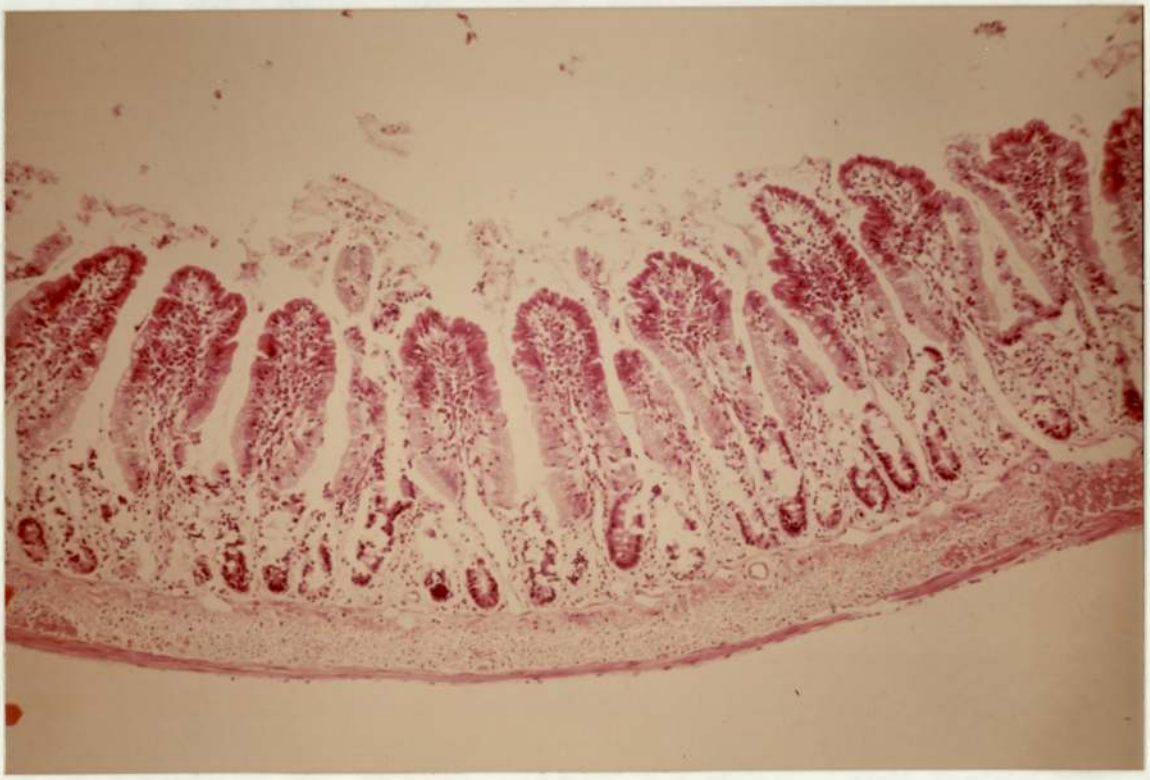


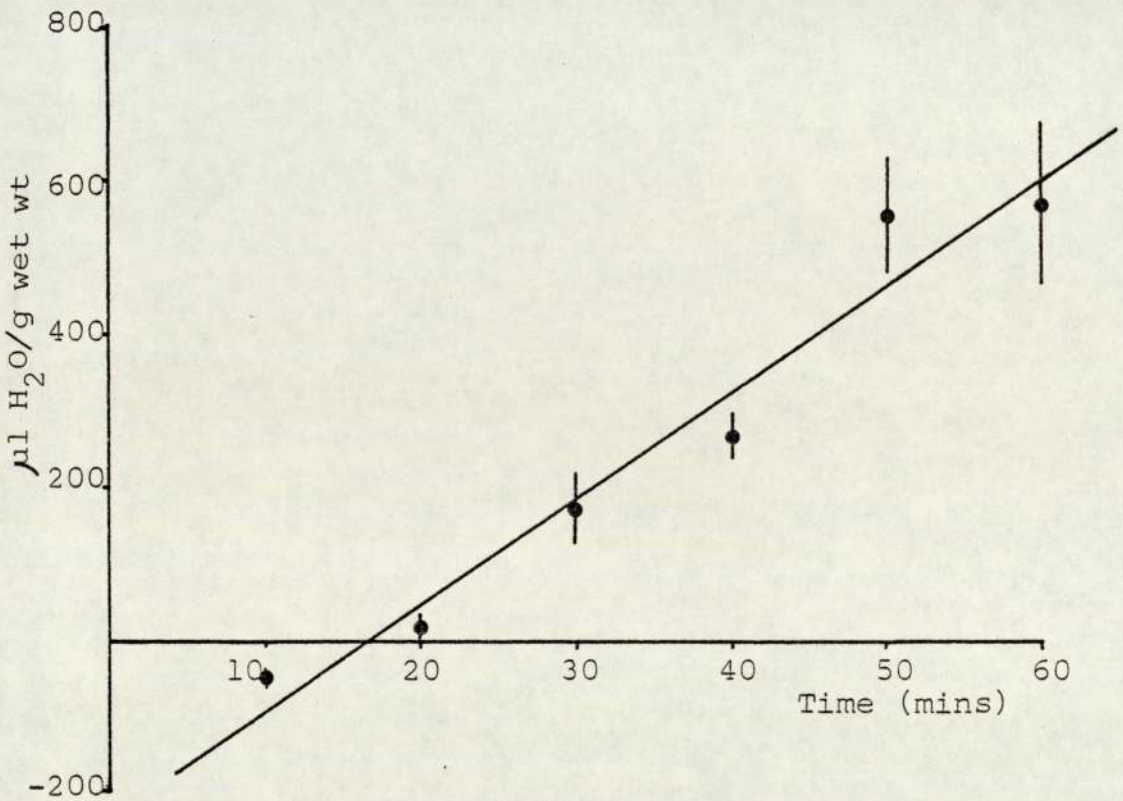
Table 3.2.

Transport of water by the everted sac over a period of sixty minutes, (μl H_2O/g wet wt).

Time (minutes)					
10	20	30	40	50	60
-49 $\pm$ 8	22 $\pm$ 16	181 $\pm$ 44	263 $\pm$ 28	551 $\pm$ 73	568 $\pm$ 105

Fig. 3.2.

Transport of water by the everted sac over a period of sixty minutes, (μl H_2O/g wet wt).



Each value is the mean $\pm$ S.E.M. of six experimental observations.

has been recognised for some years, and has been measured by many researchers in different laboratories. The measurements described in section 3.1.1. were stable, reproducible and comparable to those found by other experimenters, (Coleman, 1979; Porter,1982). For these reasons it was considered that the preparations could be regarded as viable over a 60 minute period.

The transport of glucose from the lumen of the gut into the epithelial cell is an active process, (Crane,1964), requiring the production of energy by the tissue preparation. A continuous energy supply can only be produced by a physiologically intact and functioning cell. The transport of glucose occurs against a concentration gradient, causing a higher concentration in the serosal fluid compared with the mucosal compartment. The serosal to mucosal ratio found in these studies was always greater than unity, and indicated a viable preparation capable of actively transporting glucose.

Diamond and Bossert, (1967), proposed a model for water movement in which sodium was actively pumped across the cell wall, causing an osmotic imbalance. The imbalance was then corrected by the flow of water into the cell. In this way, the flow of water across the gut wall is another indirect indication of active transport processes. The data reported demonstrate that water is transported into the serosal space at a rate of 13.8 µl/g wet wt/min, comparing

favourably with the results of Gardner, (1978), and Coogan, (1982).

Some mechanical damage to the everted sac would be expected during the incubation because of turbulence caused by gas bubbles in the incubation medium. Levine et al, (1970), criticised the everted sac technique, and reported that everted sac preparations showed noticeable disruption after only five minutes incubation, and 50-75% disintegration after thirty minutes. In the present studies, visual examination of histological preparations of everted sacs demonstrate that only a small percentage of the villi are adversely affected during a thirty minute period of incubation.

In conclusion, it may be said that the information obtained from measurements of potential difference, glucose and water transport and histological examination show that everted sacs prepared as described in section 2.5. are viable for at least sixty minutes, and that useful experimental data on the transport of lead cations may be obtained from them.

3.2. AN INVESTIGATION INTO THE CHARACTERISTICS OF LEAD TRANSPORT.

The flow of lead ions into and across the tissue of intestinal everted sac preparations has previously been measured by experimenters from different laboratories, (Cikrt,1970; Coleman,1979; Aungst and Fung,1981). Each have found a similar pattern of lead transport : a rapid uptake of the cation by the tissue followed by its gradual appearance in the serosal compartment.

The purpose of the present experiments was, (i) to confirm the rate at which lead was transported across tissue preparations and compare the results with those presented by previous researchers in this and other laboratories, (ii) to confirm that lead transport is a passive process, and,(iii) to gather information on the site of binding of the lead cation by the tissue. This last objective was achieved by using both tissue scrape preparations and preparations in which the apical glycocalyx was removed by the application of a solution of agar-agar.

3.2.1. The Transport of the Lead Cation as a Function of Time.

Everted sacs prepared as described in section 2.5. were incubated at $37^{o}C$ in Krebs-Hensleit bicarbonate buffer containing 20mM glucose and $10^{-6}M$ lead acetate labelled with ^{203}Pb for various time periods up to sixty minutes.

The serosal fluid was drained, and the radioactivity present in the tissue and fluid assessed. From this, the amount of lead present in each fraction was calculated.

The transport of lead into the serosal space was slow, (0.4 pmol/g wet wt/min), and linear with time, (r=0.9979). Extrapolation of the regression line to the 'y' axis produced an intercept above the origin. Over the sixty minute incubation period only 0.10-0.15% of the lead was transported across the everted sac preparation, (Table 3.3., Fig. 3.3(a).).

The uptake of lead cations by the tissue was of a greater order than the amount transported into the serosal compartment. The slope of the uptake curve was biphasic in character, (Fig. 3.3(b).). During the initial thirty minutes of incubation, there was a rapid uptake of the lead cation by the tissue, at a rate of 1.15 nmol/g wet wt/min, resulting in approximately 20% of the lead cations in the incubation medium being taken up by the tissue. The rate of lead uptake then slowed to 0.2 nmol/g wet wt/min. As a result of the slowed rate, only another 4% of the lead cations initially present in the incubation medium were taken up by the tissue in the following thirty minutes.

3.2.2. The Effect of a Metabolic Inhibitor on the Transport of Lead.

Everted sacs prepared as described in section 2.5. were incubated for thirty minutes at 37^{o}C in Krebs-Hensleit

Table 3.3.

The serosal transport and tissue uptake of the lead cation as a function of time.

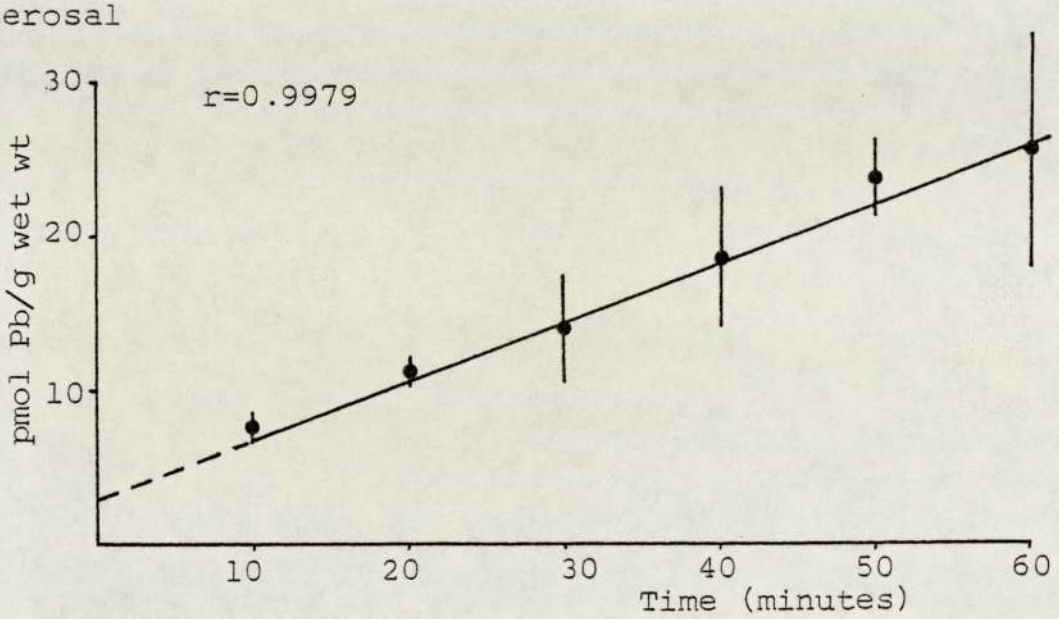
Time (mins)	Serosal (pmol/g wet wt)	Tissue (nmol/g wet wt)
10	7.46 ± 1.06	1.69 ± 0.34
20	10.95 ± 0.92	2.56 ± 0.45
30	13.79 ± 3.53	3.94 ± 0.73
40	18.54 ± 4.60	4.22 ± 0.94
50	21.25 ± 2.31	4.29 ± 0.72
60	25.50 ± 7.30	4.61 ± 1.25

Each value is the mean ± S.E.M. of six experimental observations.

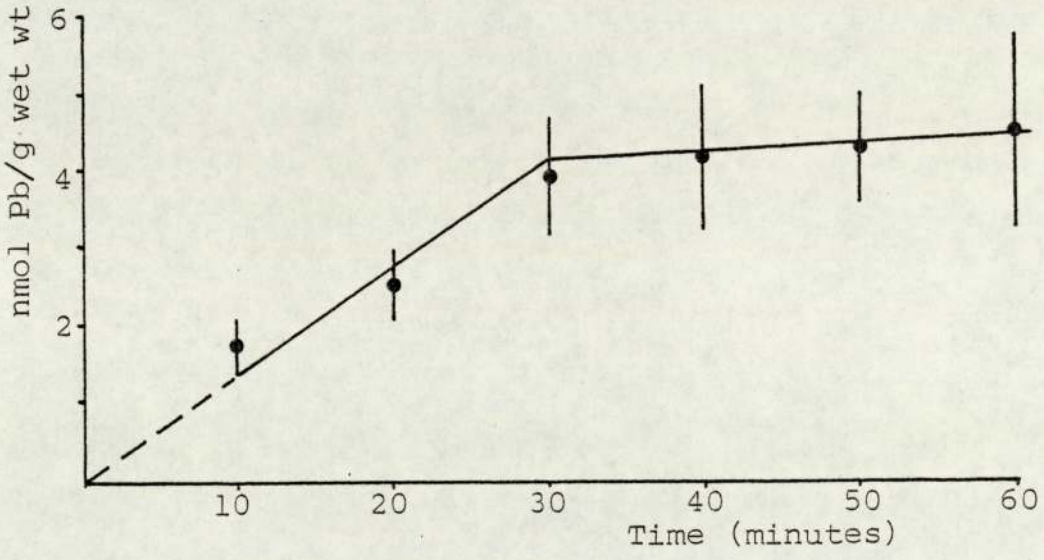
Fig. 3.3.

The serosal transport and tissue uptake of the lead cation as a function of time.

a) Serosal



b) Tissue



Each value is the mean ± S.E.M. of six experimental observations.

bicarbonate buffer containing 20mM glucose and 10^{-6}M lead acetate labelled with ^{203}Pb, and in the presence of 10^{-2}M sodium iodoacetate. A control incubation was carried out simultaneously, under the same conditions but excluding the sodium iodoacetate from the incubation medium.

Lead transport into the serosal compartment after the thirty minute incubation period was 19.9 $\pm$6.5 pmol/g wet wt in the absence of iodoacetate, and 19.0 $\pm$ 8.4 pmol/g wet wt in its presence. The tissue uptake of the lead cation was 5.93 $\pm$ 1.33 nmol/g wet wt in the former case, and 6.03 $\pm$ 1.75 nmol/g wet wt in the latter.

The data demonstrated that iodoacetate had no effect on either the serosal transport or the tissue uptake of the lead cation. The lack of effect was not due to any inefficiency of iodoacetate in suppresing metabolic activity, as water transport was significantly reduced ($p<0.001$) from 204 μl H_2O/g wet wt to -85 μl H_2O/g wet wt after the thirty minute period.

3.2.3. The Distribution of the Lead Cation between the Mucosal and Submucosal Layers.

Everted sacs prepared as described in section 2.5. were incubated at 37°C in Krebs-Hensleit bicarbonate buffer containing 20mM glucose and 10^{-6}M lead acetate labelled with ^{203}Pb for between ten and sixty minutes. After incubation the sacs were drained, and the intestinal villi scraped off

as described in section 2.6. The scraping process left only the muscle layer (Plate 3).

The mucosal and muscle layers were assessed for radioactivity separately in the N.E.8312 gamma counter, and the amount of lead present in each layer calculated by extrapolation from a standard curve. The results were expressed as a percentage of the total amount of lead found in the tissue preparation (mucosal plus muscle).

The mean distribution of the lead cation was found to be 14.33 $\pm$ 0.77% in the muscle layer and 85.67 $\pm$ 0.77% in the mucosa (Table 3.4.).

3.2.4. An Assessment of the Proportion of Tissue-Bound Lead Associated with the Apical Glycocalyx.

Ugolev *et al*, (1979), showed by histological and biochemical means that the apical glycocalyx can be removed by the application of a warm (40^{o}C) 3% solution of agar-agar. The method outlined by these investigators was used to assess the proportion of tissue-bound lead which was associated with the apical glycocalyx, and how the amount changes with time.

Rats dosed with 10^{-6}M lead acetate labelled with ^{203}Pb, in distilled water were sacrificed after periods of between ten minutes and twenty-four hours. The apical glycocalyx was removed from the mid-jejunal section of the small intestine using 3% agar-agar solution as described in

Plate 3(a).

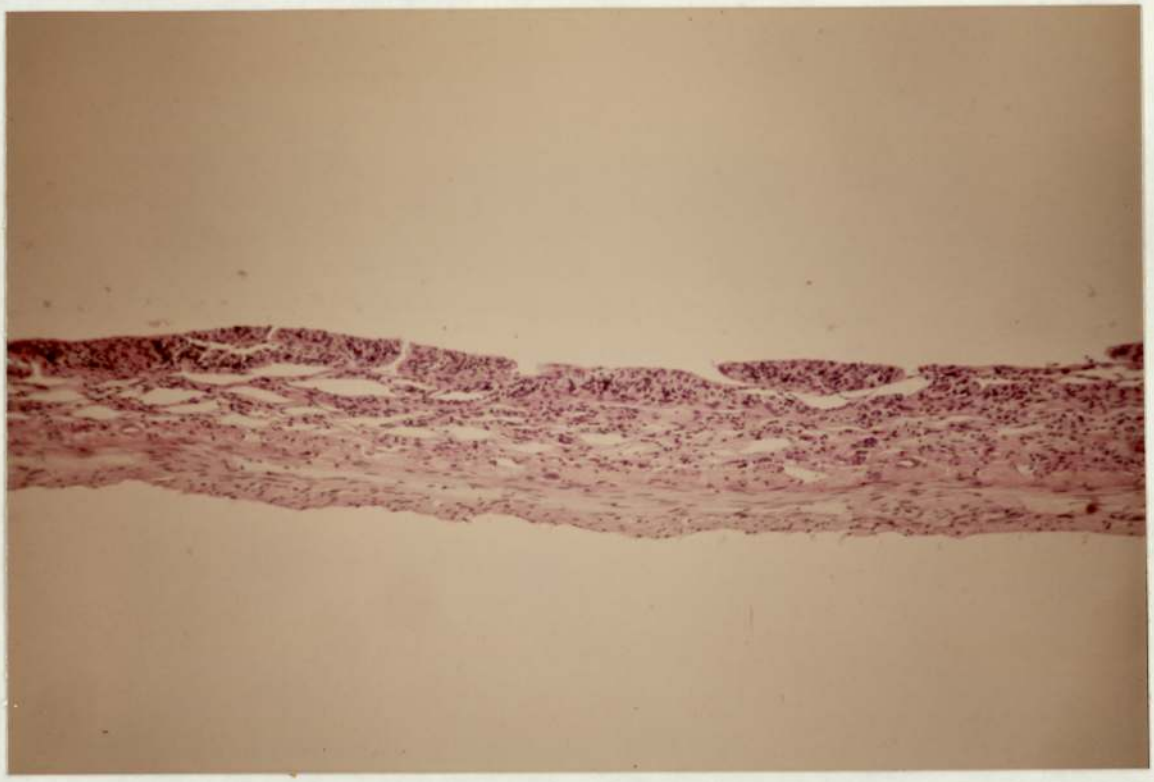
T.S. of mid-jejunal tissue after being scraped to remove the mucosal layer.
Stained with P.A.S.

Magnification x 100

Plate 3(b).

T.S. of mid-jejunal tissue after being scraped to remove the mucosal layer.
Stained with H & E.

Magnification x 100



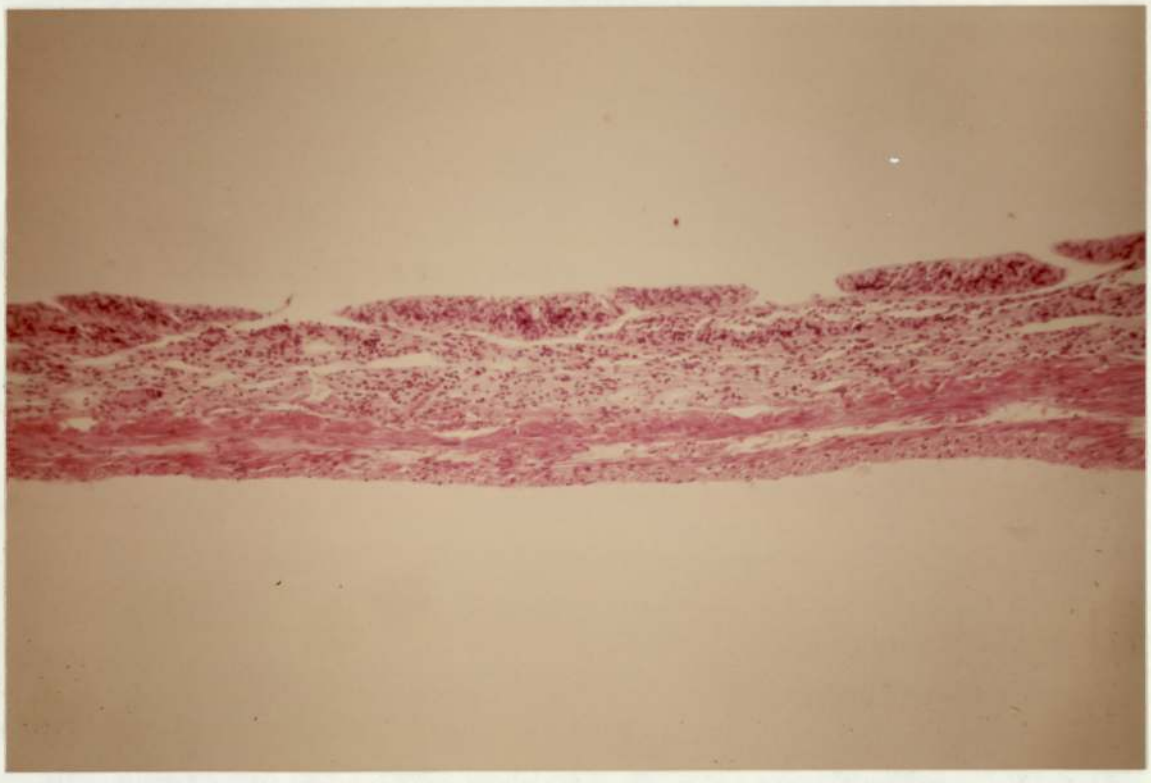


Table 3.4.

The distribution of the lead cation within the intestinal wall.

Time	Mean Percentage Distribution	
	Muscle Layer	Mucosa
10	12.33 ± 1.33	87.67 ± 1.33
20	17.33 ± 2.10	82.67 ± 2.10
30	17.33 ± 1.94	82.67 ± 1.94
40	16.50 ± 1.73	83.50 ± 1.73
50	10.50 ± 1.37	89.50 ± 1.37
60	10.00 ± 1.20	90.00 ± 1.20
Total	14.33 ± 0.77	86.67 ± 0.77

The value given at each time period is the mean ± S.E.M. of six experimental observations.

The 'total' values are the mean ± S.E.M. of thirty-six experimental observations.

section 2.11. The agar replicas and tissue were assessed for radioactivity and the proportion present in each fraction calculated.

The distribution of the lead cation between the agar and the tissue varied with the length of time between dosing and the sacrifice of the rat. In rats sacrificed ten minutes after dosing, approximately 43% of the lead present was associated with the apical glycocalyx, and 57% in the intestinal wall. However, the apical glycocalyx of rats sacrificed twenty-four hours after dosing contained only approximately 10% of the total lead found in the preparation, with 90% in the intestinal wall, (Tables 3.5(a). and 3.5(b).).

3.2.5. Discussion.

The experimental data indicate that the transport of lead into the serosal compartment is a slow, linear process, and are comparable to those reported by Coleman, (1979), Aungst and Fung, (1981), and Coogan, (1982). There appears to be a good correlation ($r=0.9682$, $p < 0.001$) between lead and water movement into the serosal compartment, (Table 3.6, Fig. 3.4.). Similar findings have been reported by Coleman, (1979), and Coogan, (1982).

Turnberg, (1978), suggested that the flow of water through the tight junctions carries small solutes with it by the process known as 'solvent drag'. It has been postulated that the tight junctions are lined with negatively

Table 3.5.

The distribution of the lead cation between the apical glycocalyx and the intestinal wall.

a) 10 - 60 minutes after dosing.

Time (mins)	Percentage Distribution	
	Apical Glycocalyx	Intestinal Wall
10	43.2 ± 11.6	56.8 ± 11.6
20	47.5 ± 8.0	52.5 ± 8.0
30	40.6 ± 4.9	59.4 ± 4.9
40	44.4 ± 5.1	55.6 ± 5.1
50	41.8 ± 6.3	58.2 ± 6.3
60	31.2 ± 5.1	68.8 ± 5.1

b) 1 - 24 hours after dosing.

Time (hrs)	Percentage Distribution	
	Apical Glycocalyx	Intestinal Wall
1	31.2 ± 5.1	68.8 ± 5.1
2	27.5 ± 2.5	72.5 ± 2.5
3	30.1 ± 1.7	69.9 ± 1.7
4	23.8 ± 2.0	76.2 ± 2.0
5	18.2 ± 2.4	81.8 ± 2.4
6	20.6 ± 2.4	79.4 ± 2.4
24	10.2 ± 0.8	89.8 ± 0.8

Each value is the mean ± S.E.M. of six experimental observations.

Table 3.6.

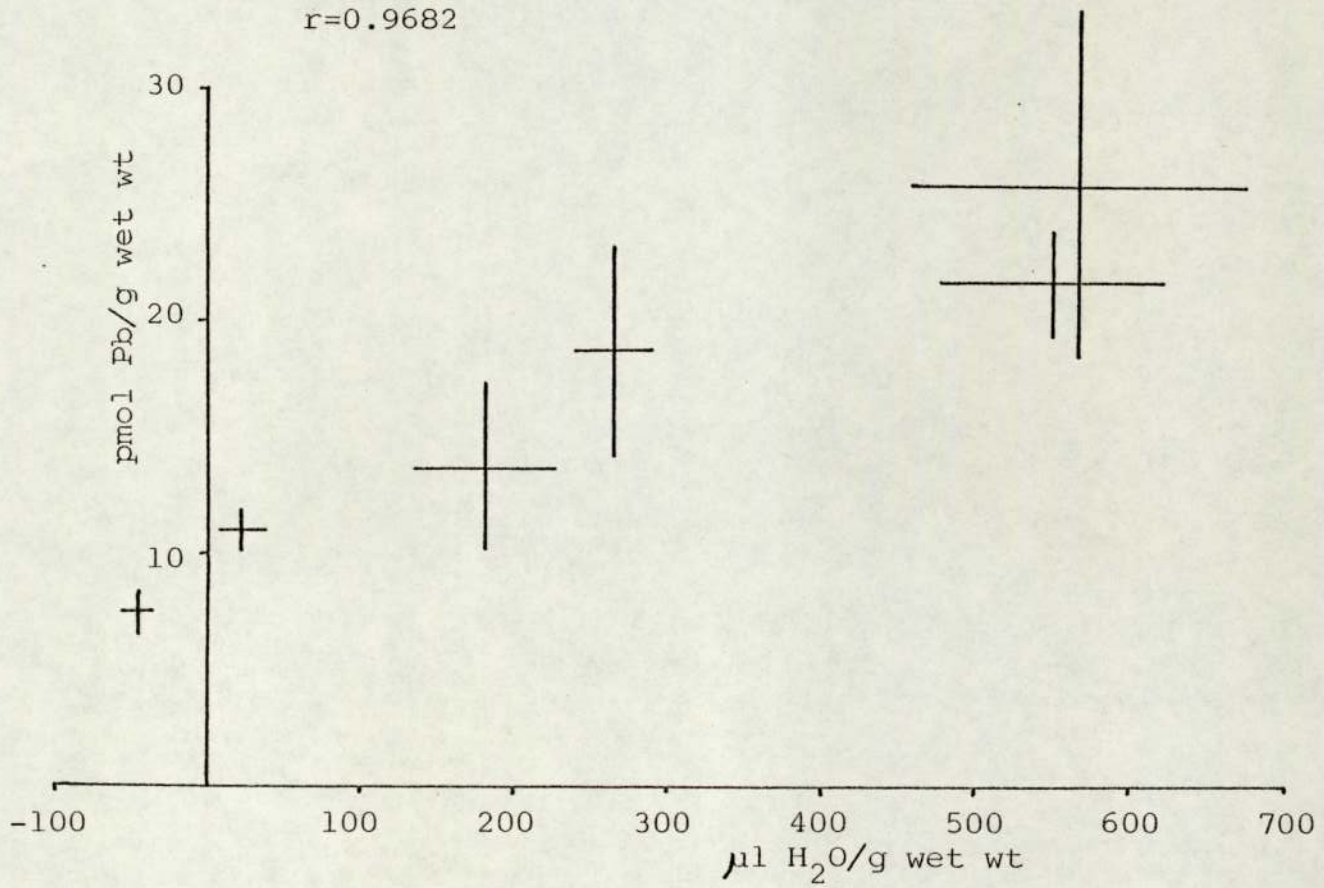
The correlation between the transport of lead and water by the everted sac, as a function of time.

Time (mins)	Pb Transport (pmol/g wet wt)	H_2O Movement (μl/g wet wt)
10	7.5 $\pm$ 1.1	-49 $\pm$ 8
20	11.0 $\pm$ 0.9	22 $\pm$ 16
30	13.8 $\pm$ 3.5	181 $\pm$ 45
40	18.5 $\pm$ 4.6	263 $\pm$ 28
50	21.3 $\pm$ 2.3	551 $\pm$ 73
60	25.5 $\pm$ 7.3	568 $\pm$ 106

Each value is the mean $\pm$ S.E.M. of six experimental observations.

Fig. 3.4.

The correlation between the transport of water and lead cations.



Each value is the mean ± S.E.M. of six experimental observations.

charged groups, and positively charged ions, including Pb^{2+}, could be transferred by this means. If so, this would explain the correlation found.

The process of 'solvent drag' cannot be the only method by which lead crosses the intestinal wall. During incubation in the presence of the metabolic inhibitor sodium iodoacetate, water transport was reversed, yet the transport of lead was unaffected. Therefore, the correlation between lead and water transport was probably an artefact of the experiment, or may indicate a causal relationship between the movement of lead cations and water.

There is some dispute as to whether lead transport is an active or a passive process. If it is an active process, it would require a carrier mechanism, which would become saturated. No evidence of saturation has been found by Coleman (1979) or Coogan (1982). Aungst and Fung,(1981), and Mykkänen and Wasserman,(1981), disagree, claiming to show at least partial saturation of lead transport at lead concentrations over 10^{-5}M. The same investigators also report a reduction in lead transport in the presence of metabolic inhibitors and in anoxic conditions. The experimental data presented in this thesis do not support these observations, and no evidence of an active transport system was found.

The tissue uptake of the lead cation was much faster than serosal transport, and progressed in two distinct

phases; initially very rapid, over the first thirty minutes, and then at a slower rate. Other experimenters have reported broadly similar results, although differing in detail. Coleman,(1979), found the rapid uptake phase lasted twenty minutes, with tissue-lead figures remaining constant thereafter, while Coogan,(1982), reported that the initial phase lasted ten minutes, with a slower accumulation after this. Mykkänen and Wasserman,(1981), working with isolated intestinal loops, claimed an even shorter initial period (only five minutes), with tissue concentrations remaining constant after this. These differences are probably due to a variation in experimental technique. The initial fast phase may be due to a rapid binding of the lead cation by the tissue. Coleman et al,(1978), suggested a surface adsorption effect, possibly with phosphate ions at the mucosal surface binding the lead cations.

The theory of surface adsorption was tested using mucosal scrapes and agar application. Data from the scrape experiments showed that the lead in the tissue was predominantly at, or near, the surface of the preparation, although a small proportion was present in the muscle layer.

Lead cations must travel through the muscle to reach the serosal space. The cations are thought to percolate through the ruptured blood and lymph vessels. During this process, lead cations may become bound to the walls of these vessels, so giving rise to the lead found in the muscle region.

The results obtained from the scrape experiments are comparable to those found by other workers for lead (Coogan, 1982), and cadmium distribution, (Porter,1982).

The proportion of tissue-bound lead associated with the apical glycocalyx was measured by using the method of Ugolev et al,(1979). These researchers have shown histologically that only the glycocalyx at the tips of the microvilli is removed after application of agar-agar gel. The percentage of tissue-bound lead removed by this method varied. When the rat was sacrificed ten minutes after being dosed with lead, 43% of the tissue-bound lead was removed by the agar-agar gel, whereas 10% was removed twenty-four hours after dosing. The proportion of the initial dose found associated with the intestinal tissue also varied with time, reaching a maximum after three hours and falling to approximately 1.5% of the maximum twenty-four hours after dosing.

From these data it can be seen that a significant proportion of the lead bound to the tissue immediately after its exposure to the lead cation is found associated with the apical glycocalyx. The lead cations appear to penetrate the preparation, but the distribution of the lead within the tissue is not clear.

It may be concluded from the experimental results that the transport of lead by the intestinal tissue is a slow, passive, linear process, and that tissue uptake is a

result of rapid binding of the lead cation onto the surface followed by a gradual movement across the preparation. The results are in agreement with the model put forward by Coleman,(1979), and contradict theories put forward by other authors who suggest an active transport mechanism for lead cations.

CHAPTER FOUR

AN ASSESSMENT OF THE ABSORPTION OF LEAD IN THE FORM OF A CHELATED COMPLEX

AND

AN INVESTIGATION INTO THE TISSUE BINDING OF LEAD CATIONS AND LEAD-EDTA.

4.1. AN ASSESSMENT OF THE ABSORPTION OF LEAD IN THE FORM OF A CHELATED COMPLEX.

It is probable that lead which is ingested as part of the diet is not fully ionised after passage through the stomach. Foodstuffs contain a number of chelating groups which can bind the cation, eg. citrate, ascorbate and various amino acids such as histidine and glycine. These dietary components would change the nature of the lead from a relatively small, positively charged hydrated species to a larger complex, either charged or uncharged, which is likely to be more lipid soluble. The transport characteristics of the complex may be different from those of the lead cation. Therefore, it is necessary to study the transport of the chelated lead species and compare it to that of the lead cation.

The presence of both the ionic and chelated lead species would complicate the interpretation of the results. Therefore, disodium calcium ethylenediaminetetraacetic acid (EDTA), which has a high dissociation constant with lead, was used in these studies.

4.1.1. The Effect of a Chelating Agent on the Serosal Transport and Tissue Uptake of Lead.

Everted intestinal sacs prepared as described in section 2.5. were incubated at $37^{o}C$ in Krebs-Hensleit bicarbonate buffer containing 20mM glucose and $10^{-6}M$ lead

acetate labelled with ^{203}Pb, and gassed with $95\%O_2:5\%CO_2$, under the following conditions: (i) for thirty minutes in the presence of various concentrations of disodium calcium EDTA ($10^{-6}M$ to $5x10^{-5}M$); (ii) for various periods up to one hour in the presence of $10^{-5}M$ disodium calcium EDTA; and (iii) for thirty minutes in the presence of $10^{-5}M$ disodium calcium EDTA and $10^{-2}M$ sodium iodoacetate, with $10^{-5}M$ disodium calcium EDTA and no iodoacetate present in the control medium. Tissue uptake and serosal transfer of lead were assessed.

From Table 4.1. and Figure 4.1. it can be seen that during the thirty minute incubation period in the presence of equimolar EDTA, the serosal transport of lead was significantly raised ($p<0.05$) to a level of 26.9 ± 3.5 pmol/gram wet weight of tissue. Further increase in concentration of EDTA resulted in a greater transport of lead, though no overall significant difference was found between the higher EDTA concentrations. Tissue uptake of lead was significantly reduced ($p<0.01$) in the presence of equimolar EDTA, and did not change significantly with increasing concentrations of EDTA.

The serosal transport of the lead-EDTA complex was linear with time ($r=0.9944$, $p<0.001$) at a rate of 0.7 pmol/gram wet weight tissue/min, approximately twice that of lead cation transport, (Table 4.2(a).,Fig.4.2(a).). The tissue uptake was also linear ($r=0.9339$, $p<0.01$) at a rate of 0.02 nmol/gram wet weight tissue/min, (Table 4.2(b).,Fig.4.2(b).). There was no evidence of the initial rapid accumulation seen

Table 4.1.

The effect of chelation with various concentrations of EDTA on the transport and tissue uptake of 10^{-6}M lead acetate.

a) Serosal Transport

EDTA concentration	0	10^{-6}M	5 x 10^{-6}M	10^{-5}M	5 x 10^{-5}M
Serosal transport (pmol/g wet wt)	13.8 ± 3.5	26.9* ± 3.5	38.5*** ± 3.8	32.2** ± 5.2	38.8** ± 5.9

b) Tissue Uptake

EDTA concentration	0	10^{-6}M	5 x 10^{-6}M	10^{-5}M	5 x 10^{-5}M
Tissue uptake (nmol/g wet wt)	3.9 ± 0.7	1.2** ± 0.2	0.9** ± 0.2	1.3** ± 0.2	0.9** ± 0.3

Each value is the mean ± S.E.M. of six experimental observations.

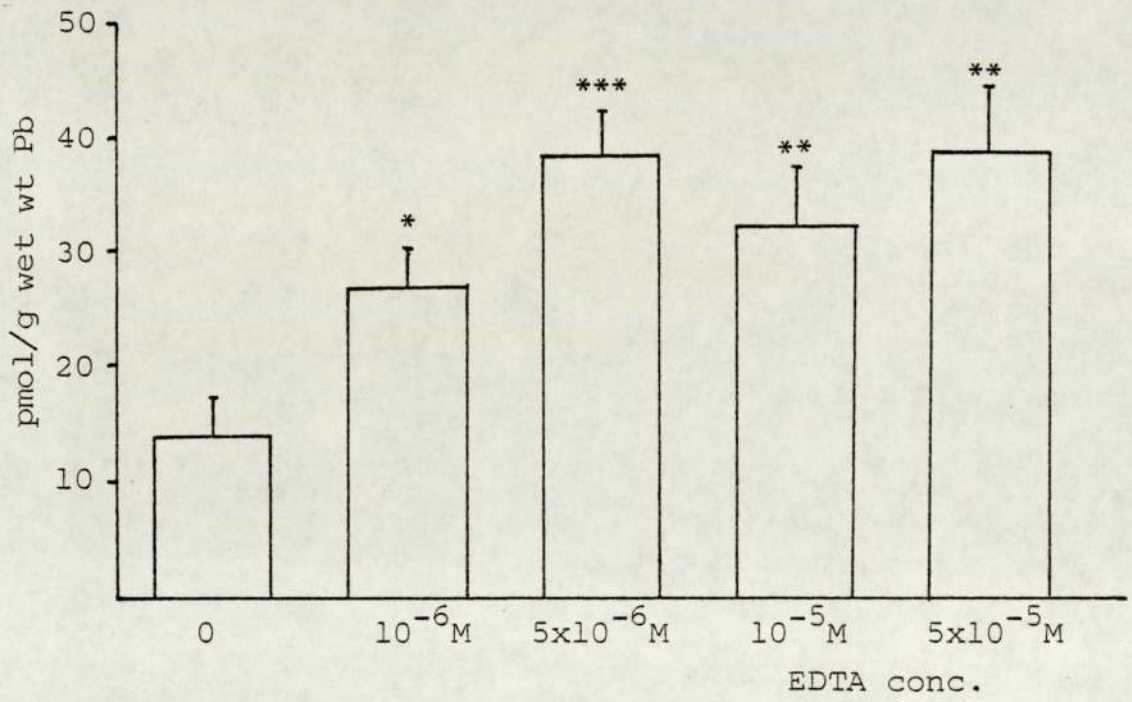
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Fig. 4.1.

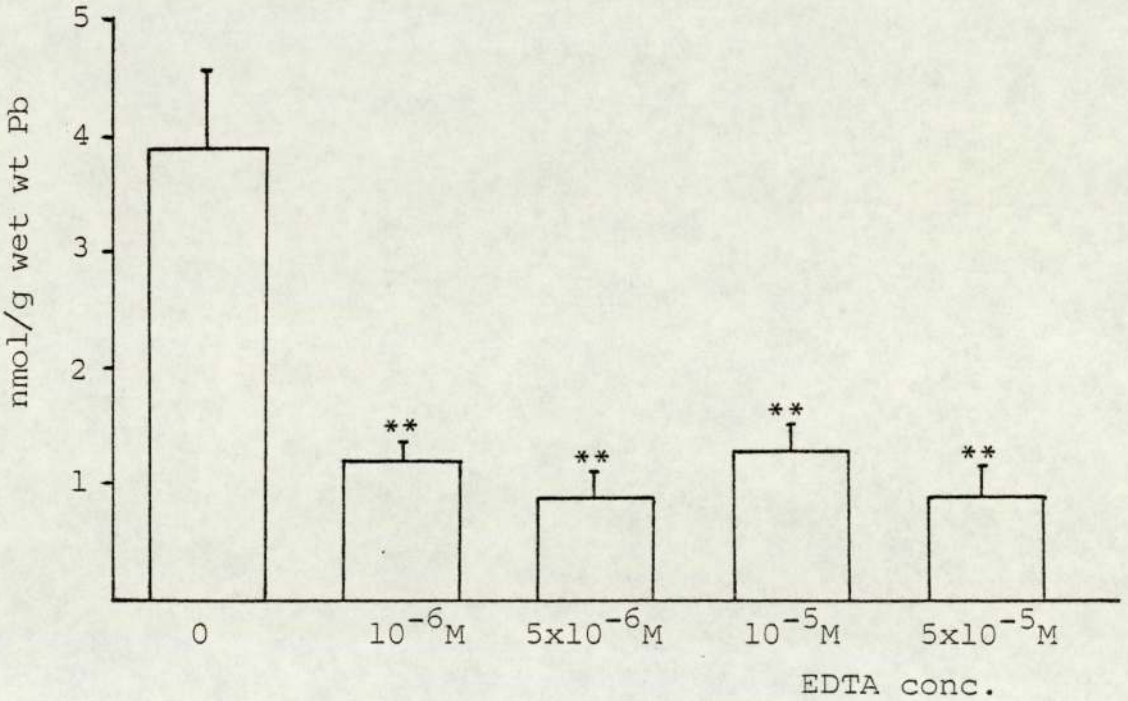
The effect of chelation with various concentrations of EDTA on the transport and tissue uptake of 10^{-6}M lead acetate.

* p 0.05 ** p 0.01 *** p 0.001

a) Serosal



b) Tissue



Each value is the mean ± S.E.M. of six experimental observations.

Table 4.2.

The transport and tissue uptake of the Pb-EDTA complex as a function of time.

a) Serosal

Time (mins)	10	20	30	40	50	60
Transport (pmol/g wet wt)	17.96 ± 6.57	24.22 ± 4.57	32.15 ± 5.16	41.58 ± 7.56	46.70 ± 9.26	51.55 ± 11.00

b) Tissue

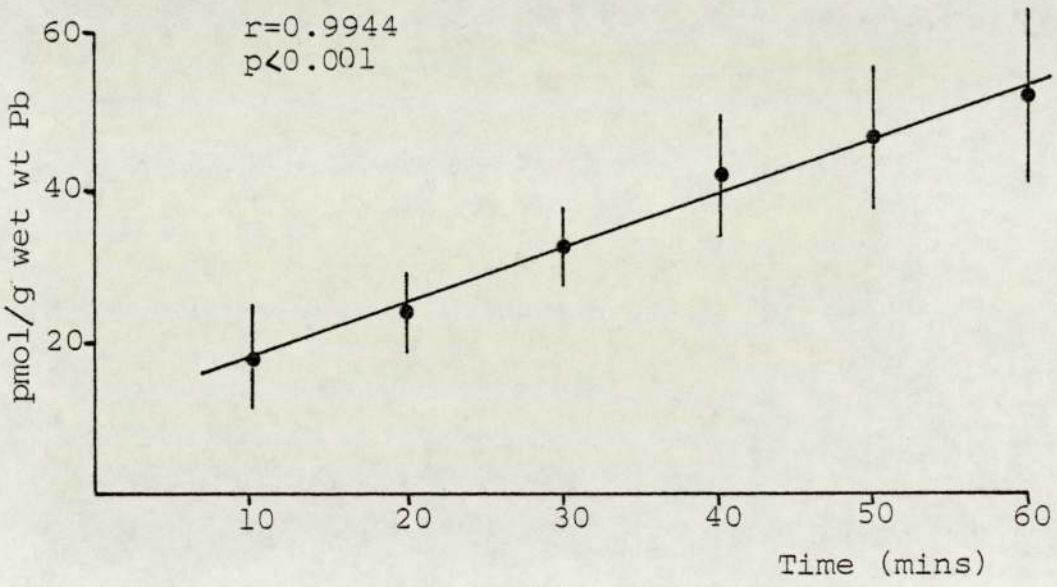
Time (mins)	10	20	30	40	50	60
Uptake (nmol/g wet wt)	0.74 ± 0.10	1.17 ± 0.25	1.30 ± 0.21	1.37 ± 0.17	1.91 ± 0.36	1.74 ± 0.41

Each value is the mean ± S.E.M. of six experimental observations.

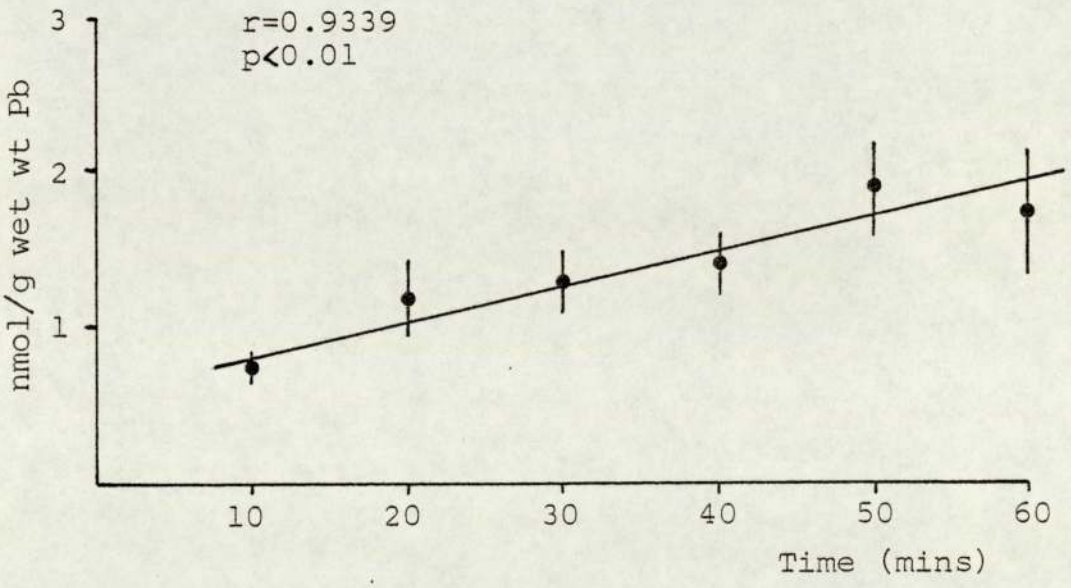
Fig. 4.2.

The transport and tissue uptake of the Pb-EDTA complex as a function of time.

a) Serosal



b) Tissue



Each value is the mean ± S.E.M. of six experimental observations.

with the lead cation alone, and no consequent slowing of tissue uptake. The lead-EDTA associated with the tissue was approximately half that found with lead cations.

The transport of lead-EDTA into the serosal space was not significantly affected by the presence of iodoacetate (Table 4.3(a).). Neither can a significant effect be found on the tissue uptake of lead-EDTA.

Water transport was unaffected by the presence of disodium calcium EDTA in the incubation media. However, the presence of iodoacetate resulted in significantly decreased water movement both into the serosal and tissue compartments (Table 4.3(b).).

4.1.2. The Transport and Tissue Uptake of Various Concentrations of the Lead-EDTA Complex.

Everted sacs prepared as described in section 2.5. were incubated for thirty minutes in concentrations of lead acetate ranging from 10^{-6}M to 10^{-1}M in Tris(hydroxymethane)-aminomethane buffer. A tenfold excess of disodium calcium EDTA was present in each case. The incubations were carried out at 37°C, and gassed with 100% O_2. The solutions were labelled with ^{203}Pb, and the tissue uptake and serosal transfer were assessed.

The appearance of the lead-EDTA complex in the serosal compartment, and that taken up by the tissue were proportional

Table 4.3.

The effect of 10^{-2}M sodium iodoacetate on the transport and tissue uptake of Pb-EDTA and water.

a) Pb-EDTA

	Serosal (pmol/g wet wt)	Tissue (nmol/g wet wt)
Control	37.9 ± 11.8	3.0 ± 0.3
10^{-2}M Na Iodoacetate	32.5 ± 7.7	3.6 ± 1.0

b) Water

	Serosal (µl/g wet wt)	Tissue (µl/g wet wt)
Control	204 ± 59	639 ± 40
10^{-2}M Na Iodoacetate	-80*** ± 10	203*** ± 33

Each value is the mean ± S.E.M. of six experimental observations.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

to its concentration in the incubation media (Table 4.4., Fig. 4.3.). No evidence of saturation was found over the concentration range studied.

4.1.3. Discussion.

A number of chelating agents have been shown to enhance lead absorption both in in vitro intestinal preparations, (Coleman,1979; Coogan,1982), and in intact animals, (Jugo et al,1975; Garber and Wei,1974). However, little is known about the mechanism by which chelated species are transported across the intestinal wall.

The present data show a significantly elevated serosal transport of the lead-EDTA complex compared to that of lead cations, possibly due to its greater lipid solubility. There is also a decrease in the amount of lead associated with the tissue. The lead-EDTA complex has a net negative charge (Fig. 4.4.). The readiness of the lead to bind to the negatively charged tissue surface would consequently be reduced, and a greater proportion of the lead in the initial incubation medium would be available for transport. This may also contribute to the increase in serosal transfer of lead when in the chelated form.

The rise in serosal transport as EDTA concentration increased may be due to a greater proportion of the lead in solution being chelated. Although the dissociation constant for lead and EDTA is high (approximately 18 at pH7), other

Table 4.4.

The transport and tissue uptake of various concentrations of the Pb-EDTA complex after incubation for thirty minutes in Tris buffer.

a) Serosal

Pb-EDTA Concentration	10^{-6}M	10^{-5}M	10^{-4}M	10^{-3}M	10^{-2}M	10^{-1}M
Transport (pmol/gwet wt)	74.8 ± 20.3	93×10^{1} ± 27×10^{1}	103×10^{2} ± 13×10^{2}	111×10^{3} ± 15×10^{3}	168×10^{4} ± 10×10^{4}	141×10^{5} ± 35×10^{5}

b) Tissue

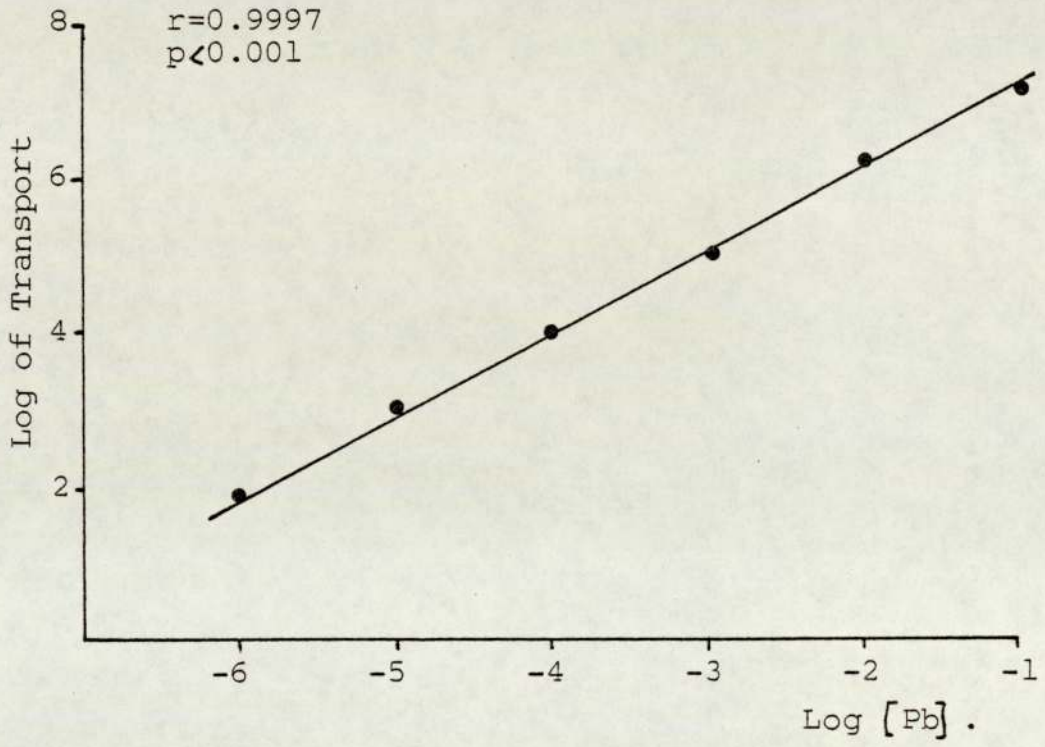
Pb-EDTA Concentration	10^{-6}M	10^{-5}M	10^{-4}M	10^{-3}M	10^{-2}M	10^{-1}M
Uptake (nmol/g wet wt)	0.8 ± 0.2	0.4×10^{1} ± 0.1×10^{1}	0.9×10^{2} ± 0.1×10^{2}	0.7×10^{3} ± 0.1×10^{3}	0.6×10^{4} ± 0.2×10^{4}	0.6×10^{5} ± 0.1×10^{5}

Each value is the mean ± S.E.M. of six experimental observations.

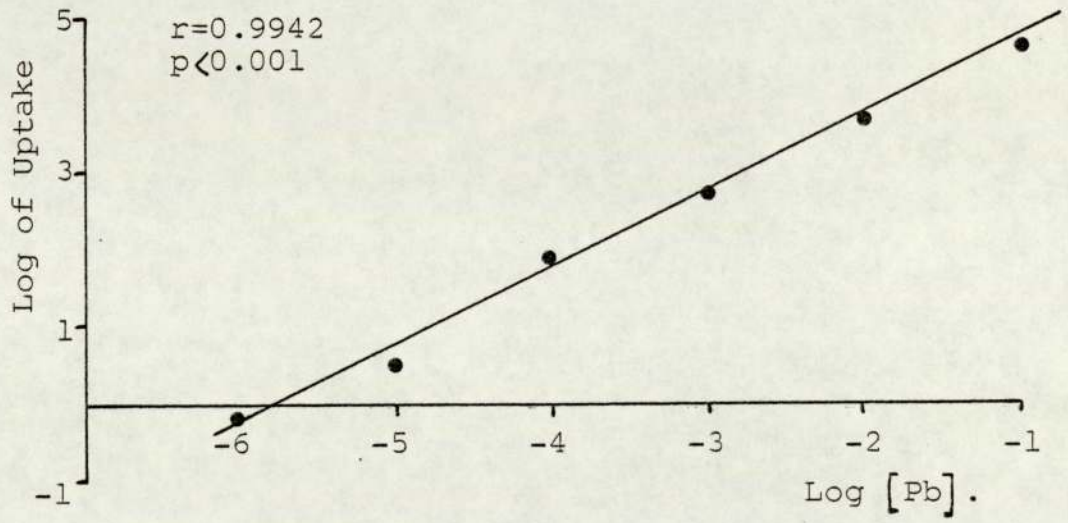
Fig. 4.3.

The transport and tissue uptake of various concentrations of the Pb-EDTA complex after incubation for thirty minutes in Tris buffer.

a) Serosal



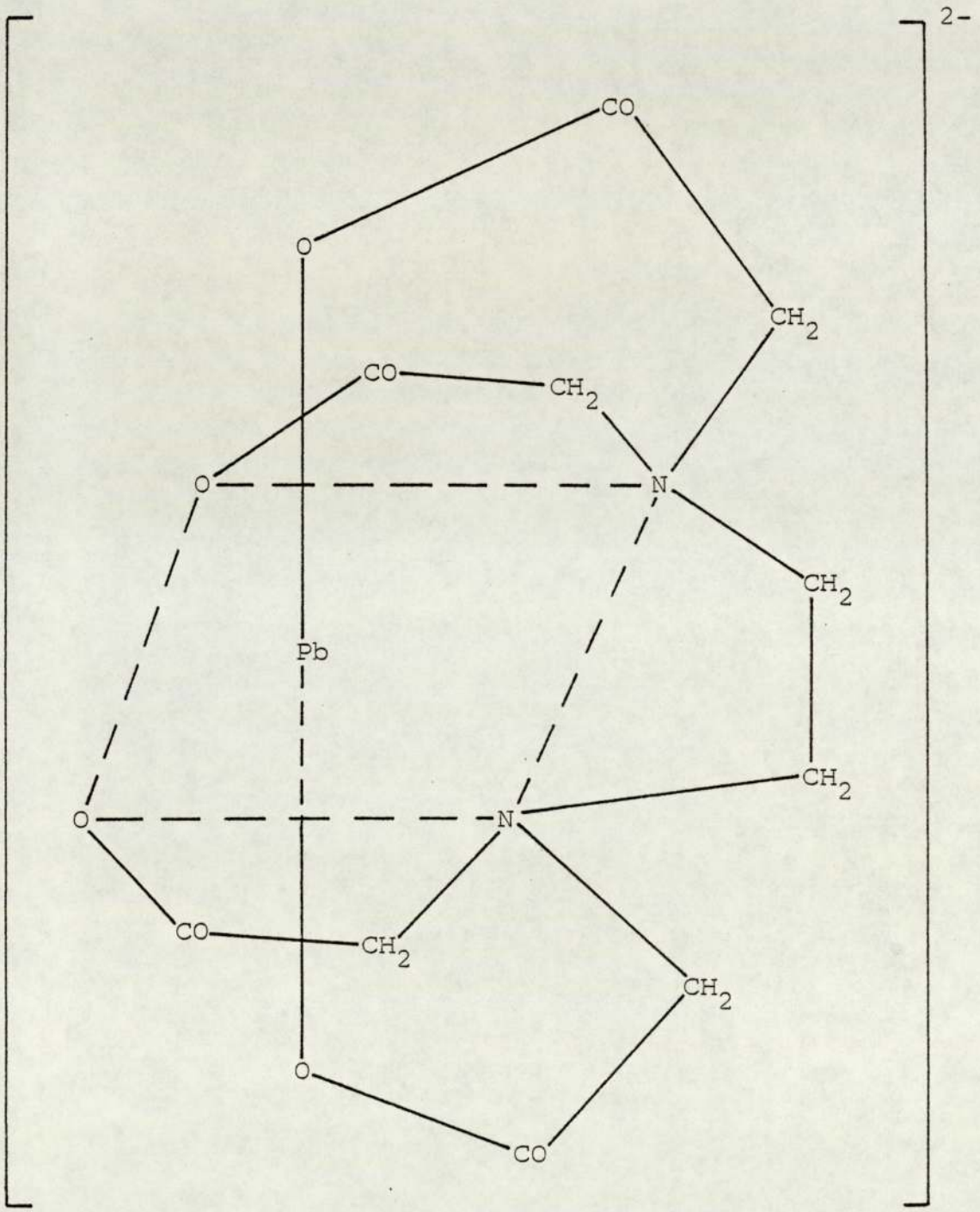
b) Tissue



Each value is the mean ± S.E.M. of six experimental observations.

Fig. 4.4.

The structural formula of the lead-EDTA complex.



cations may compete for binding sites. From the results presented, a tenfold excess of disodium calcium EDTA over lead appears to ensure maximum chelation of lead cations.

The same proportion of the lead-EDTA complex was transported into the serosal space, even with a hundred-thousand-fold increase in its concentration in the incubation medium, a fact which was also true of tissue uptake. The result points to a transport mechanism of the lead-EDTA complex which does not involve a carrier, as no evidence of saturation can be seen. Therefore, the transport process is passive.

The theory that transport is due to a passive mechanism is supported by the fact that 10^{-2}M sodium iodoacetate has no effect on either the serosal transport or the tissue uptake of lead-EDTA. Iodoacetate is a metabolic inhibitor, and its activity in this experiment is shown by the severe depression of water movement. Although the movement of water across the intestinal wall is a passive process, it relies on an increase in osmotic pressure caused by the active transport of a number of solutes, therefore it would be decreased if active transport was adversely affected.

It can be seen that the transport of the lead-EDTA complex is not dependent on water transport. Therefore, it cannot be said that lead-EDTA enters the body due to "solvent drag".

4.2. AN INVESTIGATION INTO THE TISSUE BINDING OF LEAD CATIONS AND LEAD-EDTA.

The uptake of the lead cation by the everted sac tissue is thought to be a surface adsorption effect (Coleman, 1979). It has further been suggested, by the same author, that the lead cations are covalently bound by phosphate groups on the surface of the epithelial membrane, in the form $Pb_3(PO_4)_2$. However, no experimentation has been carried out to determine if phosphate groups, or any other, have an effect on the surface adsorption of the lead cation onto the tissue.

The purpose of the experiments outlined in this section is to find factors which affect the tissue binding of lead cations, and to suggest the mechanism, or mechanisms, by which they are sequestered by the tissue.

The epithelial cells of the villi have an outer coating, known as the glycocalyx, on the luminal side. The glycocalyx is composed of glycoproteins - long chain polysaccharide molecules attached to the epithelial wall by protein filaments (Clamp et al,1978). Over the glycocalyx there is a loosely adhering coat of mucus, produced by the goblet cells on the villi, as protection from extremes of pH and the abrasive action of food residue in the lumen of the gastrointestinal tract. The mucus also contains carbohydrate-rich glycoproteins (Creeth,1978).

A number of techniques have been used to decrease the viscosity of mucous *in vitro*. Similar techniques have been employed in the following experiments in an effort to reduce the amount of glycoprotein present at the surface of the intestinal preparation. The agents used were :
(i) guanidinium chloride, a chaotrophe which denatures the protein component of the glycoprotein; (ii) N-acetyl-L-cysteine, a thiol-containing reagent which has been demonstrated to reduce the viscosity of pig gastric mucin (Sheffner,1963), by splitting disulphide bonds; and (iii) proteolytic enzymes, which cause the partial breakdown of the glycoprotein chain.

In addition to these experiments, the effect of ethanol on the transport and tissue uptake of both lead cations and lead-EDTA was studied. Ethanol has been reported to reduce the absorption of lead from isolated intestinal loops (Barton and Conrad,1978). However, the experiment was carried out in the presence of 50% ethanol (v/v). This level damages the intestinal mucosa (Beck and Dinda,1981). Therefore, the result must be viewed with some caution. The experiment presented in this section used concentrations of ethanol which may reasonably be expected to occur in the small intestine of a man drinking moderate amounts of spirits on an empty stomach.

4.2.1. The Effect of Guanidinium Chloride and N-Acetyl-L-Cysteine on the Serosal Transport and Tissue Uptake of Lead Cations.

Everted sacs prepared as described in section 2.5. were pre-incubated at 37°C for fifteen minutes in Krebs-Hensleit bicarbonate buffer containing 20mM glucose and either (i) guanidinium chloride at concentrations of 10^{-2}M or 1M, or (ii) N-acetyl-L-cysteine at a concentration of 10^{-2}M. After pre-incubation the sacs were incubated for thirty minutes in Krebs-Hensleit bicarbonate buffer containing 20mM glucose and 10^{-6}M lead acetate labelled with ^{203}Pb. The incubations were gassed with 95%O_2:5%CO_2. The serosal fluid and tissue were assessed for their lead content.

Pre-incubation in 10^{-2}M guanidinium chloride had no significant effect on either serosal transport or tissue uptake of lead cations (Table 4.5(a).). However, a significant reduction in tissue uptake was found in sacs pre-incubated in 1M guanidinium chloride. Water transport, though unaffected after pre-incubation in 10^{-2}M guanidinium chloride, was severely reduced after exposure to the molar solution (Table 4.5(b).). Visual examination of the sacs showed that unacceptable damage had occurred to the preparations, and therefore the result should be viewed with caution.

No significant difference was seen in either the serosal transport or the tissue uptake of lead cations after pre-incubation in 10^{-2}M N-acetyl-L-cysteine (Table 4.6(a).).

Table 4.5.

The effect of 10^{-2}M and 1M guanidinium chloride on the transport and tissue uptake of the lead cation.

a) Lead

	Serosal (pmol/g wet wt)	Tissue (nmol/g wet wt)
Control	20.3 ± 6.0	1.41 ± 0.18
10^{-2}M Guanidinium chloride	15.1 ± 4.8	1.10 ± 0.23
1M Guanidinium chloride	9.9 ± 3.2	0.86* ± 0.17

b) Water

	Serosal (µl/g wet wt)	Tissue (µl/g wet wt)
Control	433 ± 83	885 ± 49
10^{-2}M Guanidinium chloride	417 ± 198	899 ± 38
1M Guanidinium chloride	-151*** ± 17	1*** ± 94

Each value is the mean ± S.E.M. of six experimental observations.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 4.6.

The effect of N-acetyl-L-cysteine (NAC) on the transport and tissue uptake of the lead cation.

a) Lead.

	Serosal (pmol/g wet wt)	Tissue (nmol/g wet wt)
Control	17.8 ± 2.5	2.54 ± 0.11
10^{-2}M NAC	24.1 ± 4.5	1.85 ± 0.27

b) Water.

	Serosal (µl/g wet wt)	Tissue (µl/g wet wt)
Control	454 ± 186	725 ± 71
10^{-2}M NAC	466 ± 59	721 ± 52

Each value is the mean ± S.E.M. of six experimental observations.

Water transport was also unaffected (Table 4.6(b).).

4.2.2. The Effect of Proteolytic Enzymes on the Serosal Transport and Tissue Uptake of Lead Cations.

Everted sacs prepared as described in section 2.5. were pre-incubated at 37°C for fifteen minutes in Krebs-Hensleit bicarbonate buffer containing 20mM glucose and either (i) trypsin at concentrations ranging from 1.2 mg/ml to 10 mg/ml; or (ii) chymotrypsin at concentrations of 5 mg/ml or 10 mg/ml. After pre-incubation, the sacs were incubated for thirty minutes at 37°C in Krebs-Hensleit bicarbonate buffer containing 20mM glucose and 10^{-6}M lead acetate labelled with ^{203}Pb. Incubations were gassed with $95\%O_2:5\%CO_2$.

The tissue uptake of lead was not significantly affected after pre-incubation in trypsin at a concentration of 1.2 mg/ml. At higher concentrations, however, the lead associated with the tissue preparation was significantly reduced ($p<0.01$ for 2.4 mg/ml trypsin, $p<0.001$ for 5 mg/ml and 10 mg/ml trypsin), (Table 4.7(a).). The serosal transport of lead was not significantly altered after pre-incubation in 1.2 mg/ml, 5 mg/ml or 10 mg/ml trypsin, but was reduced ($p<0.05$) with the use of 2.4 mg/ml trypsin.

Pre-incubation in chymotrypsin solutions caused a significant decrease in the tissue uptake of lead, both at 5 mg/ml ($p<0.01$) and 10 mg/ml ($p<0.001$). Serosal transport

Table 4.7.

The effect of proteolytic enzymes on the transport and tissue uptake of the lead cation.

a) Trypsin

Enzyme Conc.	Serosal (pmol/g wet wt)	Tissue (nmol/g wet wt)
Control	16.9 ± 2.2	2.91 ± 0.32
1.2 mg/ml	15.3 ± 2.0	2.39 ± 0.33
2.4 mg/ml	10.7* ± 1.5	1.56** ± 0.13
5 mg/ml	14.9 ± 2.9	0.79*** ± 0.13
10 mg/ml	11.2 ± 4.9	0.78*** ± 0.04

b) Chymotrypsin

Enzyme Conc.	Serosal (µl/g wet wt)	Tissue (µl/g wet wt)
Control	16.4 ± 1.2	2.50 ± 0.25
5 mg/ml	17.8 ± 3.4	1.32** ± 0.18
10 mg/ml	15.0 ± 3.5	0.99*** ± 0.12

Each value is the mean ± S.E.M. of six experimental observations.

*$p<0.05$ **$p<0.01$ ***$p<0.001$

of lead was not affected by either concentration of chymotrypsin (Table 4.7(b).).

4.2.3. The Effect of Proteolytic Enzymes on the Serosal Transport and Tissue Uptake of the Lead-EDTA Complex.

Everted sacs prepared as described in section 2.5. were pre-incubated for fifteen minutes at 37°C in Krebs-Hensleit bicarbonate buffer containing 20mM glucose and either (i) 2.4 mg/ml trypsin; or (ii) 5 mg/ml chymotrypsin. The sacs were then incubated for thirty minutes at 37°C in Krebs-Hensleit bicarbonate buffer containing 20mM glucose, 10^{-6}M lead acetate labelled with ^{203}Pb and 10^{-5}M disodium calcium EDTA. Incubations were gassed with 95%O_2:5%CO_2.

Neither trypsin nor chymotrypsin had a significant effect on either the tissue uptake or the serosal transport of the lead-EDTA complex (Table 4.8.).

4.2.4. The Effect of Ethanol on the Serosal Transport and Tissue Uptake of Lead Cations.

Everted sacs prepared as described in section 2.5. were incubated for thirty minutes at 37°C in Krebs-Hensleit bicarbonate buffer containing 20mM glucose, 10^{-6}M lead acetate labelled with ^{203}Pb and either 5% or 7% (v/v) ethanol. A further experiment was carried out where sacs were pre-incubated for fifteen minutes in Krebs-Hensleit bicarbonate buffer containing 20mM glucose and 7% ethanol, before incubation

Table 4.8.

The effect of proteolytic enzymes on the transport and tissue uptake of the lead-EDTA complex.

	Serosal (pmol/g wet wt)	Tissue (nmol/g wet wt)
Control	43.2 ± 4.8	0.33 ± 0.03
2.4 mg/ml Trypsin	61.3 ± 7.8	0.35 ± 0.04
5 mg/ml Chymotrypsin	51.0 ± 11.7	0.32 ± 0.03

Each value is the mean ± S.E.M. of six experimental observations.

in buffer containing 10^{-6}M lead acetate and 7% ethanol. Incubations were gassed with 95%O_2:5%CO_2.

The serosal transport was not significantly affected by incubation in either 5% or 7% ethanol (Table 4.9(a).). However, when the sacs were pre-incubated, therefore subjecting them to forty-five minutes in the presence of 7% ethanol, a significant reduction in serosal transport was observed (Table 4.9(b).).

The tissue uptake of lead, though unaffected in the presence of 5% ethanol, was significantly elevated after incubation with 7% ethanol. After pre-incubation in 7% ethanol, however, the tissue uptake showed no sign of any increase.

4.2.5. The Effect of Ethanol on the Serosal Transport and Tissue Uptake of the Lead-EDTA Complex.

Everted sacs prepared as described in section 2.5. were incubated at 37°C for thirty minutes in Krebs-Hensleit bicarbonate buffer containing 20mM glucose, 10^{-6}M lead acetate labelled with ^{203}Pb, 10^{-5}M disodium calcium EDTA and 7% ethanol (v/v). Incubations were gassed with 95%O_2:5%CO_2.

The serosal transport of the lead-EDTA complex was significantly elevated ($p<0.05$), as was the tissue uptake ($p<0.001$), (Table 4.10.).

Table 4.9.

The effect of ethanol on the transport and tissue uptake of the lead cation.

a) Thirty minute incubation.

	Serosal (pmol/g wet wt)	Tissue (nmol/g wet wt)
Control	14.3 ± 2.8	1.68 ± 0.29
5% Ethanol	11.5 ± 4.2	1.94 ± 0.12
7% Ethanol	13.0 ± 3.9	2.97** ± 0.29

b) Fifteen minute preincubation followed by thirty minute incubation.

	Serosal (pmol/g wet wt)	Tissue (nmol/g wet wt)
Control	10.6 ± 1.9	1.93 ± 0.24
7% Ethanol	3.2** ± 0.6	1.98 ± 0.31

Each value is the mean ± S.E.M. of six experimental observations.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 4.10.

The effect of ethanol on the transport and tissue uptake of the lead-EDTA complex.

	Serosal (pmol/g wet wt)	Tissue (nmol/g wet wt)
Control	37.6 ± 6.5	1.09 ± 0.28
7% Ethanol	81.7* ± 14.7	4.30*** ± 0.59

Each value is the mean ± S.E.M. of six experimental observations.

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

4.2.6. Discussion.

Little work has been carried out to determine the roles of the glycocalyx and the mucous layer in the absorption of metals. Quarterman,(1981), reported an increase in the depth of the glycocalyx after starvation. This increase may play a part in the enhanced absorption of lead that has been found after fasting (Rabinowitz et al,1980; Chamberlain et al,1978).

The mucopolysaccharides which form the mucous layer have negatively charged end groups of sialic acid (N-acetyl- or N-glycollyl-neuraminic acid) which would readily bind any positively charged species, and may play a significant part in the binding of lead cations to the tissue surface.

Guanidinium chloride, with its denaturing action on proteins, may be expected to affect the tissue binding of lead. No evidence of this action was seen after pre-incubation with 10^{-2}M guanidinium chloride. Tissue damage was judged to be too severe after pre-incubation in molar guanidinium chloride, both by visual evidence and its effect on water transport, for a valid deduction to be made. It is probable that the non-specific chaotrophism of this agent was too strong for use with a preparation which was required to remain physiologically viable.

The use of N-acetyl-L-cysteine to break down the glycoprotein coat also seemed unsuccessful at the concentration used. A higher level of N-acetyl-L-cysteine was

considered inadvisable, in case of damage to the tissue preparation as had occurred with molar guanidinium chloride. Both these agents have been used by other investigators in successfully decreasing the viscosity of mucus and in the isolation of cells from a mucus-containing medium (Allen, 1977; Sheffner,1963). However, Allen and Sheffner gave no consideration as to the viability of the preparations. The present work indicates that these agents are unsuitable for use when viable preparations are required.

The use of proteolytic enzymes ensured both the maintenence of viability and the alteration of tissue interaction with lead cations. Although it was not possible to tell how much of the glycocalyx surface was removed by these enzymes, it can be inferred that the groups removed by proteolytic action contribute significantly to the lead-binding properties of the tissue surface.

The interaction of the lead-EDTA complex with the tissue was unaffected by the action of either trypsin or chymotrypsin, which suggests that the interaction is not a surface phenomenon. It is possible that the lead-EDTA associated with the tissue preparation is located inside the epithelial cell.

There was an increase in the amount of lead cations associated with the intestinal tissue after the everted sac had been incubated in a 7% solution (v/v) of ethanol and

Krebs-Hensleit bicarbonate buffer. The increase may have been due to a chemical reaction of the cell surface with ethanol, possibly an effect of alcohol metabolism by alcohol dehydrogenase. Serosal transport was decreased after pre-incubation in 7% ethanol, while tissue uptake was not affected. Barton and Conrad,(1978), found a decrease in lead absorption from ligated loops after exposure to 50% ethanol (v/v). Concentrations of this magnitude have been shown to damage intestinal tissue (Beck and Dinda,1981). Therefore, the decreased serosal transport found after forty-five minutes incubation in 7% ethanol may also be due to tissue damage.

In the present study, ethanol had an entirely different effect on the uptake and transport of lead-EDTA. Here, there was an increase in both serosal transport (by a factor of two), and tissue uptake (by a factor of four). EDTA is a lipid soluble compound, and ethanol can act as a solvent for lipids. Therefore, it is possible that the ethanol interacts with the lipid-rich cell membrane, resulting in some solubilization of the epithelial cell wall. The lead-EDTA complex would be able to cross the membrane more easily, and there would be a consequent increase in tissue uptake and serosal transport.

The transport of the lead cation and the lead-EDTA complex are two entirely different mechanisms, reacting in different ways to the conditions to which they are exposed.

The data indicate that the interaction of lead cations with the intestinal tissue is a phenomenon occurring mainly at the surface. This interaction takes place, to a significant extent, with the end groups of glycoproteins attached to the epithelial cell surface. These groups may be removed by the use of proteolytic enzymes.

The association between the tissue and lead-EDTA does not take place at the surface, but may be as a result of transport of the complex into the epithelial cell.

CHAPTER FIVE

A COMPARISON BETWEEN THE TRANSPORT OF LEAD, IRON AND SODIUM

AND

THE EFFECT OF DIETARY AND PHYSIOLOGICAL FACTORS ON THE ABSORPTION OF LEAD, TOGETHER WITH SOME COMPARISON WITH IRON.

5.1. INTRODUCTION.

The everted intestinal sac is a useful investigative procedure for the study of transport in the gastrointestinal tract. However, critics of the technique argue that it is an artificial system with a short period of usefulness, and does not truly reflect the situation in the living animal. Whole body studies, in which the experimental animal remains alive during the period of study, may produce more relevant information on the transport of substances by the intestine. Although it is not possible to obtain direct measurements, one can obtain information about absorption by measuring the rate of recovery of an administered dose. The amount of a substance in the body may also be assessed by studying the recovery in the organs.

The liver, spleen, kidneys and heart were chosen for the experiments presented in this thesis. The organs were chosen because: (i) the liver is the main storage and detoxification organ; (ii) the spleen is a reservoir for blood; (iii) the kidneys are the main excretory organs; and (iv) the heart is a distinct example of body muscle. Since the skeleton is the principal retention area for lead, the organs studied do not account for the total body burden of the ion studied, but give an estimation of the scale of absorption.

This chapter deals with the effect on absorption of a number of factors commonly found in the diet. The effect

of various components of food, eg. energy sources, soluble chelating agents and fibre, are studied, together with other dietary substances which may affect the absorption of lead, eg. caffeine and iron.

A comparison is made between the absorption of lead and that of two other metal ions: sodium and iron. Sodium, an element essential to life, enters the body via a number of routes, both active and passive. It is actively pumped out of the epithelial cells through the basolateral membrane, in order to maintain osmotic balance. Iron, also an essential metal, has an active transport mechanism, and is thought by some to compete with lead for a transport route, (Flanagan et al, 1979).

5.2. A COMPARISON BETWEEN THE TRANSPORT OF LEAD, IRON AND SODIUM.

5.2.1. The Absorption of Lead, Iron and Sodium from the Intestine of the Intact Rat.

Rats (170 - 200g) which had been deprived of food for 18 hours were dosed as described in section 2.10. with (i) 0.3ml 10^{-6}M lead acetate, labelled with ^{203}Pb, in distilled water; (ii) 0.3ml 10^{-6}M iron (III) chloride, labelled with ^{59}Fe, in distilled water; or (iii) 0.3ml 100mM sodium chloride, labelled with ^{22}Na, in distilled water. The rats were kept without food, but with free access to water, for 2,4 or 6 hours in gridded cages, or for 24 hours in glass metabowls.

At the end of the experimental period, the rats were sacrificed and the intestine removed as previously described (section 2.10.). The small intestine was flushed through with 0.9% saline solution, and the sections of intestine, washings and faeces and urine (where applicable) were assessed separately for their radioactivity content. The percentage of the original dose found in each section was calculated by extrapolation from a standard curve.

Table 5.1. shows the progressive movement of lead along the gastrointestinal tract with time. Four hours after the dose of lead, the stomach had expelled over 99% of the dose. The lead associated with the small intestinal tissue did not change significantly between four and six hours after the dose. The proportion of the dose in the washings, which contained lead not directly in association with the tissue and also that which was only loosely adhering to the intestinal wall, decreased with time. There was a concomitant increase in the percentage of the dose found in the large intestine.

Recovery of the initial dose of lead after six hours was 78.81 $\pm$ 7.50%. No faeces had been passed over this period, therefore, using the equation presented in section 2.10., it could be calculated that 21.19 $\pm$ 7.50% of the dose of lead had been absorbed.

Twenty-four hours after dosing, over 90% of the lead

recovered was located in the faeces or the large intestine. The urine contained 0.73% ± 0.40% of the initial dose, indicating that only a very small proportion of the lead absorbed was excreted in the urine during the first 24 hours after the dose.

The clearance of a dose of iron from the stomach, and its association with the small intestine (Table 5.2.) is very similar to that found with lead. However, a significantly lower amount ($p<0.05$) appeared in the large intestine. The low trcovery rate (29.71% - 42.45%) which resulted implied a rapid absorption of the iron cations from the intestine.

Twenty-four hours after the dose of iron the recovery rate was still significantly lower than that of lead. Only 0.08% ± 0.05% of the dose was excreted in the urine in the twenty-four-hour experimental period, less than the percentage of the lead dose eliminated over the same time period, implying that the iron which was absorbed was stored in the body for subsequent use.

Table 5.3. shows the very significant drop in recovery ($p<0.001$) after a dose of sodium ions when compared with lead ions, (Table 5.1.). Two hours after the dose, approximately 90% had been absorbed by the rat, which suggested a very rapid absorption fate for sodium. After twenty-four hours, 2.02% ± 0.72% of the dose had been excreted in the urine, more than either lead or iron. Thus, sodium does not appear to be stored in the body of the rat to the same extent as

Table 5.1.

The distribution of lead in the intestine of the intact rat as a function of time.

Section of Intestine	Percentage of initial dose			
	2 hours	4 hours	6 hours	24 hours
Stomach	7.46 ± 4.43	0.43 ± 0.07	0.63 ± 0.18	0.10 ± 0.03
Small Intestine	30.53 ± 8.30	14.40 ± 5.19	14.84 ± 5.05	1.18 ± 0.64
Washings	21.38 ± 6.17	14.64 ± 5.78	3.43 ± 1.44	0.22 ± 0.15
Large Intestine	22.10 ± 16.28	56.72 ± 15.44	59.89 ± 13.57	41.63 ± 15.24
Faeces	-	-	-	31.21 ± 14.82
Urine	-	-	-	0.73 ± 0.40
Recovery	81.47 ± 9.89	86.19 ± 6.70	78.79 ± 7.50	75.07 ± 7.39

Each value is the mean ± S.E.M. of six experimental observations.

Table 5.2.

The distribution of iron in the intestine of the intact rat as a function of time.

Section of Intestine	Percentage of initial dose			
	2 hours	4 hours	6 hours	24 hours
Stomach	1.93 ± 1.31	0.62 ± 0.16	0.38 ± 0.09	1.20 ± 0.87
Small Intestine	19.50 ± 2.27	13.57 ± 1.46	14.47 ± 3.07	8.08 ± 1.12
Washings	19.59 ± 7.30	3.35 ± 1.72	2.04 ± 1.12	1.12 ± 0.35
Large Intestine	1.43 ± 0.47	11.16 ± 3.54	21.78 ± 7.14	15.92 ± 4.25
Faeces	-	-	-	7.82 ± 2.95
Urine	-	-	-	0.08 ± 0.05
Recovery	42.45 ± 8.92	28.70 ± 5.28	38.67 ± 9.37	34.22 ± 4.10

Each value is the mean ± S.E.M. of six experimental observations.

Table 5.3.

The distribution of sodium in the intestine of the intact rat as a function of time.

Section of Intestine	Percentage of initial dose			
	2 hours	4 hours	6 hours	24 hours
Stomach	3.73 ± 1.14	2.57 ± 1.24	0.81 ± 0.01	0.68 ± 0.04
Small Intestine	2.08 ± 0.08	2.12 ± 0.08	2.25 ± 0.15	1.82 ± 0.07
Washings	1.39 ± 0.06	1.25 ± 0.08	1.79 ± 0.53	1.19 ± 0.17
Large Intestine	3.05 ± 0.39	3.95 ± 0.41	3.56 ± 0.32	3.51 ± 0.42
Faeces	-	-	-	0.41 ± 0.16
Urine	-	-	-	2.02 ± 0.72
Recovery	10.25 ± 1.01	9.89 ± 1.10	8.41 ± 0.35	9.63 ± 1.00

Each value is the mean ± S.E.M. of six experimental observations.

does lead and iron.

The liver, spleen, kidneys and heart were removed from rats sacrificed six hours after the dose of either lead or iron cations. The recovery figures presented in table 5.4. show that 5.69% $\pm$ 1.12% of the dose of lead and 12.47% $\pm$ 1.46% of the dose of iron was present in the organs removed. The balance of the absorbed dose was probably in the blood, muscle and skeleton of the experimental animals.

After lead administration the liver and kidneys retained approximately equal proportions of the initial dose, and together contained over 98% of the lead found in the organs studied. When recovery of the dose was calculated per gram wet weight of tissue, it could be seen that there was a five-fold greater concentration of lead in the kidneys compared to the liver.

When iron was administered the distribution of the recovered dose differed from that of lead. The liver and spleen contained the majority of the dose recovered, while the kidneys and heart had a lower iron content. When the recovery was calculated per gram wet weight, it was seen that the spleen retained by far the highest concentration of iron. The liver, heart and kidneys contained approximately equal concentrations of iron in comparison to that in the spleen.

Table 5.4.

The percentage of the initial dose of either 10^{-6}M lead acetate or 10^{-6}M iron (III) chloride found in the organs six hours after the dose.

Organ	Percentage of initial dose			
	10^{-6}M Lead acetate		10^{-6}M Iron (III) chloride	
	per organ	per g wet wt	per organ	per g wet wt
Liver	2.70 ± 0.62	0.32 ± 0.07	6.82 ± 0.94	0.82 ± 0.11
Spleen	0.67 ± 0.02	0.11 ± 0.02	4.47 ± 0.80	7.36 ± 1.32
Kidneys	2.89 ± 0.50	1.60 ± 0.46	0.86 ± 0.19	0.48 ± 0.10
Heart	0.04 ± 0.01	0.05 ± 0.01	0.32 ± 0.06	0.35 ± 0.06
Total Organs	6.30 ± 1.12		12.47 ± 1.46	

Each value is the mean ± S.E.M. of six experimental observations.

5.2.2. The Effect of Increased Concentrations of Lead and Iron on Their Absorption from the Intestine of the Intact Rat.

Rats (170 - 200g) which had been deprived of food for 18 hours were dosed as described in section 2.10. with (i) 0.3ml of either 10^{-4}M or 10^{-3}M lead acetate, labelled with ^{203}Pb, in distilled water; or (ii) 0.3ml of either 10^{-4}M or 10^{-2}M iron (III) chloride, labelled with ^{59}Fe, in distilled water. The rats were kept in gridded cages without food, but with free access to water, for six hours. At the end of the experimental period the rats were sacrificed and the intestine, liver, spleen, kidneys and heart removed. The small intestine was flushed through with 0.9% saline solution, and the sections of intestine, organs and washings were assessed separately for their radioactivity content. The percentage of the original dose found in each section was calculated by extrapolation from a standard curve.

Table 5.5. shows that the percentage of the dose of lead in each section of intestine, and in the washings, when rats were dosed with either 10^{-4}M or 10^{-3}M lead acetate was not significantly different from rats dosed with 10^{-6}M lead acetate. Recovery of the dose was slightly, but not significantly, lower. The lead content of the organs (Table 5.6.) was raised after dosing with both 10^{-4}M and 10^{-3}M lead acetate, significantly in the case of 10^{-3}M ($p<0.01$). The increase was seen chiefly in the liver and kidneys, and implied a greater absorption of the dose. However, since

Table 5.5.

The effect of increased concentrations of lead acetate on its distribution in the intestine of the intact rat six hours after dosing.

Section of Intestine	Percentage of initial dose	
	10^{-4}M Lead acetate	10^{-3}M Lead acetate
Stomach	4.13 ± 2.59	0.82 ± 0.05
Small Intestine	10.07 ± 2.51	8.10 ± 0.21
Washings	11.15 ± 6.25	8.75 ± 7.00
Large Intestine	46.90 ± 7.84	55.30 ± 4.66
Recovery	72.25 ± 9.07	72.97 ± 6.51

Each value is the mean ± S.E.M. of six experimental observations.

Table 5.6.

The effect of increased concentrations of lead acetate on the percentage of the initial dose found in the organs six hours after the dose.

Organ	Percentage of initial dose			
	10^{-4}M Lead acetate		10^{-3}M Lead acetate	
	per organ	per g wet wt	per organ	per g wet wt
Liver	3.64 ± 1.25	0.44 ± 0.15	5.33 ± 0.90	0.64 ± 0.10
Spleen	0.09 ± 0.03	0.15 ± 0.05	0.09 ± 0.02	0.15 ± 0.03
Kidneys	2.96 ± 1.19	1.63 ± 0.65	4.95 ± 0.48	2.73 ± 0.24
Heart	0.06 ± 0.02	0.07 ± 0.02	0.06 ± 0.01	0.07 ± 0.01
Total Organs	6.75 ± 2.47		10.43 ± 1.06	

Each value is the mean ± S.E.M. of six experimental observations.

the recovery was unaffected, the phenomenon may be explained by a difference in distribution of lead in the rat, due to the large amount of lead cations absorbed.

The transit times of both 10^{-4}M and 10^{-2}M iron (III) chloride (Table 5.7.) were similar to that of 10^{-6}M iron (III) chloride (Table 5.2.). However, the washings taken from the small intestine of rats dosed with 10^{-2}M iron (III) chloride contained a greater percentage of the initial dose than washings from rats dosed with either 10^{-6}M or 10^{-4}M iron (III) chloride, and the recovery figure for 10^{-2}M iron (III) chloride was significantly higher. The figures imply that a smaller proportion of the dose of 10^{-2}M iron (III) chloride was absorbed than of doses of 10^{-4}M or 10^{-6}M iron (III) chloride, indicating saturation of the iron transport mechanism. The percentage of the dose found in the organs (Table 5.8.) was significantly lower ($p<0.05$) in rats dosed with 10^{-2}M iron (III) chloride when compared with organs of rats dosed with 10^{-4}M iron (III) chloride, supporting the suggestion of a reduction in absorption of the dose.

5.2.3. The Absorption of Lead-EDTA and Iron-EDTA from the Intestine of the Intact Rat.

Rats (170 - 200g) which had been deprived of food for 18 hours were dosed as described in section 2.10. with (i) 0.3ml 10^{-6}M lead acetate chelated with 10^{-5}M disodium calcium ethylenediaminetetraacetic acid, labelled with ^{203}Pb,

Table 5.7.

The effect of increased concentrations of iron (III) chloride on its distribution in the intestine of the intact rat six hours after dosing.

Section of Intestine	Percentage of initial dose	
	10^{-4}M Iron (III) chloride	10^{-2}M Iron (III) chloride
Stomach	0.41 ± 0.05	0.77 ± 0.40
Small Intestine	10.27 ± 1.45	19.47 ± 3.69
Washings	1.37 ± 0.31	30.58 ± 13.07
Large Intestine	13.65 ± 3.57	31.03 ± 11.97
Recovery	25.70 ± 2.87	81.85 ± 5.36

Each value is the mean ± S.E.M. of six experimental observations.

Table 5.8.

The effect of increased concentrations of iron (III) chloride on the percentage of the initial dose found in the organs six hours after the dose.

Organ	Percentage of initial dose			
	10^{-4}M Iron (III) chloride		10^{-2}M Iron (III) chloride	
	per organ	per g wet wt	per organ	per g wet wt
Liver	6.41 ± 0.64	0.77 ± 0.07	6.50 ± 2.90	0.78 ± 0.35
Spleen	9.45 ± 1.56	15.57 ± 2.29	2.29 ± 0.52	3.77 ± 0.86
Kidneys	1.01 ± 0.19	0.56 ± 0.09	0.44 ± 0.07	0.24 ± 0.04
Heart	0.68 ± 0.14	0.76 ± 0.14	0.20 ± 0.03	0.22 ± 0.03
Total Organs	17.55 ± 1.86		9.43 ± 3.05	

Each value is the mean ± S.E.M. of six experimental observations.

or (ii) 0.3ml 10^{-6}M iron (III) chloride chelated with 10^{-5}M disodium calcium ethylenediaminetetraacetic acid, labelled with ^{59}Fe. The rats were kept in gridded cages without food but with free access to water for (i) 2,4 or 6 hours, in the case of rats dosed with lead-EDTA, or (ii) 6 hours in the case of rats dosed with iron-EDTA. At the end of the experimental period, the rats were sacrificed and the intestine removed. The liver, spleen, kidneys and heart were removed from rats sacrificed 6 hours after the dose of either lead-EDTA or iron-EDTA. The small intestine was flushed through with 0.9% saline solution, and the sections of intestine, organs and washings were assessed separately for their radioactivity content. The percentage of the dose found in each section was calculated by extrapolation from a standard curve.

No significant difference was seen in the percentage of the dose found in each section of intestine at any time period between rats dosed with lead-EDTA (Table 5.9.) or unchelated lead cations (Table 5.1.). However, there was a difference in the ratio of lead associated with the tissue to that found in the washings. In the case of rats dosed with lead cations the ratio was approximately 1.5:1, 1:1 and 4:1 for 2,4 and 6 hours respectively. In rats dosed with lead-EDTA the ratio was approximately 1:2, 1:3 and 1:1 for 2,4 and 6 hour results. At each time period, a smaller proportion of the lead in the small intestine adhered to the tissue in rats dosed with lead-EDTA. This gives rise to the

Table 5.9.

The distribution of the lead-EDTA complex in the intestine of the intact rat as a function of time.

Section of Intestine	Percentage of initial dose		
	2 hours	4 hours	6 hours
Stomach	8.12 ± 5.09	3.32 ± 1.89	0.54 ± 0.21
Small Intestine	23.32 ± 6.49	16.68 ± 2.85	7.24 ± 1.33
Washings	59.69 ± 5.33	44.21 ± 9.27	8.15 ± 3.31
Large Intestine	3.31 ± 3.46	26.10 ± 10.18	72.35 ± 5.67
Recovery	94.44 ± 5.62	90.31 ± 4.03	88.28 ± 3.51

Each value is the mean ± S.E.M. of six experimental observations.

conclusion that the availability of lead for transport was reduced by chelation with disodium calcium EDTA. The percentage of the dose found in the organs six hours after dosing (Table 5.10.) was lower than after a dose of unchelated lead cations (Table 5.4.), although the reduction was not significant.

Table 5.11. shows that although the transit time of iron-EDTA was no different from that of unchelated iron cations (Table 5.2.), a greater percentage of the dose of iron-EDTA was found in the large intestine compared to iron cations, giving rise to a significantly increased ($p<0.01$) recovery rate. The high recovery rate implied that iron-EDTA was not absorbed as readily as iron cations, and was supported by the significantly lower ($p<0.01$) iron content of the organs studied (Table 5.12.).

5.2.4. The Effect of the Presence of 10^{-4}M Iron (III) Chloride on Lead Absorption.

Rats (170 - 200g) which had been deprived of food for 18 hours were dosed as described in section 2.10. with 0.3ml 10^{-6}M lead acetate labelled with ^{203}Pb in either (i) distilled water, or (ii) a 10^{-4}M solution of iron (III) chloride. Six hours after dosing, the rats were sacrificed and the intestine, liver, spleen, kidneys and heart removed. The small intestine was flushed through with 0.9% saline solution, and the washings, organs and sections of intestine assessed separately

Table 5.10.

The percentage of an initial dose of lead-EDTA found in the organs six hours after dosing.

Organ	Percentage of initial dose per organ	per g wet wt
Liver	1.88 ± 0.27	0.23 ± 0.03
Spleen	0.04 ± 0.01	0.07 ± 0.01
Kidneys	1.92 ± 0.40	1.06 ± 0.22
Heart	0.03 ± 0.01	0.03 ± 0.01
Total Organs	3.87 ± 0.64	

Each value is the mean ± S.E.M. of six experimental observations.

Table 5.11.

The distribution of iron cations and iron-EDTA in the intestine of the intact rat six hours after dosing.

Section of Intestine	Percentage of initial dose	
	10^{-6}M Iron (III) chloride	10^{-6}M Iron-EDTA
Stomach	0.40 ± 0.06	0.29 ± 0.06
Small Intestine	21.03 ± 4.16	23.06 ± 1.80
Washings	3.42 ± 0.86	6.15 ± 1.29
Large Intestine	14.73 ± 4.33	43.77** ± 6.60
Recovery	39.58 ± 8.14	73.27** ± 6.35

Each value is the mean ± S.E.M. of six experimental observations.

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Table 5.12.

The percentage of a dose of iron-EDTA and of iron (III) chloride found in the organs six hours after the dose.

Organ	Percentage of initial dose			
	10^{-6}M Iron (III) chloride		10^{-6}M Iron-EDTA	
	per organ	per g wet wt	per organ	per g wet wt
Liver	4.49 ± 0.31	0.54 ± 0.03	1.94*** ± 0.30	0.23 ± 0.04
Spleen	4.18 ± 1.11	6.88 ± 1.64	1.49* ± 0.47	2.45 ± 0.78
Kidneys	1.02 ± 0.10	0.56 ± 0.05	0.39*** ± 0.05	0.21 ± 0.03
Heart	0.39 ± 0.03	0.43 ± 0.03	0.20* ± 0.06	0.23 ± 0.07
Total Organs	10.08 ± 1.41		4.02** ± 0.78	

Each value is the mean ± S.E.M. of six experimental observations.

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

for radioactivity. The percentage of the dose found in each section was calculated by extrapolation from a standard curve.

Administration of iron (III) chloride together with lead cations had no significant effect on the percentage of the dose found associated with the stomach or the tissue of the small intestine (Table 5.13.). There was, however, a significantly lower percentage ($p<0.05$) associated with the washings, with a concommittant rise ($p<0.05$) in the percentage found in the large intestine. There appears to be an increase in transit time due to the presence of iron (III) chloride, but since the recovery figures are similar, and there is no significant difference in the percentage of the dose in the organs (Table 5.14.), lead absorption appears unaffected by the presence of a hundredfold excess of iron (III) chloride.

5.3. THE EFFECT OF DIETARY AND PHYSIOLOGICAL FACTORS ON THE ABSORPTION OF LEAD, TOGETHER WITH SOME COMPARISON WITH IRON.

5.3.1. The Effect of the Presence of Food in the Gastrointestinal Tract on the Absorption of Lead and Iron.

Rats (170 - 200g) which had been allowed free access to food and water before experimentation were dosed as described in section 2.10. with 0.3ml 10^{-6}M lead acetate, labelled with ^{203}Pb, in distilled water. The rats were

Table 5.13.

The effect of the presence of 10^{-4}M iron (III) chloride on the distribution of lead in the intestine of the intact rat six hours after dosing.

Section of Intestine	Percentage of initial dose	
	10^{-6}M Lead acetate	10^{-6}M Lead acetate & 10^{-4}M Iron (III) chloride
Stomach	2.60 ± 1.92	0.34 ± 0.07
Small Intestine	13.41 ± 2.69	11.82 ± 2.46
Washings	17.64 ± 6.04	5.87* ± 1.97
Large Intestine	41.29 ± 8.22	60.78* ± 4.87
Recovery	74.94 ± 9.76	78.81 ± 4.14

Each value is the mean ± S.E.M. of six experimental observations.

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Table 5.14.

The effect of the presence of 10^{-4}M iron (III) chloride on the percentage of a dose of 10^{-6}M lead acetate found in the organs six hours after dosing.

Organ	Percentage of initial dose			
	10^{-6}M Lead acetate		10^{-6}M Lead acetate & 10^{-4}M $FeCl_3$	
	per organ	per g wet wt	per organ	per g wet wt
Liver	2.96 ± 0.78	0.27 ± 0.10	3.40 ± 0.88	0.41 ± 0.10
Spleen	0.07 ± 0.02	0.11 ± 0.03	0.11 ± 0.04	0.18 ± 0.06
Kidneys	3.47 ± 1.17	1.92 ± 0.58	3.81 ± 1.01	2.11 ± 0.56
Heart	0.16 ± 0.07	0.18 ± 0.08	0.07 ± 0.02	0.08 ± 0.03
Total Organs	6.66 ± 1.90		7.39 ± 1.91	

Each value is the mean ± S.E.M. of six experimental observations.

housed in gridded cages for 2,4 or 6 hours, or for 24 hours in glass metabowls. Free access to food and water was permitted at all times. In a further experiment, rats were dosed with 0.3ml 10^{-6}M iron (III) chloride, labelled with ^{59}Fe, in distilled water, and kept for 6 hours, also with free access to food and water at all times.

At the end of the experimental period, the rats were sacrificed and the intestines removed as previously described. The small intestine was flushed through with 0.9% saline solution, and the washings, sections of intestine, and faeces and urine (where applicable) were assessed separately for radioactivity. The liver, spleen, kidneys and heart were removed from rats which had been kept for 6 hours after dosing with either lead or iron cations, and assessed for radioactivity. The percentage of the dose found in each section was calculated by extrapolation from a standard curve.

The transit time of lead was slower in rats given free access to food than when no food was present in the intestine (section 5.2.1.), giving rise to a significantly higher percentage of the dose found in the stomach 2,4 and 6 hours after administration of the dose (Table 5.15.). The large intestine contained a significantly lower percentage of the dose after 2 and 4 hours, but the difference was not significant after 6 hours. The transit time over a 24-hour

Table 5.15.

The effect of food on the distribution of lead in the intestine of the intact rat, as a function of time.

Section of Intestine	Percentage of initial dose			
	2 hours	4 hours	6 hours	24 hours
Stomach	28.69 ± 6.16	14.68 ± 3.91	4.55 ± 1.45	0.02 ± 0.01
Small Intestine	1.24 ± 0.24	2.12 ± 1.09	0.38 ± 0.11	0.11 ± 0.02
Washings	61.91 ± 6.13	50.23 ± 2.56	8.56 ± 1.59	0.09 ± 0.03
Large Intestine	0.24 ± 0.12	22.55 ± 4.62	79.68 ± 3.13	3.08 ± 0.38
Faeces	-	-	-	92.77 ± 0.44
Urine	-	-	-	0.21 ± 0.06
Recovery	92.08 ± 1.65	89.58 ± 2.29	93.17 ± 1.17	96.28 ± 1.18

Each value is the mean ± S.E.M. of six experimental observations.

period was faster in animals with access to food, with 96.86% ± 0.44% of the dose in the faeces compared to 39.29% ± 14.82% in the absence of food. The total levels of lead found in the small intestine did not differ by more than a factor of two between rats starved for 18 hours and those with food ad libitum. However, the amount of lead associated with the small intestinal tissue was widely different. The lead in the dose was probably sequestered by the food residue in the intestine, rendering it unavailable for transport into the body, and was flushed out in the washings. This is highlighted by a ratio of approximately 1:23 between the tissue and washings 4 and 6 hours after dosing, compared with 1:1 and 4:1 for the same time periods in rats starved for 18 hours. The severely reduced association of the lead with the tissue was coupled with a highly significant reduction in the percentage of the dose absorbed by 6 hours, evidenced by the drop ($p<0.001$) in the recovery of lead fron the organs (Table 5.16.) when compared with starved rats (Table 5.4.). The presence of food in the gastrointestinal tract, therefore, significantly reduced the absorption of a dose of lead.

A similar pattern was found when the rats were dosed with iron. There was a slower rate of loss from the stomach (Table 5.17a.) and a changed distribution of the iron between the small intestinal tissue and the washings, from approximately 9:1 in favour of the tissue (Table 5.2.) to about 1:2 in favour of the washings. There was a high recovery rate

Table 5.16.

The effect of food on the percentage of an initial dose of lead found in the organs.

Organ	Percentage of initial dose per organ	per g wet wt
Liver	0.22* ± 0.01	0.03 ± 0.01
Spleen	0.01 ± 0.004	0.02 ± 0.01
Kidneys	0.18** ± 0.01	0.10 ± 0.01
Heart	0.01 ± 0.005	0.01 ± 0.004
Total Organs	0.42*** ± 0.02	

Each value is the mean ± S.E.M. of six experimental observations.

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Table 5.17.

The effect of food on the distribution of iron in the intestine and the organs six hours after dosing.

a) Intestine

$^{*}p < 0.05$
$^{**}p < 0.01$
$^{***}p < 0.001$

Section of Intestine	Percentage of initial dose
Stomach	$3.91^{**} \pm 0.99$
Small Intestine	$4.11^{**} \pm 0.69$
Washings	$7.80^{*} \pm 2.16$
Large Intestine	$64.63^{***} \pm 6.97$
Recovery	$80.45^{**} \pm 5.94$

b) Organs

Organ	Percentage of initial dose per organ	Percentage of initial dose per g wet wt
Liver	$1.79^{***} \pm 0.38$	0.21 ± 0.05
Spleen	$0.82^{***} \pm 0.23$	1.35 ± 0.38
Kidneys	$0.19^{**} \pm 0.03$	0.11 ± 0.01
Heart	$0.10^{**} \pm 0.01$	0.11 ± 0.01
Total Organs	$2.90^{***} \pm 0.61$	

Each value is the mean $\pm$ S.E.M. of six experimental observations.

of the dose, 80.45% ± 5.94%, and a significant reduction ($p<0.001$) in the percentage found in the organs studied (Table 5.17b.). The food in the intestine appears to have reduced the availability of iron cations for transport, as it did for lead cations.

5.3.2. The Effect of Glucose on the Absorption of Lead and Iron Cations.

Rats (170 - 200g) which had been deprived of food for 18 hours were dosed as described in section 2.10. with (i) 0.3ml 10^{-6}M lead acetate, labelled with ^{203}Pb, in solution with 20mM, 100mM or 1M glucose; or (ii) 0.3ml 10^{-6}M iron (III) chloride, labelled with ^{59}Fe, in solution with 100mM glucose. The rats were housed in gridded cages for 6 hours, without food but with free access to water. At the end of the experimental period, the rats were sacrificed and the intestines, liver, spleen, kidneys and heart removed. The small intestine was flushed with 0.9% saline solution, and the washings, sections of intestine and organs were assessed for radioactivity. The percentage of the dose found in each section was calculated by extrapolation from a standard curve.

Table 5.18. shows a large variation in the rate of recovery of the dose of lead. When 20mM glucose was present the recovery in the intestine was 50.41% ± 4.20%, which, together with a recovery of the dose from the organs of

Table 5.18.

The effect of various concentrations of glucose on the distribution of lead in the intestine of the intact rat six hours after dosing.

Section of Intestine	Percentage of initial dose		
	20mM Glucose	100mM Glucose	1M Glucose
Stomach	1.02 ± 0.50	0.33 ± 0.06	1.45 ± 0.91
Small Intestine	17.81 ± 2.60	12.65 ± 3.00	10.44 ± 2.95
Washings	4.52 ± 0.55	6.06 ± 1.25	6.01 ± 2.13
Large Intestine	27.07 ± 3.97	60.20 ± 8.14	61.18 ± 6.32
Recovery	50.42 ± 4.20	79.24 ± 4.24	79.08 ± 6.19

Each value is the mean ± S.E.M. of six experimental observations.

12.71% ± 1.24% (Table 5.19.) suggests an enhanced absorption of lead. However, when lead was administered in solution with 100mM and 1M glucose, this effect was not found. Therefore, it must be said that no conclusive effect was found of glucose on lead absorption.

The presence of 100mM glucose had no effect on the absorption of iron (Table 5.20a.), although transit time was slightly reduced. No significant difference was found in the percentage of the dose of iron cations present in the organs (Table 5.20b.).

5.3.3. The Effect of Calcium Phytate and Caffeine on the Absorption of Lead Cations.

Rats (170 - 200g) which had been deprived of food for 18 hours were dosed as described in section 2.10. with either (i) 0.25g calcium phytate suspended in approximately 0.4ml distilled water; or (ii) 0.3ml 2 x 10^{-2}M caffeine in distilled water. Thirty minutes later, the rats were dosed with 0.3ml 10^{-6}M lead acetate, labelled with ^{203}Pb, in distilled water. The rats were kept without food but with free access to water for 2,4 or 6 hours in gridded cages or 24 hours in glass metabowls in the case of calcium phytate dosed animals, and 6 hours in gridded cages after dosing with caffeine. A control experiment was carried out simultaneously, where rats were dosed with 0.3ml distilled water thirty minutes before administration of the lead dose, and kept for 6 hours.

Table 5.19.

The effect of various concentrations of glucose on the percentage of a dose of lead found in the organs six hours after dosing.

Organ	Percentage of initial dose (per organ)		
	20mM Glucose	100mM Glucose	1M Glucose
Liver	6.06 ± 0.57	3.39 ± 0.83	3.04 ± 1.25
Spleen	0.21 ± 0.02	0.12 ± 0.03	0.13 ± 0.04
Kidneys	5.77 ± 0.70	2.32 ± 0.57	3.06 ± 1.12
Heart	0.13 ± 0.02	0.07 ± 0.02	0.05 ± 0.02
Total Organs	12.17 ± 1.24	5.90 ± 1.43	6.28 ± 2.37

Each value is the mean ± S.E.M. of six experimental observations.

Table 5.20.

The effect of glucose on the distribution of iron in the intestine and the organs six hours after dosing.

a) Intestine

Section of Intestine	Percentage of initial dose
Stomach	0.24 ± 0.02
Small Intestine	8.66 ± 0.69
Washings	1.12 ± 0.11
Large Intestine	49.02 ± 7.71
Recovery	59.04 ± 7.63

b) Organs

Organ	Percentage of initial dose per organ	per g wet wt
Liver	3.62 ± 0.53	0.43 ± 0.06
Spleen	3.97 ± 0.70	6.54 ± 1.15
Kidneys	0.49 ± 0.07	0.27 ± 0.04
Heart	0.30 ± 0.09	0.33 ± 0.10
Total Organs	8.38 ± 1.29	

Each value is the mean ± S.E.M. of six experimental observations.

At the end of the experimental period, the rats were sacrificed and the intestine removed. The small intestine was flushed through with 0.9% saline solution. The liver, spleen, kidneys and heart were removed from rats sacrificed 6 hours after the dose of lead, and were assessed for radioactivity with the sections of intestine, washings and faeces and urine where applicable. The percentage of the dose found in each section was calculated by extrapolation from a standard curve.

The transit time of the lead dose following administration of calcium phytate (Table 5.21a.) was slower than that of rats given lead with no previous dose of calcium phytate, and was similar to rats which had had free access to food. Recovery of the lead dose was over 90% up to 6 hours after its administration, and the distribution of lead between the tissue of the small intestine and the washings was approximately 1:20, again similar to results obtained from rats given food ad libitum. The percentage of the dose found in the organs after 6 hours was significantly lower ($p<0.001$) than in the control rats (Table 5.21b.), indicating a reduction in lead absorption. The effect of a single dose of calcium phytate persisted up to 24 hours after administration (Table 5.21a.).

No significant effect of caffeine on the absorption of lead was found (Table 5.22.). There was no difference in transit time, recovery or organ content (Table 5.23.) of lead between the control and test rats.

Table 5.21a.

The effect of calcium phytate on the distribution of lead in the intestine of the intact rat, as a function of time.

Section of Intestine	Percentage of initial dose			
	2 hours	4 hours	6 hours	24 hours
Stomach	47.37 ± 8.35	20.51 ± 11.26	10.27 ± 5.85	0.80 ± 0.75
Small Intestine	3.46 ± 0.74	4.70 ± 2.28	1.94 ± 0.72	1.00 ± 0.52
Washings	44.11 ± 6.31	48.82 ± 8.64	34.32 ± 9.51	1.28 ± 0.55
Large Intestine	0.01 ± 0.01	19.25 ± 12.62	46.82 ± 12.00	46.76 ± 8.06
Faeces	-	-	-	37.11 ± 8.74
Urine	-	-	-	0.11 ± 0.03
Recovery	94.95 ± 5.44	93.28 ± 3.78	93.35 ± 2.65	87.06 ± 3.92

Each value is the mean ± S.E.M. of six experimental observations.

Table 5.21b.

The effect of calcium phytate on the percentage of an initial dose of lead found in the organs six hours after dosing.

Organ	Percentage of initial dose per organ	per g wet wt
Liver	$0.23^{*} \pm 0.01$	0.03 ± 0.01
Spleen	0.01 ± 0.004	0.02 ± 0.01
Kidneys	$0.20^{**} \pm 0.01$	0.11 ± 0.01
Heart	0.01 ± 0.007	0.01 ± 0.01
Total Organs	$0.45^{***} \pm 0.02$	

Each value is the mean $\pm$ S.E.M. of six experimental observations.

$* p < 0.05$

$** p < 0.01$

$*** p < 0.001$

Table 5.22.

The effect of $5x10^{-2}$M caffeine on the distribution of lead in the intestine of the intact rat six hours after dosing.

Section of Intestine	Percentage of initial dose	
	10^{-6}M Lead acetate	10^{-6}M Lead acetate & $5x10^{-2}$M Caffeine
Stomach	0.97 ± 0.48	0.28 ± 0.06
Small Intestine	17.05 ± 0.13	14.64 ± 2.88
Washings	11.70 ± 3.06	9.91 ± 2.59
Large Intestine	38.33 ± 6.54	40.98 ± 7.35
Recovery	68.05 ± 4.16	65.81 ± 5.81

Each value is the mean ± S.E.M. of six experimental observations.

Table 5.23.

The effect of $5x10^{-2}$M caffeine on the percentage of a dose of 10^{-6}M lead acetate found in the organs six hours after dosing.

Organ	Percentage of initial dose			
	10^{-6}M Lead acetate		10^{-6}M Lead acetate & $5x10^{-2}$M Caffeine	
	per organ	per g wet wt	per organ	per g wet wt
Liver	3.25 ± 0.62	0.39 ± 0.07	2.91 ± 0.61	0.35 ± 0.07
Spleen	0.12 ± 0.03	0.19 ± 0.04	0.08 ± 0.02	0.14 ± 0.03
Kidneys	4.03 ± 0.65	2.23 ± 0.36	3.79 ± 0.82	2.09 ± 0.41
Heart	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.06 ± 0.02
Total Organs	7.44 ± 1.28		6.83 ± 1.42	

Each value is the mean ± S.E.M. of six experimental observations.

5.3.4. The Effect of Age on the Absorption of Lead from the Intestine of the Intact Rat.

Rats (50 - 60g, 23 - 28 days old) which had been deprived of food for 18 hours were dosed as described in section 2.10. with 0.2ml 10^{-6}M lead acetate, labelled with ^{203}Pb, in distilled water. The rats were housed in gridded cages for 2,4 or 6 hours, or in glass metabowls for 24 hours, without food but with free access to water. At the end of the experimental period, the rats were sacrificed and the intestine, liver, spleen, kidneys and heart removed. The small intestine was flushed through with 0.9% saline solution. The sections of intestine, washings, organs, and faeces and urine(where applicable) were assessed separately for radioactivity. The percentage of the original dose found in each section was calculated by extrapolation from a standard curve.

Table 5.24. shows that the transit time in weanling rats was slightly slower than in adult (10 week old) rats, and that a greater percentage of the dose was found to be associated with the small intestinal tissue at all time periods. The recovery of the dose is not significantly different from adult rats either 2 or 4 hours after dosing, but is significantly reduced 6 hours after the dose ($p<0.05$), implying a greater absorption of the lead dose. The reduction in recovery persisted until at least 24 hours after administration of the lead dose, together with the elevation in the

Table 5.24.

The distribution of lead in the intestine of young rats (23-28 days old) as a function of time.

Section of Intestine	Percentage of initial dose			
	2 hours	4 hours	6 hours	24 hours
Stomach	1.12 ± 0.22	1.00 ± 0.24	0.68 ± 0.14	0.34 ± 0.18
Small Intestine	40.86 ± 2.86	24.41 ± 2.52	19.90 ± 1.35	4.88 ± 0.36
Washings	36.69 ± 5.24	38.31 ± 11.04	10.66 ± 2.15	3.20 ± 1.48
Large Intestine	0.96 ± 0.63	10.90 ± 4.62	21.50 ± 8.26	29.31 ± 6.45
Faeces	-	-	-	6.79 ± 2.25
Urine	-	-	-	1.20 ± 0.17
Recovery	79.63 ± 3.23	74.62 ± 5.64	52.74 ± 6.73	45.72 ± 4.57

Each value is the mean ± S.E.M. of six experimental observations.

lead associated with the small intestinal tissue.

The percentage of the dose found in the organs was significantly higher ($p<0.001$) than that found in adult rats 6 hours after administration of the dose (Table 5.25.). As in adult rats, the kidney contained the highest percentage of the dose (13.72% $\pm$ 1.85%) per gram wet weight, which is approximately 20 times the concentration in the kidneys of adult rats.

Table 5.25.

The percentage of an initial dose of lead found in the organs six hours after dosing - 23-28 days old.

Organ	Percentage of initial dose per organ	per g wet wt
Liver	8.67 ± 1.33	4.89 ± 0.08
Kidneys	0.50 ± 0.27	2.42 ± 1.31
Spleen	8.82 ± 1.17	13.72 ± 1.85
Heart	0.17 ± 0.05	0.55 ± 0.16
Total Organs	18.16*** ± 2.22	

Each value is the mean ± S.E.M. of six experimental observations.

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

5.4. DISCUSSION.

Lead absorption was compared with the absorption of two other metal ions - iron and sodium. Lead was absorbed slowly, approximately 20% of the dose had entered the body six hours after administration. Compared with lead, the absorption of sodium and iron were relatively fast. Approximately 65% of the iron dose had been absorbed after six hours, and about 90% of the dose of sodium in only two hours.

It has been suggested that lead shares a transport route with iron (Flanagan et al,1980; Barton et al,1981). However, no inhibition of lead absorption was found in whole body experiments using a hundredfold excess of iron over lead. Therefore, there was no competition for a common transport mechanism at these concentrations.

Lead absorption was radically reduced by the presence of food in the intestine (Section 5.2.). Three different components of food were tested individually ie. an energy source (glucose), a soluble chelating agent (disodium calcium ethylenediaminetetraacetic acid) and fibre (calcium phytate). Fibre has been found to reduce lead absorption in the form of pectate (Paskins-Hurlburt et al,1977). Wise,(1981), suggested that phytate may cause precipitation of lead, thus rendering it unavailable for transport. When rats were given phytate in the present studies, lead absorption was reduced by a similar extent as when food was present in the intestine.

Neither glucose nor disodium calcium diethyleneaminetetraacetic acid had a significant effect on lead absorption. Therefore, it may be concluded that the reduction of lead absorption in the presence of food is largely due to its fibre content.

Iron absorption was also reduced in the presence of food. However, unlike lead, iron absorption was also reduced by chelation with disodium calcium diethyleneamine-tetraacetic acid. The iron-EDTA chelate would be unable to bind to the carrier for iron, therefore, the complex entered the body via another, slower route. The lead-EDTA chelate would also enter the body via a different route to lead ions, but since lead cations are absorbed slowly, a change in the transport route to another slow, passive process would not be expected to affect the overall rate of lead absorption.

The presence of glucose in the dose of lead had no effect on absorption, therefore, the availability of energy on the luminal side of the epithelial wall did not alter transport. However, this cannot be taken as evidence for passive transport as iron absorption was also unaffected by the presence of glucose.

The presence of caffeine in the intestine had no effect on lead absorption. Coogan,(1982), found that when everted sacs were incubated with lead in the presence of caffeine, lead transport was reduced though tissue interaction was unchanged. He suggested that the effect of caffeine was as a result of inhibition of phosphodiesterase, with a consequent

increase of the intracellular concentration of cyclic-AMP. The resultant reversal of flow of electrolytes caused the collapse of intercellular spaces *in vitro*, but this did not occur in the intact animal.

Iron transport showed saturation kinetics. Absorption was reduced from approximately 65% of the administered dose of 10^{-6}M iron (III) chloride, to approximately 18% when rats were given 10^{-2}M iron (III) chloride. By contrast, when the concentration of lead cations was increased, no evidence of saturation was seen. The same percentage of the administered dose was absorbed at concentrations of 10^{-4}M and 10^{-3}M lead acetate as was absorbed when the dose contained 10^{-6}M lead acetate. There was some difference in the distribution of absorbed lead in the body, however. The liver and kidneys contained a larger proportion of the dose of 10^{-3}M lead acetate than either 10^{-4}M or 10^{-6}M lead acetate. The total lead content of the organs was significantly raised ($p<0.01$) at this concentration. The results indicate a passive transport process for lead absorption, and do not agree with those reported by Mykkänen and Wasserman (1981), who claim to show evidence of a saturable carrier transport mechanism for lead.

Several authors have reported that lead absorption is greater in young rats than in adult rats (Quarterman and Morrison,1978; Kostial *et al*,1971; Mykkänen and Wasserman, 1981). The present results agree with the findings of these

researchers. Keller and Doherty,(1980), suggested that pinocytosis may play some part in lead absorption in weanling animals, thus resulting in greater absorption. No experiments to measure pinocytosis have been carried out in the present research, however. It is possible that the greater lead absorption in the young rat is solely due to the general enhancement of nutrient absorption during a period of rapid growth. The figures for absorption of lead by weanling rats are broadly similar to those published by other workers, (Kostial et al,1971; Bushnell and DeLuca,1981).

The accumulation of lead in the liver and kidneys is a widely reported feature. In all cases the kidneys contained approximately five times the concentration of lead than the liver, despite large differences in percentage absorption of the original dose. The one exception was in the experiment using weanling rats, where the distribution was approximately 3:1 in favour of the kidneys.

The tissue distribution of iron was different from that of lead, and was probably a reflection of blood content rather than the accumulation of the cation. Liver and spleen contain large stores of blood while the kidneys and heart, though exposed to a large blood volume, do not store it in any way.

The ratio between the percentage of the dose found in the washings from the small intestine and that associated with the small intestinal tissue may be an indication of the

extent of lead absorption. In cases where little lead was absorbed, eg. in the presence of food or calcium phytate, the tissue:washings ratio was approximately 1:20. The absorption of lead from the intestine of rats starved overnight was higher and the tissue:washings ratio was approximately 4:1 after six hours. The total lead content of the small intestine (tissue plus washings) was similar in each case. Tissue interaction may be the determining factor in absorption rather than apparent availability in the intestine. This questions the theory put forward by Blair et al,(1979), who suggested that tissue interaction acts as a protection against lead absorption, and will be discussed further in the following chapter.

CHAPTER SIX

DISCUSSION

6.1. DISCUSSION

A model for lead transport put forward by Blair *et al*, was broadly supported by the experimental results in this thesis. The present investigation has shown that lead movement into the serosal space of everted intestinal sacs is a slow, linear process, unaffected by the presence of the metabolic inhibitor sodium iodoacetate. In the whole animal, no evidence was found for competition for transport between iron and lead cations *in vivo*, contradicting reports by Flanagan *et al*., (1979). These data suggested that lead was transported across the intestinal wall via a different route from that taken by iron.

It was confirmed using the everted sac technique that the interaction of lead cations with the tissue is biphasic in character, with an initial rapid uptake followed by a slower accumulation of lead cations by the tissue. Removal of a significant fraction of the tissue-bound lead by the application of agar-agar gave support to the suggestion that the interaction was mainly a surface phenomenon, as did the reduction of tissue interaction after the everted sac preparation was pre-treated with proteolytic enzymes, to remove part of the glycoprotein coat of the epithelial cells.

The exact nature of the tissue interaction is not known. Coleman *et al*., (1978), suggested that lead cations

were bound by phosphate groups on the membrane surface, but no evidence for this was put forward. The present experimental results showed that the glycoprotein coat plays a significant role in determining the amount of lead cations that are bound to the tissue. Quarterman (1981) suggested that the increase in thickness of the glycocalyx after starvation may be related to the concommittant increase in metal absorption. A significant proportion of the end-groups of intestinal glycoproteins are composed of sialic acids eg. N-acetylneuraminic acid and N-glycollylneuraminic acid, which have an axial carboxyl group and are an important source of negative charge for many mucus glycoproteins. It is therefore equally possible that lead cations are bound by carboxyl groups.

The postulation by Coleman (1979) that the interaction of lead with the tissue gives a protective effect against lead absorption by reducing the amount of lead available must be also questioned. The theory may have been prompted by the observation that the enhanced serosal transport of lead, when chelated with disodium calcium EDTA, was coupled with a reduction of tissue binding. No account was taken, however, of the fact that the transport routes of lead cations and lead-EDTA may be different. The present experimental results show that the reduction of tissue interaction with lead cations, using proteolytic enzymes, had no effect on serosal transport. Conversely, *in vivo* experimentation demonstrated that there was a similar increase in tissue bound level with

increased lead transport into the body, possibly by keeping the lead in the small intestine for a longer period and thus increasing the time available for transport. It is probable, therefore that far from tissue binding having protective effect, interaction of lead cations with the tissue may contribute to a greater lead absorption.

Another possibility is that, in the *in vivo* situation, the amount of lead found associated with the tissue is merely an indication of the amount in the process of being transported into the body cavity, and the absorption is influenced either by physical factors, eg. the presence of food or chemicals, or physiological factors, eg. age. It is likely that the full explanation lies in a mixture of these two theories.

Whole body animal studies demonstrated that the presence of food in the intestine had a major effect on lead absorption. This effect appears largely due to the fibre content, as the administration of calcium phytate with the lead dose gave a very similar result. The soluble components of food, eg. glucose, would not appear to influence the absorption of lead, although chelating agents other than disodium calcium EDTA may alter it.

The extrapolation of results gained from rats to predict effects in humans must be carried out with care, as

there are some very significant differences between the responses of the two species. For example, data from rats indicate that approximately 20% of the administered dose of lead was absorbed after overnight starvation. Flanagan et al., (1982) reported that humans starved for the same period absorbed approximately 60% of a lead dose. Therefore, it can be seen that one cannot extrapolate directly from rat to man. However, it is possible to make educated predictions on the effects of substances on lead absorption in humans by studying their effects in rats, bearing in mind the physiological and dietary differences between the two species.

Having obtained information on factors affecting lead absorption, we can use this to try to minimize the amount of lead absorbed from dietary sources. Since lead taken on an empty stomach is absorbed far more readily than when taken with food, it can be seen that drinks made with tap water (which may have a high lead content) should be avoided before eating a morning meal.

A diet rich in fibre would appear to be beneficial in reducing lead absorption, although the precise nature of the fibre component may be important as some have been reported to enhance lead uptake from the diet (Weimer and Keis, 1981).

Environmental factors also play a part in the amount

of lead absorbed, therefore it would be expedient to avoid situations where one would encounter high levels of lead. This is not always practicable, however, eg. if one works in a city centre or a factory where lead is used in manufacturing processes. Young children, who have a higher capacity for lead absorption, should be especially protected by being given regular meals to counteract the greater number of risk factors to which they are exposed, eg. play areas near busy roads and a greater number of hand-to-mouth actions. Elimination of the risk of lead absorption is, of course, impossible in a mechanised society, but it is important to endeavour to reduce those risks as far as one is able.

6.2. FURTHER RESEARCH

1. Attempting to account for 100% of the administered dose by whole body assessment of radioactivity.

2. Extending the age range of the experimental animals to cover lead absorption in new-born rats to senility.

3. Further investigation on the chemical interaction of lead cations with the tissue surface.

4. Identification of the binding factor(s) on the tissue surface, possibly by gel filtration.

5. Studying the role of precipitation of lead by various dietary components, and their effect on absorption.

6. Looking at the biochemical effects of lead in different body tissues in an effort to relate them with the toxic effects of lead.

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