THE GASTROINTESTINAL ABSORPTION OF LEAD

A thesis submitted for the degree of

Doctor of Philosophy

by

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Summary

The transport of environmentally relevant concentrations of the lead cation has been investigated using the everted sac preparation. Only a small percentage of lead was transported into the serosal compartment but there was a rapid and massive uptake on to the tissue. There was no significant difference in the amount of lead transported across different regions of the small intestine. Both rate of transport into the serosal compartment and the tissue uptake increased linearly with increased concentration of lead. Little evidence for saturation of serosal transport or tissue uptake was found. Lead transport into the serosal compartment appeared to be related to water movement but was little affected by changes in glucose concentration, temperature or anoxic conditions. In the absence of calcium ions, or in the presence of excess hydrogen ions, transport of lead into the serosal compartment was increased, therefore lead may passively traverse the intestinal epithelium via the zonulae occludentes. Increased lead transport may proceed by an intracellular route after interaction with a chelating agent. The interaction between lead ions and the intestinal tissue was extremely tenacious and displayed characteristics of covalent bonding. Variations in pH markedly influenced the lead-tissue interaction which supports the hypothesis that tissue phosphate ions are responsible for removing lead ions from the lumen. Excess bile salts and DTPA decreased the lead-tissue interaction whilst decreased luminal volume increased the interaction. The observations permit the development of a simple model which a) elucidates the mechanism of lead transport, b) demonstrates that the intestinal lumen protects the animal against excessive exposure to lead by governing the quantity of lead that appears in blood, c) predicts the conditions in which lead absorption may be markedly affected.

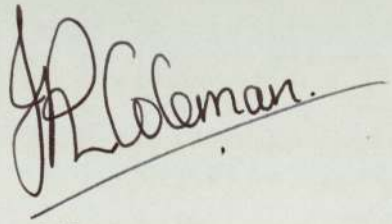
Key Words

Intestine
Lead
Zonulae Occludentes
Phosphate

DECLARATION

This work was carried out between October 1975 and September 1978 in the Department of Chemistry, University of Aston in Birmingham.

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



I.P.L. Coleman

To my wife and family

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CHAPTER 1

INTRODUCTION

1.1 Introduction

The adverse effects of lead on health have been known for centuries, but the biological interest in the element was only initiated as a consequence of the recognition of lead as a potential industrial hazard. As a result, the amounts of lead that produce different clinical symptoms of poisoning are well documented (Goyer, 1971; Goyer and Rhynne, 1973; Kehoe, 1976). With stricter industrial control cases of lead poisoning due to occupational exposure have occurred less frequently in recent years. In contrast the problems associated with the long term exposure to amounts of lead that do not result in obvious clinical symptoms are not fully understood.

There are two main routes whereby lead can enter the body; by ingestion or inhalation. Some organolead compounds may be absorbed by the subcutaneous route but the amount passing into the body is relatively small and may be ignored (Waldron and Stöfen, 1974). Industrial or occupational exposure to lead occurs mainly as a result of inhalation. However, normally the greatest contribution to man's body burden of lead is furnished by the oral intake of the element (Hilburn, 1977).

1.2 The Ingestion of Lead

The main sources of dietary lead are food, water, paint and dust. The daily intake of lead from water is usually 15-20 µg but may be greater in areas where domestic plumbing utilises lead piping. Acidic conditions increase the solubility of lead salts; hence where the water supply is soft and acid, an excess of lead may be consumed. Total dietary intake of lead has fallen over the last thirty years. In 1949 the Food Standards Committee reported that lead was a serious contaminant in food and drink (Monier-Williams, 1949) and at that time the mean daily figure for ingested lead was 250 µg. By 1961 regulations had been introduced to control the limits of lead in

food and drink thus reducing the level of contamination to a present day ingested figure of approximately 140 µg lead per day from the food (MAFF, 1975), plus a further 60 µg lead from water and dust (Hilburn, 1977).

The amount of lead ingested as a part of the diet varies considerably and depends upon a number of factors:-

i) the total amount of food ingested

ii) the source of the food, i.e. fresh vegetables, tinned foods.

iii) the amount of intrinsic lead - that is lead absorbed from the soil by plants, or extrinsic lead - from dust or deposited pesticides, (MAFF, 1975).

1.3 The Gastrointestinal Tract

In order to investigate the mechanism of lead absorption following oral ingestion, it is necessary to be familiar with the morphology of the gastrointestinal tract. The small intestine may be regarded as a hollow cylinder through which dietary contents pass en route to the caecum and large intestine. Classically, it is divided into three regions, the duodenum, jejunum and ileum. The duodenum begins at the pyloric sphincter and extends in the adult rat for about 15 cm to the ligature of Trietz. The upper part of the small intestine below the duodenum is called the jejunum and the lower part is termed the ileum, although there is no distinct anatomical boundary between the two.

Water, nutrients, vitamins and ions are absorbed from the intestinal lumen, particularly across the jejunum, and in order to create a large surface area for absorption the mucosal surface displays a number of adaptations. Throughout the length of the small intestine, the mucous membrane is covered by projections termed villi. Each villus contains capillaries into which are absorbed the products of carbohydrate and protein digestion and a central lymphatic vessel into which

fats are absorbed. Light microscopy reveals that the surface of the absorptive cells of the villi consist of a specialised "brush border" of minute parallel rod-like projections, termed microvilli, which greatly increase the area available for absorption. The microvilli are covered with a surface coat of fine filaments, the glycocalyx, thought to be made up of glycoproteins. The region of the glycocalyx is also thought to be the location of a hydrogen ion gradient. The existence of such an acid microclimate is thought to influence the transport of various species, (Blair and Matty, 1974).

A number of routes and mechanisms are available for the transport of substances across the gastrointestinal tract. Molecules and ions may traverse the intestinal epithelial sheet by either an extracellular route, believed to be situated at the zonulae occludentes or a transcellular route which involves the movement of molecules across two membranes in series.

1.4 The Amount of Lead Absorbed from the Intestinal Lumen

There have been a number of studies to establish the amount of ingested inorganic lead that is absorbed. However, most of the studies determined absorption by indirect methods. Such experiments provide no information concerning the site of lead absorption or, more importantly, the mechanism involved. They do, however, provide limited information with regard to factors which influence absorption.

Kehoe (1961) performed long term balance studies in man and measured the amount of inorganic lead excreted in the urine. He concluded that approximately 10% of the total intake of lead from food and beverages was absorbed from the gastrointestinal tract. Other studies by mass spectrometric measurement of the stable isotope ^{204}Pb or using radioisotopes ^{203}Pb or ^{212}Pb have resulted in similar mean figures but there was considerable scatter in the distribution of the individual observations reported (Rabinowitz, Wetherill and Kopple,

1973; Hursch and Suomela, 1968; Blake, 1975).

Absorbed lead is distributed throughout the body via the blood stream and subsequently excreted, mainly in the urine, although a small amount is secreted into the bile, sweat and hair. Continuous exposure to lead results in a gradual accumulation in the skeleton. However, the concentration of lead in various soft tissues and blood remains relatively stable throughout adult life. Rabinowitz, Wetherill and Kopple (1976) suggest that the total body burden of lead can be divided into three major pools.

1. A relatively slow, non diffusible pool of dense bone and teeth.
2. Lead accumulated in skin, muscle and bone marrow which has an intermediate rate of exchange.
3. Lead in soft tissue and blood which is rapidly exchanged with other storage areas.

The amount of lead absorbed following ingestion appears to be influenced by a number of factors such as age, health of the animal, the physical and chemical forms of lead and the effects of other dietary compounds.

1.5 Effect of Age on the Absorption of Lead

The young of many species are considered to be more susceptible to the effects of lead than the adult (Barltrop, 1969), and it has been suggested that this may be a result of a greater absorption of lead from the gastrointestinal tract. Kostial, Simonovic and Pisonic (1971) measured the whole body retention of ^{203}Pb and estimated that almost 55% of the lead administered orally to 5-7 day old rats was absorbed. Only 1% of the administered lead was absorbed by 4 month old rats. No information was presented with regard to a specific site or mechanism for lead absorption. Studies by Forbes and Reina (1972) demonstrated that young animals absorbed almost 100% of the dose of

lead administered and that the high absorption declined as the animals matured. The authors suggested that the animal may develop some form of rejection mechanism during maturation but offered no evidence to support the theory. Clark (1959) has shown that the young animal possesses a pinocytotic mechanism which declines in efficacy 18 days after birth. Forbes and Reina (1972) also suggest that the loss of this mechanism may account for the decrease in lead absorption with maturation.

1.6 The Effect of Calcium and Other Dietary Minerals on the Intestinal Absorption of Lead

The effect of calcium upon the absorption, retention and toxicity of dietary lead has been investigated by a number of researchers. Six and Goyer (1970) found that by firstly lowering the level of dietary calcium and then feeding with 200 parts per million (ppm) lead in the drinking water, the rats possessed a higher body burden of the element than normal. As a result the authors suggested that the low calcium levels caused an increase in gastrointestinal absorption.

Quarterman and Morrison (1975) reported that rats maintained on a low diet of calcium and phosphate increased their retention of orally administered lead. However, the retention of lead, given intraperitoneally, was unaffected by changes in dietary calcium or phosphate which suggested that dietary calcium and phosphate influence the absorption of lead from the intestine.

These studies give limited information concerning factors affecting the absorption of lead. More direct evidence has been obtained by Barltrop and Khoo (1976). By the use of whole body and ligated intestinal loop studies they demonstrated that low dietary calcium and phosphate increased the absorption of ^{203}Pb, from the gastrointestinal tract. High dietary levels of these minerals decreased lead absorption. The increase in the absorption of lead in the presence of low levels of minerals was further enhanced by a

high fat diet. Low fat diets had no effect on lead absorption. It is possible that lead ions interact with fatty acids, to form a complex which may be more easily absorbed. However, the site and possible mechanism of lead absorption was not revealed by these studies.

Their observations do however support the suggestion of Kello and Kostial (1972) that the protein and fat constituents of milk enhance the absorption of lead from the intestine and that this effect is as important as the influence of calcium in milk.

Although further explanations are difficult to deduce due to the short duration (48 hours) of Barltrop and Khoo's experiments (Barltrop and Khoo, 1976), their main conclusion was that there is some form of competition between calcium and lead for absorption across the intestine. Their conclusion is supported by the indirect observations of Meredith, Moore and Goldberg (1977) who indicated from measurement of whole body retention of lead that a doubling of dietary calcium reduced the absorption of orally administered lead. The same amount of calcium given intraperitoneally had no effect on lead absorption. Whole body and tissue retention of intraperitoneally administered lead was unaffected by either orally or intraperitoneally administered calcium.

Meredith et al (1977) therefore suggested that calcium influenced the gastrointestinal absorption of lead, as a result of the proposed existence of a "common pathway" for the absorption of calcium, lead and other minerals. The hypothesis of a "common pathway" is supported by Gruden, Stantic and Buben (1974) who have demonstrated that dietary lead reduced the absorption of calcium. However, the concept of a "common pathway" is rather vague and it is difficult to speculate as so little is understood with regard to the mechanism of lead transport across the intestine.

1.7 The Effect of Various Dietary Cations on the Intestinal Absorption of Lead

Various cations have been shown to influence the body retention, and by inference, the absorption of lead. Six and Goyer (1972) have reported that lead retention is increased in iron deficient rats and suggest that this is the result of an increase in the absorption of lead across the gastrointestinal tract.

Pollack, George, Beba, Kaufman and Crosby (1965), using rats, have shown that an iron-deficient diet increased the absorption of some metals such as cadmium, but not calcium. Their observations suggest that the passage of cations across the intestine is a specific event and questions the possibility of a "common pathway" for the movement of all ions across the membrane.

Supplementation of the diet with other cations has been shown to lower the absorption of lead. Fine, Barth, Sheffet and Lavenhar (1975) have reported that increased dietary levels of magnesium resulted in a twofold decrease in the absorption of lead. Cerklewski and Forbes (1976) have reported a similar effect using zinc. Although the evidence indicates that other dietary cations may influence the absorption of lead, no suggestion as to the manner in which a mechanism for lead transport may be susceptible to a competitive effect has been postulated.

1.8 The Effect of Chelating Agents on the Intestinal Absorption of Lead

Chelating agents may interfere with the process of lead absorption by either promoting or reducing the movement of lead across the gastrointestinal tract. Garber and Wei (1974) reported that oral administration of D-penicillamine, nitriloacetic acid, citric acid and sodium citrate increased the intestinal absorption of lead. The same authors also demonstrated that although both ethylenediaminetetraacetic acid (EDTA) and diethyltriaminepentaacetic acid (DTPA)

are poorly absorbed (Foreman and Trujillo, 1954), their presence enhanced lead transport.

Jugo, Maljkovic and Kostial (1975) showed that parenteral and oral administration of chelating agents such as EDTA increased the absorption of lead across the gastrointestinal tract but the precise physiological mechanism of the action is unclear. Both groups of authors have carried out their experimentation *in vivo* and no account is made of the influence of dietary components on the action of chelating agents administered.

1.9 The Site and Mechanism of the Absorption of Lead

Despite a number of studies on the influence of various dietary components on the absorption of lead, few authors have attempted to elucidate the site and mechanism of transport of the lead cation across the gastrointestinal tract. The few studies reported are far from rigorous and provide only limited information concerning the intestinal absorption of lead.

Gerber and Deroo (1975), injected 7.5 mg/ml ^{210}Pb into the intestinal lumen of the rat and measured its rate of absorption by sampling the radioactivity appearing in the portal vein. Their observations indicated that more lead was absorbed by the jejunum than the colon or duodenum; and that young rats absorbed at least twice as much lead as adult rats. Lead absorption across the ileum was not measured. The intestinal absorption of lead was independent of the quantity of lead administered.

Few studies of lead absorption by *in vitro* techniques have been reported. Cikrt (1970) measured the intestinal absorption of 10^{-6} M and 10^{-5} M carrier free ^{212}Pb, from the mucosal solution, using the everted sac preparation. Lead in the tissue and serosal fluid was assessed after 60 minutes incubation. Loss of activity from the bathing medium was measured by sampling at 5 minute intervals during the course of the incubation.

Cikrt (1970) reported a rapid loss of lead from the bathing solution during the first fifteen minutes of incubation after which a consistent rate was achieved. Little lead was present in the serosal fluid and there was no significant difference in the amount of lead transported across the duodenum and the ileum. However, a significantly greater amount of lead was taken up by the ileal tissue than the duodenal tissue. The same trend of a loss of activity from the mucosal bathing medium was seen for both concentrations. There were no observations reported with regard to the transfer of lead across the jejunum or the physiological viability of the intestinal preparation in the presence of lead.

Interpretation of Cikrt's data is difficult as

1. Removal of aliquots from the medium during incubation reduces the amount of lead available to the tissue.
2. Serosal contents and tissue lead were only measured after 60 minutes incubation, therefore only an indirect assessment of the tissue uptake of lead at different time intervals has been made.
3. The effect of the interaction between lead and tissue on the movement of lead into the serosal compartment was not defined.

Gruden and Stantic (1975) using radioactive carrier free ^{203}Pb, at equal concentrations (0.7×10^{-6} M) in both the serosal and mucosal bathing media, measured serosal fluid/mucosal fluid ratios of lead after incubation of everted sacs for one time period only (90 min). No test for the physiological viability of the everted sacs was reported. They observed no accumulation of lead in the serosal medium for any region of the intestine investigated.

However, the reports are a little superficial as no kinetic analysis of either serosal transfer of tissue uptake was performed. Nor was

there any suggestion as to the mechanism or route of transfer of lead across the gastrointestinal tract.

1.10 Aims of the Present Study

Most of the previous studies concerning the absorption of lead have been performed at concentrations far in excess of the normal dietary levels of the element. It is doubtful whether the effects of low levels of lead can be assessed by extrapolation from observations made using high lead concentrations. Although various authors have suggested that comparatively little lead passes across the intestinal barrier, no indication is given of the site or mechanism for the transport of lead.

Consequently the aim of the present study was to measure various aspects of lead transport, at concentrations which are within the usual dietary range (10^{-7} M to 5×10^{-5} M - lead). The everted sac preparation of Wilson and Wiseman (1954) was chosen for the following investigations:-

1. The rate of the uptake of lead by the tissue and its transfer into the serosal compartment in the presence of different mucosal concentrations of lead.
2. To define a preferential site(s), if any, of absorption.
3. To attempt to elucidate the mechanism of transport of the lead cation.
4. To identify factors which influence the movement of lead across the gastrointestinal barrier and elucidate their mechanism of action.
5. To indicate the importance of the gastrointestinal tract as the first physiological barrier between the environment and the body.

CHAPTER 2

METHODS AND MATERIALS

2.1 Animals

The animals used in this study were male Wistar rats, between 190 g and 220 g in weight, bred by Bantin and Kingman Limited. They were maintained on Heygates 4 lb diet and water ad libitum in an animal house at 20°C, and starved of food but not water, 24 hr prior to sacrifice. The food and water was analysed for lead content by atomic absorption (A. Coombs, City of Birmingham Analyst) and was found to contain less than 0.1 parts per million (ppm).

2.2 Physiological Buffer Solution

Bicarbonate buffer solution was used throughout this work (Krebs and Henseleit, 1932). The solution contained sodium chloride (112 mM), potassium chloride (4.5 mM), calcium chloride (2.4 mM), potassium dihydrogen phosphate (1.12 mM), magnesium sulphate (1.12 mM) and was buffered to pH 7.4 with sodium bicarbonate (2.4 mM). The sodium bicarbonate solution was treated with carbon dioxide until acid to phenolphthalein before mixing, to prevent formation and precipitation of calcium carbonate. This solution, with the addition of glucose (20 mM) is referred to as Krebs-Henseleit bicarbonate buffer throughout the text. The reagents were analysed for lead content by atomic absorption (A. Coombs, City of Birmingham Analyst) and the total lead content was found to be less than 0.1 ppm and can be considered as negligible.

Analar grade reagents, obtained from Fisons Limited, Sigma Company Limited, BDH Limited and Ciba-Geigy Limited, were used throughout these investigations.

2.3 Preparation of the Everted Sac for Transport Studies

Inactin (5-sec butyl - 5 - ethyl - 2 - thiobarbituric acid, Promonta) used to anaesthetise the animals, was administered intraperitoneally in 0.154 mM saline, to give an injected dose of 100 mg/Kg bodyweight. The abdomen was opened with a mid-line incision and the

duodenum removed in two portions by severing at the pyloric sphincter, the ligature of Trietz and the point of entry of the bile duct. Both duodenal sections were immediately transferred to 25 ml of oxygenated Krebs-Henseleit bicarbonate buffer at 0°C. The remainder of the small intestine, from the ligature of Trietz to the ileo-caecal junction was removed into 150 ml of oxygenated Krebs-Henseleit bicarbonate buffer at 0°C. Debris was removed from the intestinal lumen by flushing through with Krebs-Henseleit bicarbonate buffer.

A maximum of three sacs were prepared from each animal, representative of the distal duodenum, mid-jejunum and distal ileum (equivalent to sacs D2, V and XI described in Chapter 3.4). Each section selected for investigation was everted over a glass rod (1.5 mm diameter) as described by Wilson and Wiseman (1954), ligatured at one end and gently blotted on Whatman's No. 1 filter paper saturated with ice cold saline. Care was taken to ensure that the everted sac never came into contact with dry filter paper as this could be seen to damage the mucosal surface. The sac was weighed (W1) on a torsion balance (Whites Elec. Inst. Co.). Gripping the open end of the sac with fine forceps, approximately 0.5 ml of Krebs-Henseleit bicarbonate buffer was introduced into the sac via a blunt-needled 1 ml disposable syringe. The needle was withdrawn and a second ligature tied to seal the sac. The sac was reweighed (W2) and incubated in a 25 ml Erlenmeyer flask containing 10 ml of Krebs-Henseleit bicarbonate buffer. $95\%O_2/5\%CO_2$ was continuously delivered to the incubation solution via a polythene tube. Incubation temperature was maintained at 37°C in a Techne Temperor shaking incubator running at 80 oscillations per minute.

After incubation the sac was weighed (W3) and the serosal contents allowed to drain into a sample vial. The empty sac was then blotted and reweighed (W4). Fluid movement into the sac was assessed from the four weighings.

W1 = Weight of empty sac + 1 ligature thread

W2 = Weight of filled sac + 2 ligature threads

W3 = Weight of filled sac + 2 ligature threads after incubation

W4 = Weight of empty sac + 2 ligature threads after incubation

Then

W3 - W2 = Total fluid uptake

W4 - W1 = Tissue fluid uptake

(W3 - W2) - (W4 - W1) = Serosal fluid uptake

The weight of the cotton threads used to ligature the sacs was estimated by determining the average weight of dry and wet threads of standard length. These were found to be negligible in comparison to the weight of the sac (< 1%) and ignored in calculation of water movement.

2.4 Preparation of Cannulated Sacs for Measurement of Transmural Potential Difference

Transmural potential difference was measured by the method of Barry, Dikstein, Matthews, Smyth and Wright (1964). A section of everted intestine, approximately 8 cm in length was ligatured to a flared hollow glass rod as illustrated in Fig. 2.1. The lower end was tied with a cotton ligature and a small weight attached in order that the sac remained vertical during the experiment. Krebs-Henseleit bicarbonate buffer was introduced into the sac with a syringe, via the glass cannula, care being taken to exclude bubbles and ensure a continuous column of fluid. The filled sac was immersed in 30 ml of Krebs-Henseleit bicarbonate buffer and continuously gassed with $5\%CO_2/95\%O_2$. Electrical contact between serosal and mucosal solutions was maintained via 3M KCl-Agar salt bridges led away to calomel half cells. The salt bridges were prepared using polythene tubing of constant internal diameter and care was taken to ensure that no bubbles were trapped in the agar gel. Transmural potential difference

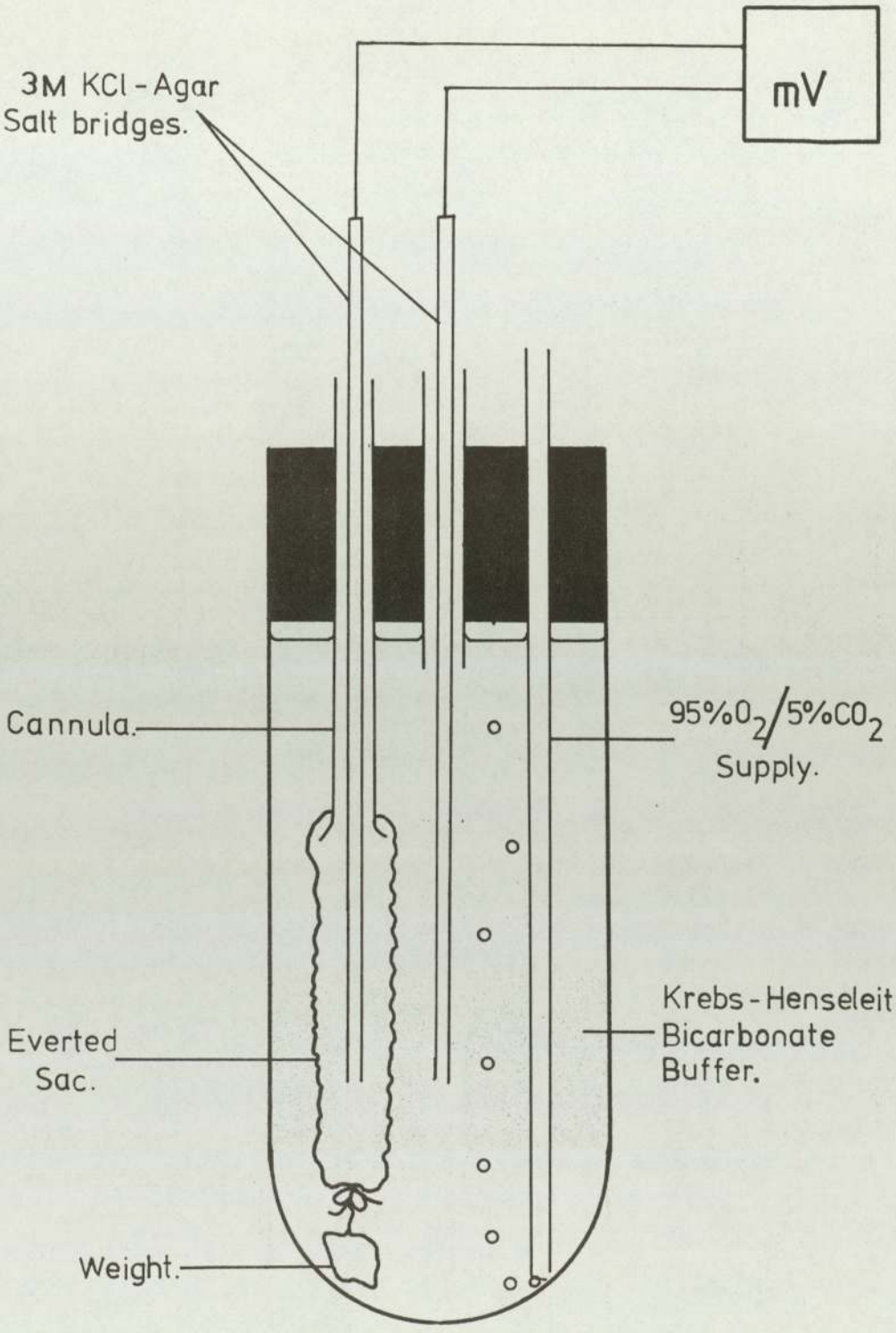


Figure 2.1.

Apparatus for measurement of transmural potential difference

readings were measured in millivolts from a Pye Unicam digital voltmeter. At the end of 60 min incubation serosal and mucosal fluid were sampled for subsequent assay of glucose.

2.5 Treatment and Assay of Fluid and Tissue Samples

Glucose Estimation

Glucose in serosal and mucosal solutions was estimated colormetrically by an automated version (Salway, 1969) of the ferricyanide reduction method (Hoffman, 1937) which measures total reducing sugar. A calibration curve constructed from glucose standards was used to estimate the amount of glucose in the serosal and mucosal solutions. Glucose transport was expressed as the ratio of serosal glucose concentration to mucosal glucose concentration (S/M ratio).

Estimation of ^{14}C

^{14}C labelled, folic acid, and 5 - methyltetrahydrofolic acid obtained from the Radiochemical Centre, Amersham, were used to measure the transport of these species across the intestine. The radioactivity present in serosal fluid samples was estimated by pipetting 400 µl of the serosal solution and 1 ml of distilled water into 10 ml of scintillant, (constituents:- toluene, 1 H; Emulsifier No 1 Mix, (500 ml) (Fisons Limited); 2,5 - diphenyloxazole (PPO), 5 g and 1,4 - bis (5 - phenyloxazol - 2 - yl benzene (POPOP), 0.1 g, (Koch-Light Laboratories). The samples were counted for 10 min or up to a maximum of 200,000 counts in a Nuclear Enterprises 8312 counter employing the β counting facility. Counts obtained were corrected using the external standard ratio method.

After incubation, the everted intestinal tissue was dried in an oven at 100°C for 48 hr. The tissue sample was then oxidised in a Beckman Biological Material Oxidiser. The equipment was allowed to reach its thermostatically controlled operating temperature (900°C) and primed in accordance with the manufacturers instructions using

500 mg aliquots of mannitol. The oxygen flow rate was kept steady throughout by means of an adjustable flow meter, and the apparatus was checked and standardised periodically using known amounts of ^{14}C activity.

Samples were burnt in boats supplied by Beckman Limited in an electrically heated combustion chamber under a continuous stream of oxygen, and the oxidation completed over a high temperature catalyst bed. The final effluent gas consisted entirely of carbon dioxide and water vapour. Care was taken to keep the sample weight well within the manufacturer's recommended range (0 - 150 mg). The $^{14}CO_2$ effluent gas was collected in 15 ml of ^{14}C absorber - P (Fisons Limited) and counted in the Nuclear Enterprises 8312 as described above.

Estimation of ^{203}Pb

The radioisotope ^{203}Pb, obtained from the Medical Research Council Cyclotron Unit, Hammersmith Hospital, was used as the tracer in all lead transport studies. (Originally it was intended to use the isotope ^{210}Pb (Radiochemical Centre, Amersham) but for the reasons outlined in the appendix, ^{203}Pb was preferred to ^{210}Pb for these studies). As ^{203}Pb is a γ emitter the radioactivity of both fluid and tissue samples can be directly assessed without using scintillants or tissue oxidation techniques. 400 μl fluid samples were counted in capped tubes for 10 min or up to a maximum of 200,000 counts, in a Nuclear Enterprises 8312 counter using the γ facility. Radioactivity in intestinal tissue was estimated by placing the entire sac in an insert tube and counting as described above,

The half-life of ^{203}Pb is only 52.4 hr. From the equation

$$A = Aoe^{-\lambda t}$$

where A is the counting rate at time t

Ao is the counting rate at time to.

the theoretical decrease in activity, with time was calculated. Using this information the sample counts were corrected to compensate for the decay in activity.

2.6 Calculation of Results

All results presented are the mean of six observations and are expressed as mean $\pm$ S.E. of mean. Student's t test was used to analyse the results and regression lines were achieved using the method of least squares. Statistical calculations were performed on an Olivetti Programma 101 Computer. $p < 0.05$ denoted by a single asterisk (*) $p < 0.001$ denoted by a double asterisk (**).

CHAPTER 3

VIABILITY OF THE EVERTED SAC PREPARATION

3.1 Introduction

No single *in vivo* or *in vitro* technique is entirely satisfactory to study the mechanism of transport of a cation across the gastrointestinal tract. However of the numerous techniques available, the everted sac preparation described by Wilson and Wiseman (1954) is relatively easy to prepare and allows a number of experimental parameters to be measured at the same time. The main advantages of this preparation are: the ready sampling of serosal, mucosal and tissue contents and the complete control over conditions on both sides of the intestinal wall. For these reasons the technique was selected to investigate the passage of the lead cation across the gastrointestinal tract.

As the intestinal tissue has been removed from its normal physiological environment, it must receive adequate oxygenation and sufficient nutrition from the incubation solution to remain viable. Viability of an everted sac preparation may be assessed by measuring metabolic processes and active transport mechanisms. Several investigators have measured transmural potential difference, oxygen consumption or transport of glucose and suggested that their preparations are viable for several hours after sacrifice of the animal (Barry et al, 1964; Bamford, 1966; Jordana and Ponz, 1969; Robinson and Felber, 1966).

There have been comparatively few studies regarding the structural integrity of the everted sac preparation. Levine, McNary, Kornguth and Leblanc (1970) examined the histological appearance of everted sacs of rat and hamster intestine after periods of incubation at different temperatures. They concluded that a structural dissolution of the mucosal epithelium began after a few minutes incubation at 37°C which led eventually to complete destruction after one hour. The effect was thought to be the result of

water absorption by the tissue.

Gibaldi and Grunhofer (1972) showed that over a two hour period of incubation the transport of neutral, lipid soluble species remained constant but that the sac permeability to ionised species increased continuously. It was suggested that the effect could be a result of the destruction of mucosal epithelial cells as these represent a rate limiting barrier to ionised species.

3.2 Effect of Temperature

Fisher and Parsons (1949), Ahren and Haglund (1973) demonstrated that damage can occur to intestinal tissue in which the oxygen supply has been interrupted for a period of time. As oxygen is supplied to the intestine via the blood system, some degree of restricted oxygenation is unavoidable during the eversion and preparation of sacs. The problem may be alleviated by lowering the temperature of preparation of the tissue, so that oxidative metabolism is minimised and physiological processes leading to tissue damage are reduced. The bathing medium could also be well oxygenated throughout all stages of preparation.

A number of investigators have carried out successful physiological observations using tissue prepared in ice cold, oxygenated medium and the practice has therefore been adhered to throughout this study (Bamford and Ingham, 1976; Blair, Johnstonand Matty, 1974; 1976).

3.3 Method of Sacrifice:- Anaesthetisation or Stunning

Levin and Syme (1976) and more recently Gardner (1978) have preferred the use of an anaesthetic, as opposed to stunning and decapitation as a means of sacrifice. The authors considered that stunning caused severe shock and gave rise to massive vasoconstriction and blood flow away from the intestinal region. For the present investigation, the thiobarbituarate Inactin was used. In common with other thiobarbituarate drugs Inactin possesses a high degree of lipid

solubility due to the presence of a >C = S bond. This confers on thiobarbituarates an ideal property of a rapid onset of action.

Barry et al (1964) have shown that a difference in electrical potential exists across the intestinal wall, maintained by the flow of ions across the epithelial sheet. Potential difference is stimulated by the presence of glucose and is a readily measured feature of a viable in vitro preparation. Therefore to assess the effect of method of sacrifice on the viability of the everted sacs, potential difference and glucose transport were measured across sacs prepared from both stunned and anaesthetised animals.

Cannulated everted sacs, prepared as described in Chapter 2.4, were selected from the proximal jejunum only, of animals sacrificed either by stunning or anaesthetisation with inactin. Potential difference measurements were made every 10 min for a period of 1 hr during incubation in Krebs-Henseleit bicarbonate buffer at 37°C. Glucose transport was assessed by measuring the concentration of glucose in both the serosal and mucosal solutions after 1 hr incubation, as described in Chapter 2.5.

Preparations from both anaesthetised and stunned rats maintained a transmural potential difference of approximately 5 mV over a period of 40 min. The slight drop in potential difference after 40 min incubation was not significant but was more marked in sacs prepared from stunned animals (Fig. 3.1). Cannulated sacs prepared from anaesthetised animals had a higher mean potential difference, but the result was not significantly greater than that from stunned animals (Table 3.1). Glucose transport expressed as a serosal-mucosal ratio, was also greater across sacs prepared from anaesthetised animals (Table 3.1) but again the difference was not statistically significant.

Neither method of sacrifice appeared to affect the viability of

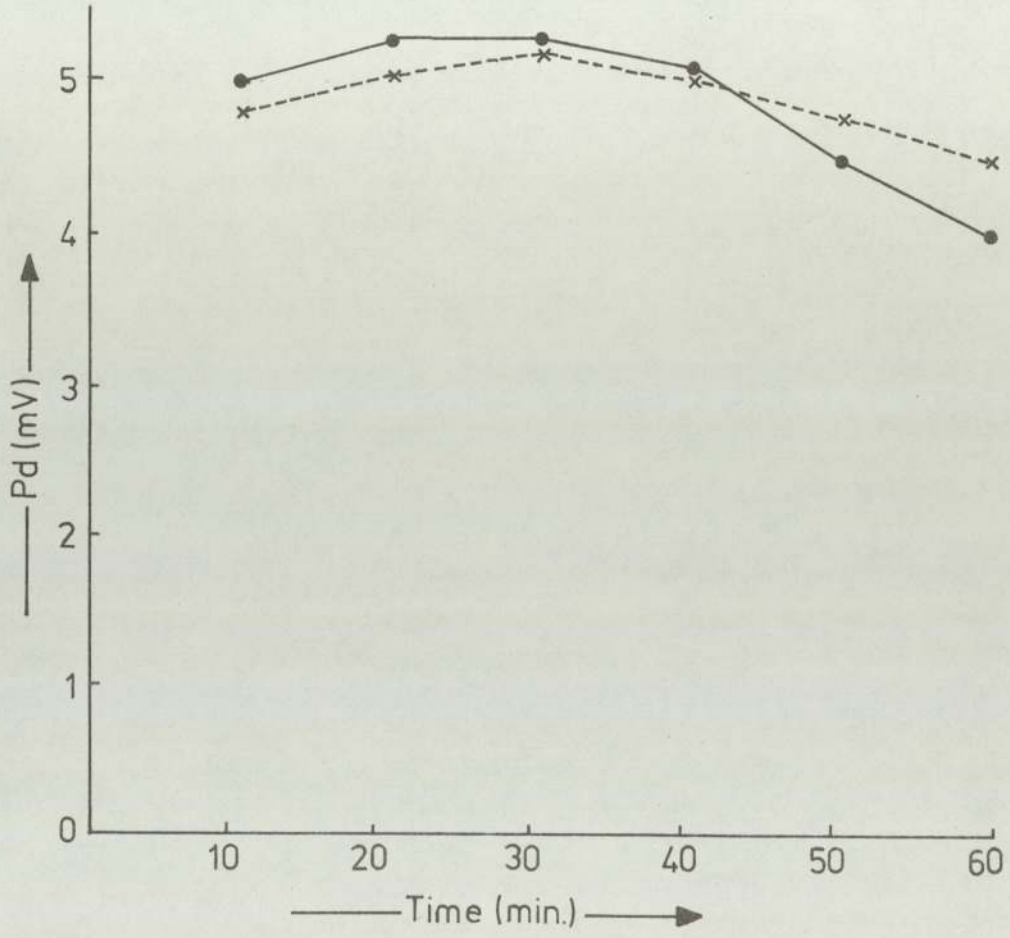


Figure 3.1.

Potential difference across cannulated jejunal sacs prepared from stunned animals (●) and anaesthetised animals (x).

Method of Sacrifice	P.D. (mV)
Stunning	4.86 ± 0.53
Anaesthesia	5.28 ± 0.24

	Glucose Transport (S/M ratio)
Stunning	0.96 ± 0.07
Anaesthesia	1.23 ± 0.14

Table 3.1

Effect of method of sacrifice on mean averaged potential difference and glucose transport across the jejunum.

the preparations. However, throughout the present investigations the anaesthetic, inactin was used as there was a consistently higher potential difference and greater transport of glucose across sacs prepared from anaesthetised animals. The use of anaesthesia was also considered to cause less stress to the experimental animals than other methods of sacrifice.

3.4 The Effect of Lead on Transmural Potential Difference, Glucose Transport and Water Transport

Wapnir, Exeni, McVicar and Lifshitz (1977) have shown that rats, fed a diet which contained 0.1 M lead acetate suffered malabsorption of glucose and amino acids. Sodium transport and mucosal ATP-ase activity was also lowered in lead treated rats. Wapnir et al (1977) suggested therefore that physiological damage occurred in tissues which were subjected to high concentrations of lead. The concentration of lead used in the present investigation was far lower than 0.1 M, nevertheless the physiological viability of the preparation was still assessed in the presence of lead (10^{-6} M, 10^{-5} M) by measurement of transmural potential difference, glucose and water transport.

Potential Difference and Glucose Transport

Potential difference and glucose transport were measured across cannulated sacs prepared from the duodenum (Sacs D1, D2) and twelve consecutive sections of the small intestine (Sacs I - XII) in the presence of 0 and 10^{-6} M-lead. The effect of higher concentrations of lead (5×10^{-6} M, 10^{-5} M) on potential difference and glucose was further investigated using only selected regions of the intestine (distal duodenum, D2; proximal jejunum, II; mid-jejunum, V; proximal ileum, VIII, and distal ileum, XI). Potential difference was measured every 10 min for 1 hr during incubation in Krebs-Henseleit bicarbonate buffer at 37°C, containing 0, 10^{-6} M,

5×10^{-6} M or 10^{-5} M-lead ions. At the end of 1 hr incubation the glucose concentration in both serosal and mucosal fluid was measured, (Chapter 2.5).

Over a period of 1 hr, transmural potential difference was maintained in the presence of lead ions. There was a slight depression in potential difference over the middle regions of the small intestine (Sacs I - VIII) and a slight elevation at the proximal (D1, D2) and distal regions (IX - XII). The only region where a consistent significant effect was observed was the duodenum ($p < 0.01$, Table 3.2). Over the same range of lead ion concentrations there was generally no diminution of glucose transport apart from the proximal ileum where a depression was observed ($p < 0.05$, Table 3.3).

Water Transport

The active accumulation of a solute initiates a local osmotic gradient within the epithelial tissue which in turn results in absorption of water and an elevated hydrostatic pressure which is the force for further transport of water and solute into the serosal compartment. The microvilli of the brush border and the intracellular channels of the mucosal epithelium have been suggested as sites for the establishment of each standing osmotic gradient in the intestine (Diamond and Bossert, 1967; Jackson and Cassidy, 1970). The transport of water across the mucosal surface is therefore a feature of a viable preparation and consequently the ability of the everted sac to transport water was investigated in the presence of lead ions.

Water movement was measured across everted sacs prepared from the distal duodenum (D2), the mid-jejunum (V) and the distal ileum (XI). The sacs were incubated in Krebs-Henseleit bicarbonate buffer at 37°C for either 10, 20, 30, 45 or 60 min in the presence of 0, 10^{-6} M or 10^{-5} M-lead ions.

Over an incubation period of up to 60 min, water movement into

		LEAD CONCENTRATION		
SAC	CONTROL	10^{-6} M	5×10^{-6} M	10^{-5} M
D1	4.86 ± 0.37	5.56 ± 0.26		
D2	4.40 ± 0.33	5.18 ± 0.32	5.44 ± 0.43*	6.07 ± 0.28*
I	4.65 ± 0.19	3.50 ± 0.37*		
II	5.28 ± 0.24	4.71 ± 0.44	6.41 ± 0.64	5.63 ± 0.58
III	7.11 ± 0.83	5.67 ± 0.46		
IV	9.05 ± 0.29	8.88 ± 0.52		
V	8.14 ± 0.55	8.72 ± 0.60	9.04 ± 0.22*	8.90 ± 0.34
VI	8.66 ± 0.53	8.49 ± 0.59		
VII	9.56 ± 0.38	8.88 ± 0.13		
VIII	8.84 ± 0.53	8.57 ± 0.33	8.78 ± 0.50	7.08 ± 0.67*
IX	7.29 ± 0.51	7.57 ± 0.97		
X	5.93 ± 0.56	6.60 ± 0.95		
XI	5.27 ± 0.42	7.19 ± 0.99*	6.78 ± 0.39*	6.77 ± 0.91
XII	4.79 ± 0.30	7.53 ± 0.72*		

Table 3.2

Mean averaged potential difference (over 1 hr) (mV) across cannulated everted sacs in the presence of lead ions.

		LEAD CONCENTRATION		
SAC	CONTROL	10^{-6} M	5×10^{-6} M	10^{-5} M
D1	1.17 ± 0.05	1.20 ± 0.03		
D2	1.27 ± 0.04	1.16 ± 0.06	1.35 ± 0.07	1.34 ± 0.06
I	1.15 ± 0.09	1.31 ± 0.03		
II	1.23 ± 0.14	1.29 ± 0.06	1.42 ± 0.06	1.30 ± 0.02
III	1.41 ± 0.18	1.40 ± 0.10		
IV	1.48 ± 0.05	1.62 ± 0.12		
V	1.76 ± 0.10	1.62 ± 0.19	1.46 ± 0.09*	1.42 ± 0.13*
VI	1.62 ± 0.09	1.63 ± 0.19		
VII	2.03 ± 0.05	1.91 ± 0.13		
VIII	1.79 ± 0.07	1.85 ± 0.13	1.50 ± 0.10*	1.31 ± 0.14*
IX	1.47 ± 0.04	1.41 ± 0.08		
X	0.97 ± 0.09	1.00 ± 0.05		
XI	0.86 ± 0.07	0.92 ± 0.06	1.00 ± 0.03	0.88 ± 0.04
XII	0.72 ± 0.04	0.90 ± 0.04		

Table 3.3

Effect of lead on serosal/mucosal ratio of glucose across cannulated everted sacs after 1 hr incubation.

the serosal compartment proceeded linearly for all concentrations of lead (Fig. 3.2). Tissue uptake of water proceeded linearly over the first 30 min incubation, however after longer periods of incubation, evidence of a slowing of water movement was observed (Fig. 3.3). Lead ions appeared not to affect the viability of the preparation (Table 3.4).

3.5 Conclusions

The method of preparation used throughout this study produced a physiologically viable everted sac. The potential difference, glucose transport and water movement measurements were similar to those reported by Barry et al (1964), Blair et al, (1974), Barry, Matthews and Smyth, (1961) and Gardner, (1978). Despite reports of the toxicity of lead at high concentrations (Wapnir et al, 1977) the concentrations of lead to be used in this investigation had no significant effect on the parameters of viability examined.

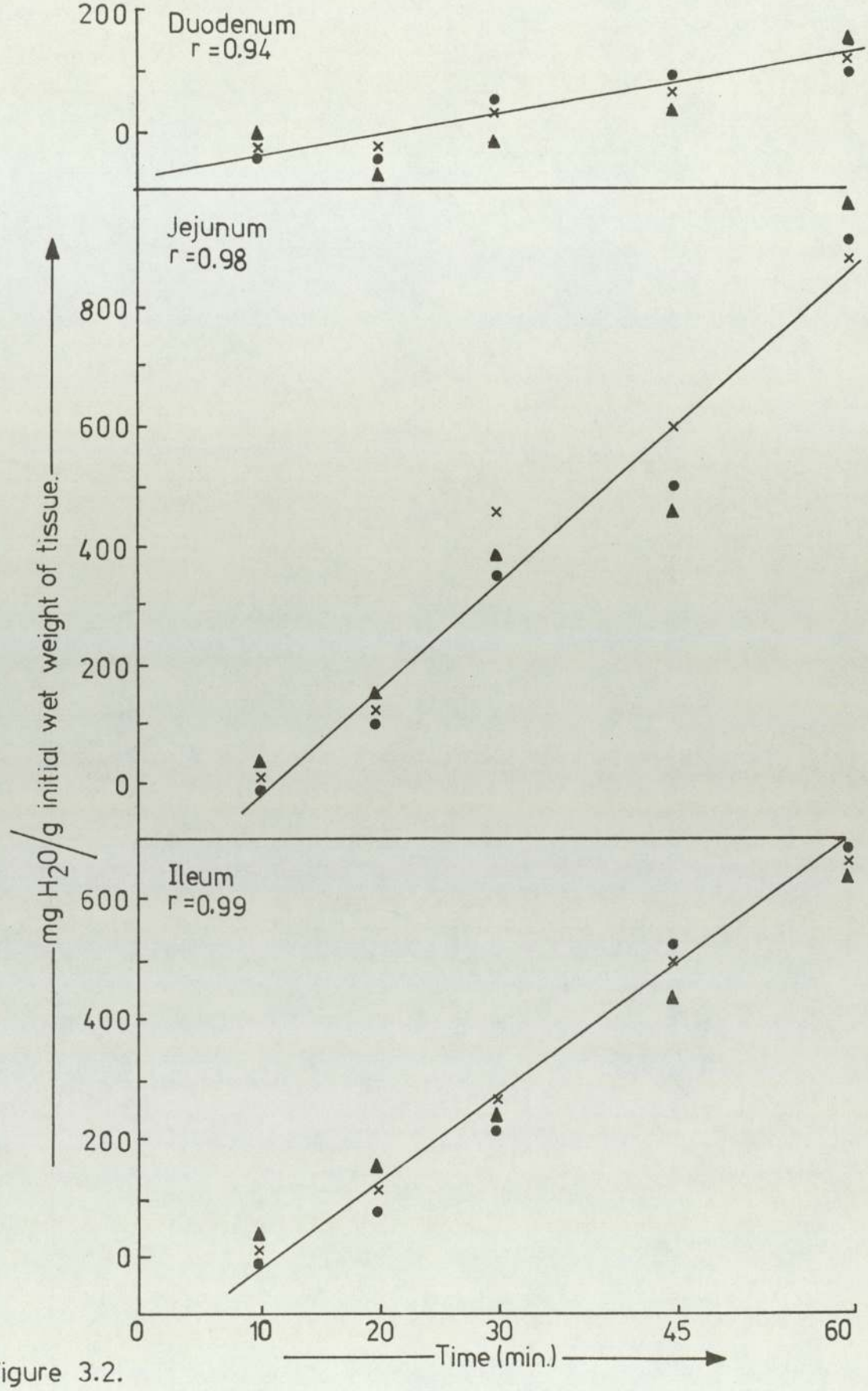


Figure 3.2.

Effect of lead on water movement into serosal compartment

Control (●) 10^{-6} M Pb (x) and 10^{-5} M Pb (▲).

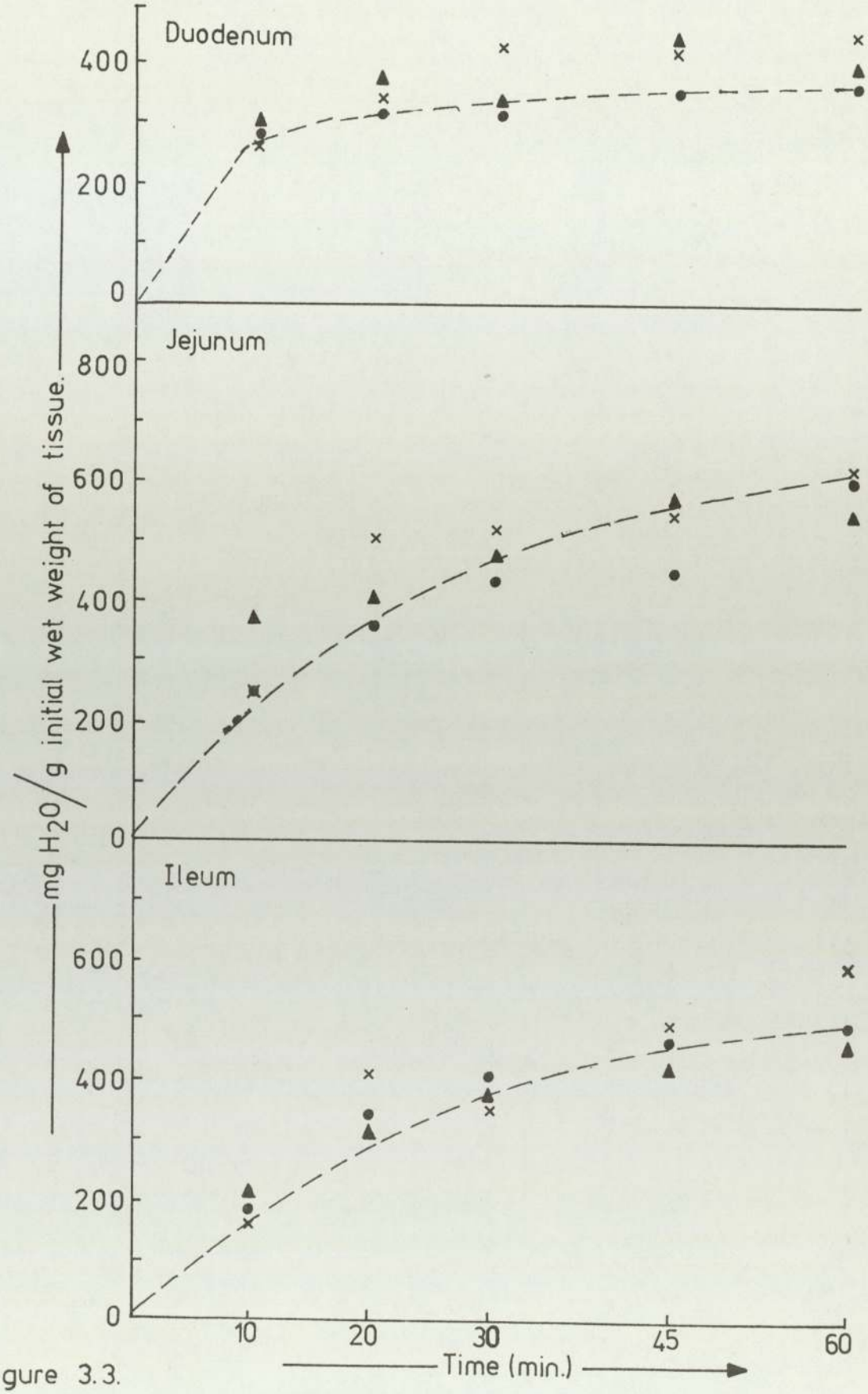


Figure 3.3.

Effect of lead on water movement into tissue compartment

Control (●) 10^{-6} M Pb (x) and 10^{-5} M Pb (▲).

	LEAD CONCENTRATION		
SEROSAL TRANSFER	CONTROL	10^{-6} M	10^{-5} M
Duodenum (D2)	110 ± 46	130 ± 35	155 ± 44
Jejunum (V)	928 ± 85	889 ± 98	984 ± 68
Ileum (XI)	712 ± 63	704 ± 79	635 ± 63
TISSUE UPTAKE	CONTROL	10^{-6} M	10^{-5} M
Duodenum (D2)	369 ± 40	488 ± 45	404 ± 43
Jejunum (V)	625 ± 32	633 ± 33	552 ± 32
Ileum (XI)	490 ± 65	607 ± 51	488 ± 49
TOTAL UPTAKE	CONTROL	10^{-6} M	10^{-5} M
Duodenum (D2)	479 ± 63	578 ± 52	559 ± 71
Jejunum (V)	1553 ± 107	1524 ± 111	1536 ± 81
Ileum (XI)	1202 ± 82	1311 ± 115	1123 ± 86

Table 3.4

Effect of 10^{-6} M and 10^{-5} M-lead on water movement across everted sacs after 60 minutes incubation.

CHAPTER 4

TRANSPORT OF LEAD ACROSS THE GASTROINTESTINAL TRACT

4.1 Rate of Uptake of Lead and its Subsequent Passage into the Serosal Compartment

Time-based studies were carried out to measure the amount of lead transferred across the gastrointestinal tract and the rate at which the transfer took place. Everted sacs prepared as in Chapter 2.3, from the duodenum, jejunum and ileum, were incubated for either 10, 20, 30, 45 or 60 min in Krebs-Henseleit bicarbonate buffer at 37°C containing 10^{-6} M-lead ions labelled with ^{203}Pb. Lead was estimated in the total serosal fluid and the entire sac, as described in Chapter 2.5.

A very small percentage (0.3% - 0.5%) of the lead initially available was transferred from the mucosal bathing medium into the serosal compartment (Fig. 4.1). Although generally the amount of lead transported across the duodenum was greater than across other regions, this difference was not statistically significant. Regression analysis demonstrated that over a 60 min incubation period there was a good linear relationship between time and the entry of lead ions into the serosal compartment for all regions of the intestine (Fig. 4.1). Extrapolation of the best-fit line gives an intercept of the y axis and indicates that there may be a faster initial rate of entry of lead ions into the serosal compartment during approximately the first 10 min of incubation. The amount of lead transported per minute declines with increased time of incubation (Fig. 4.2). However the large standard errors of means in Figures 4.1 and 4.2 precludes further detailed analysis of the data.

Simultaneous measurement of water across all sacs demonstrated that a good correlation ($r > 0.92$) existed between the entry of water and lead into the serosal compartment (Fig. 4.3)

Approximately 10 - 15% of the lead initially available in the mucosal bathing medium was taken up by the intestinal tissue, over

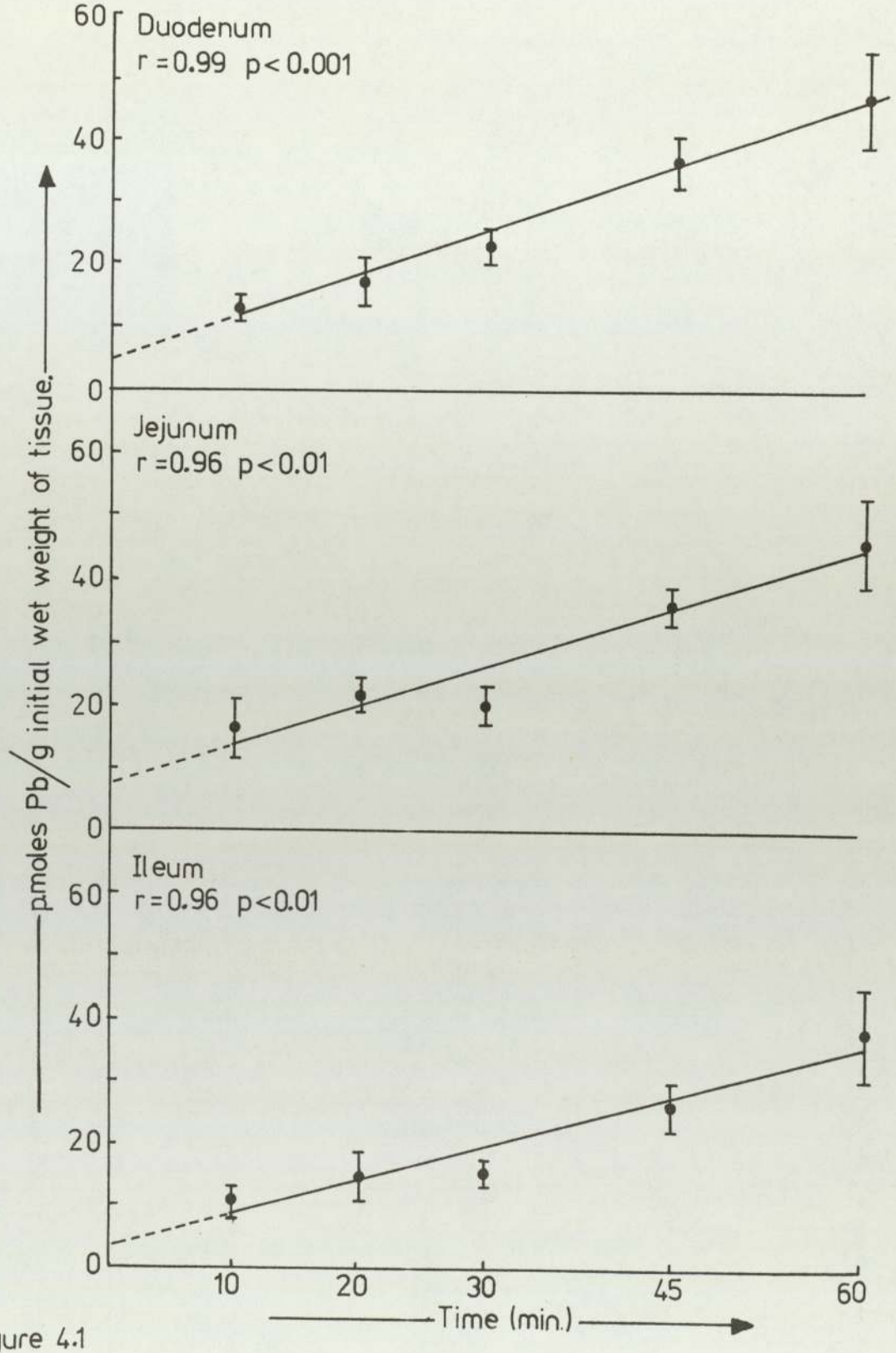


Figure 4.1

Transport of 10^{-6} M-lead into the serosal compartment of the everted sac after incubation for periods up to 60 min at 37°C. Standard error of mean represented by vertical bars.

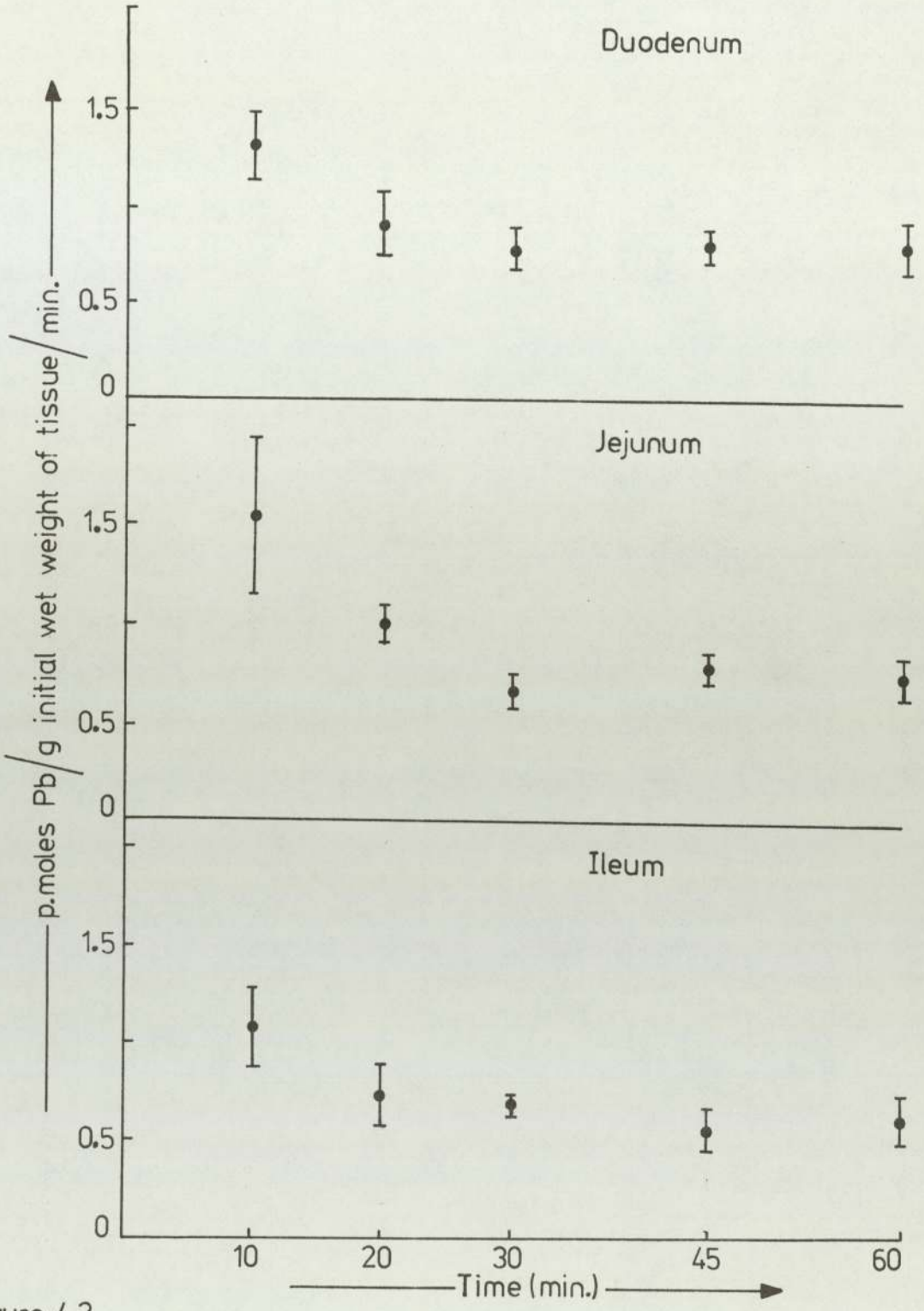


Figure 4.2

Decline in rate of transport of 10^{-6} M-lead into the serosal compartment of the everted sac after incubation for periods up to 60 min at 37°C. Standard error of mean represented by vertical bars.

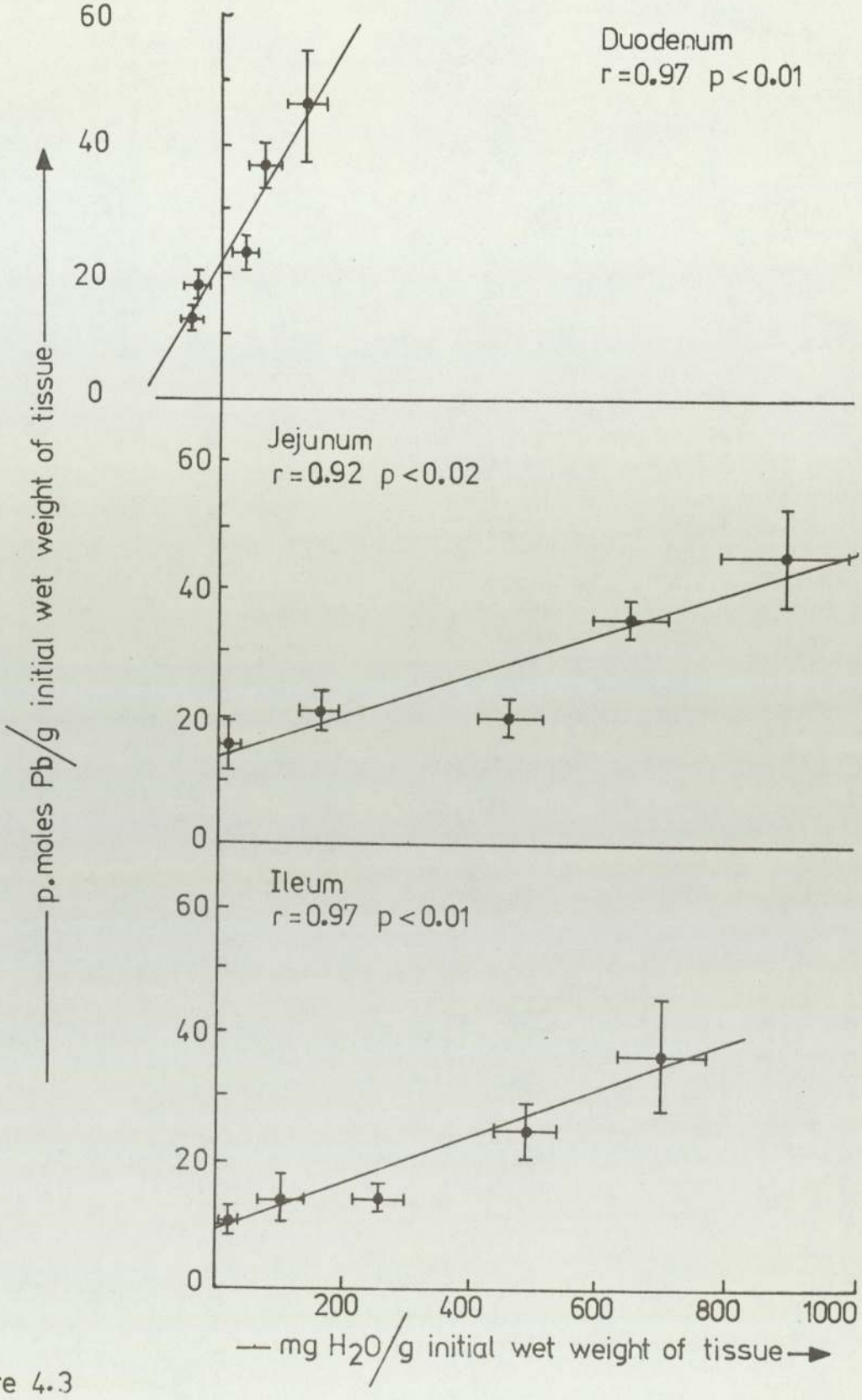


Figure 4.3

Correlation between the transport of water and lead into the serosal compartment after incubation periods of 10, 20, 30, 45 or 60 min at 37°C in 10^{-6} M-lead. Standard error of mean appropriately represented by horizontal and vertical bars.

60 min incubation. Over the first 20 min of incubation there was a rapid rate of uptake after which there appeared to be no further increase in the amount of lead taken up by the tissue (Fig. 4.4). The jejunum was observed to take up more lead on to the tissue than other sacs but again the difference was not significant.

4.2 Effect of Different Mucosal Concentrations of Lead on Tissue Uptake and Movement of Lead into the Serosal Compartment

Experiments carried out to establish the rate of both tissue uptake and serosal entry of lead were repeated under similar conditions of incubation to those described previously. The initial mucosal bathing medium concentrations were 10^{-7} M, 5×10^{-7} M, 5×10^{-6} M 10^{-5} M and 5×10^{-5} M-lead.

At all mucosal concentrations of lead investigated, the same pattern of lead uptake by the tissue and movement into the serosal compartment was observed as illustrated in Figures 4.1 and 4.4. At all mucosal concentrations of lead, the good correlation ($r > 0.92$) between the entry of water and lead into the serosal compartment was maintained. There was an excellent linear correlation ($r = 0.99$) between the initial concentration of lead in the bathing medium and both the uptake of lead by the tissue and the passage of lead into the serosal compartment after 60 min incubation (Figs. 4.5 and 4.6).

Michaelis-Menton kinetic analysis of the data for both tissue uptake and entry into the serosal compartment gave Lineweaver-Burke plots of excellent linearity ($r = 0.99$). The plots passed through the origin, giving Km and Vmax values of infinity which precludes the suggestion that lead is transported by a saturable carrier-mediated system.

The data presented are comparable to those of Sahagion, Harding-Barlow and Perry (1967) who demonstrated by means of an *in vitro* perfusion of the intestine that there is a rapid uptake of zinc, cadmium

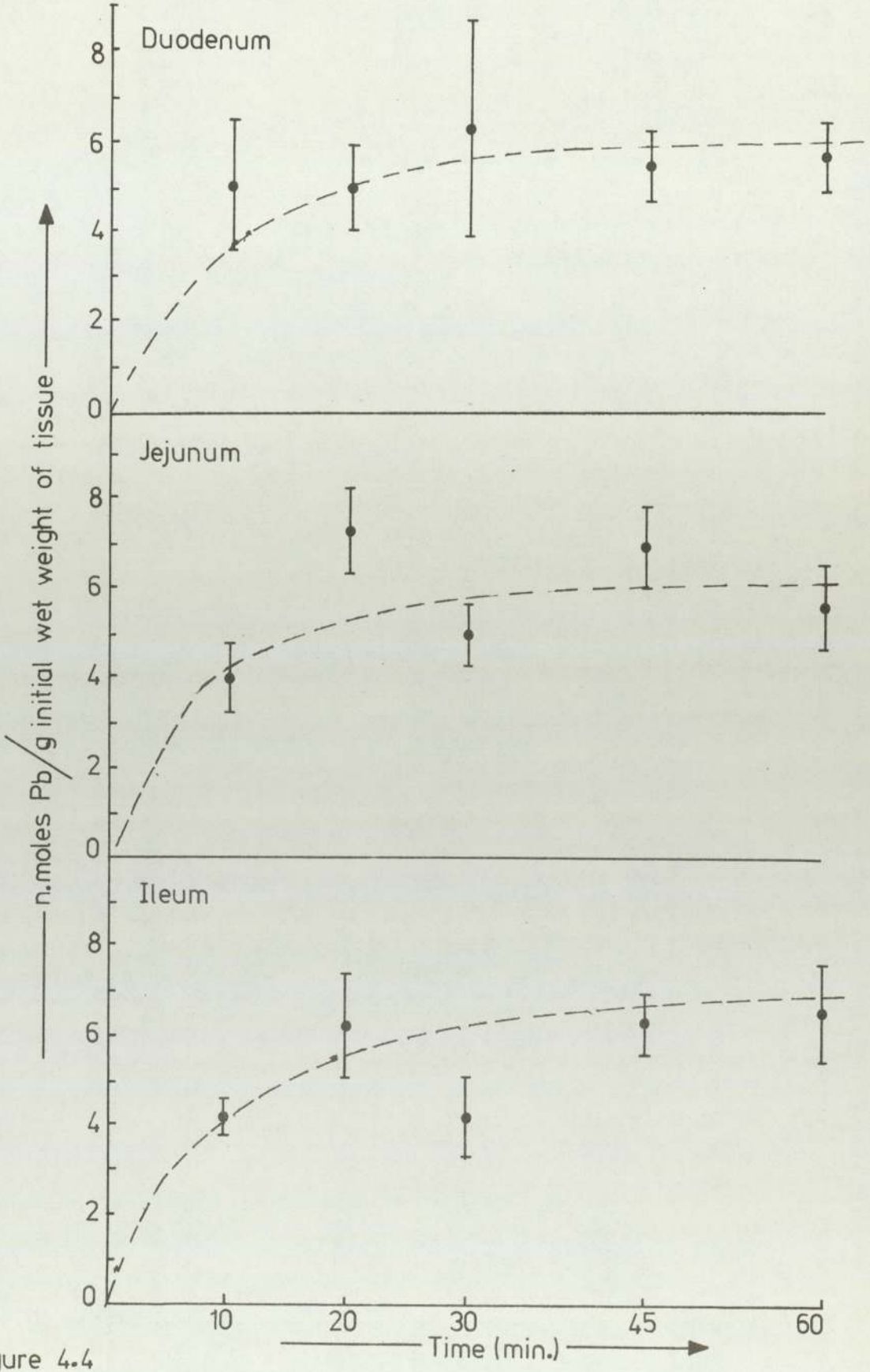


Figure 4.4

Uptake of 10^{-6} M-lead by the intestinal tissue after incubation for time periods up to 60 min at 37°C. Standard error of mean represented by vertical bars.

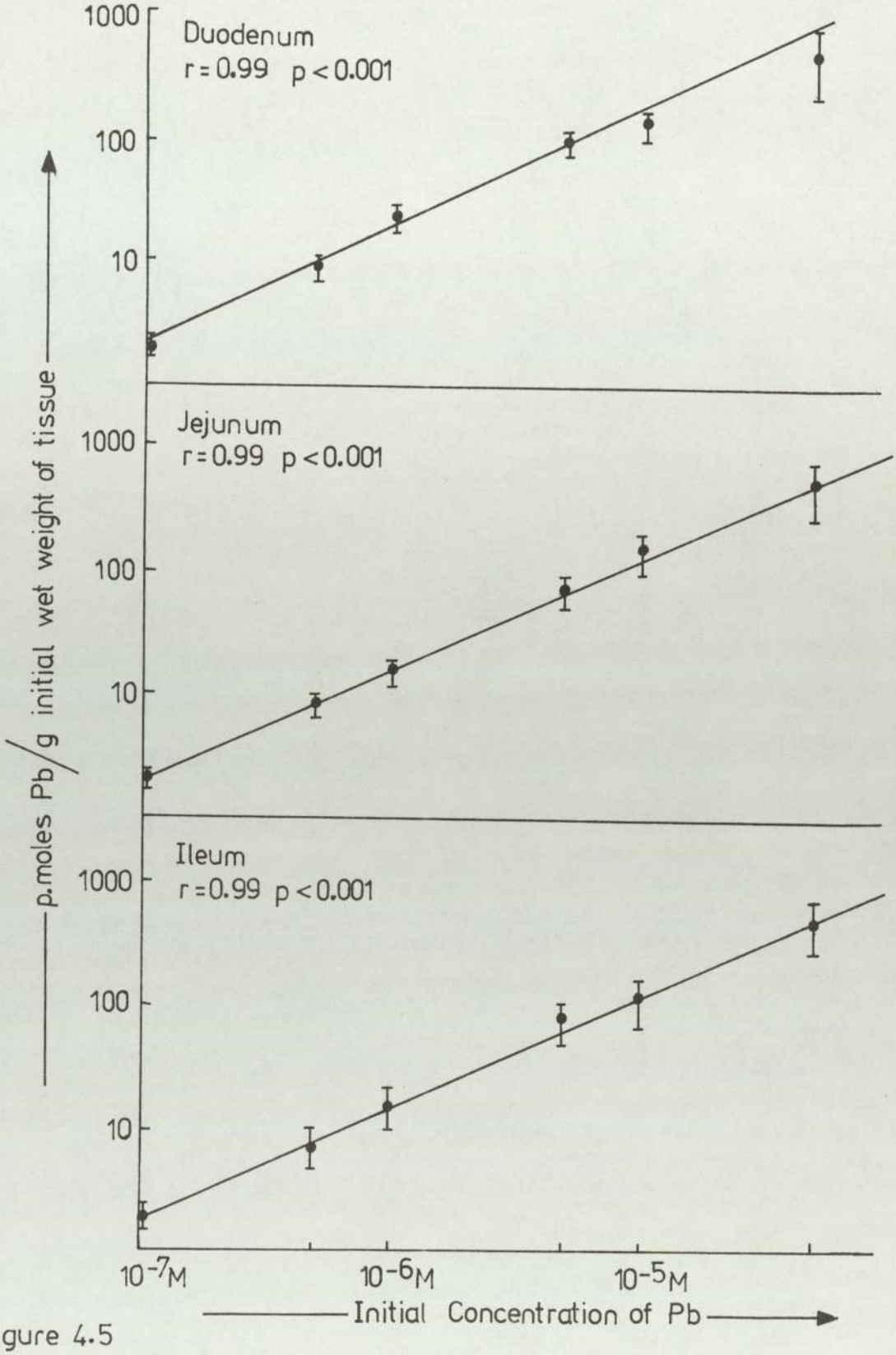


Figure 4.5

Transport of lead into the serosal compartment after 30 min incubation at 37°C in Krebs-Henseleit bicarbonate buffer containing 10^{-7} - 5 x 10^{-5} M lead. Standard error of mean represented by vertical bars.

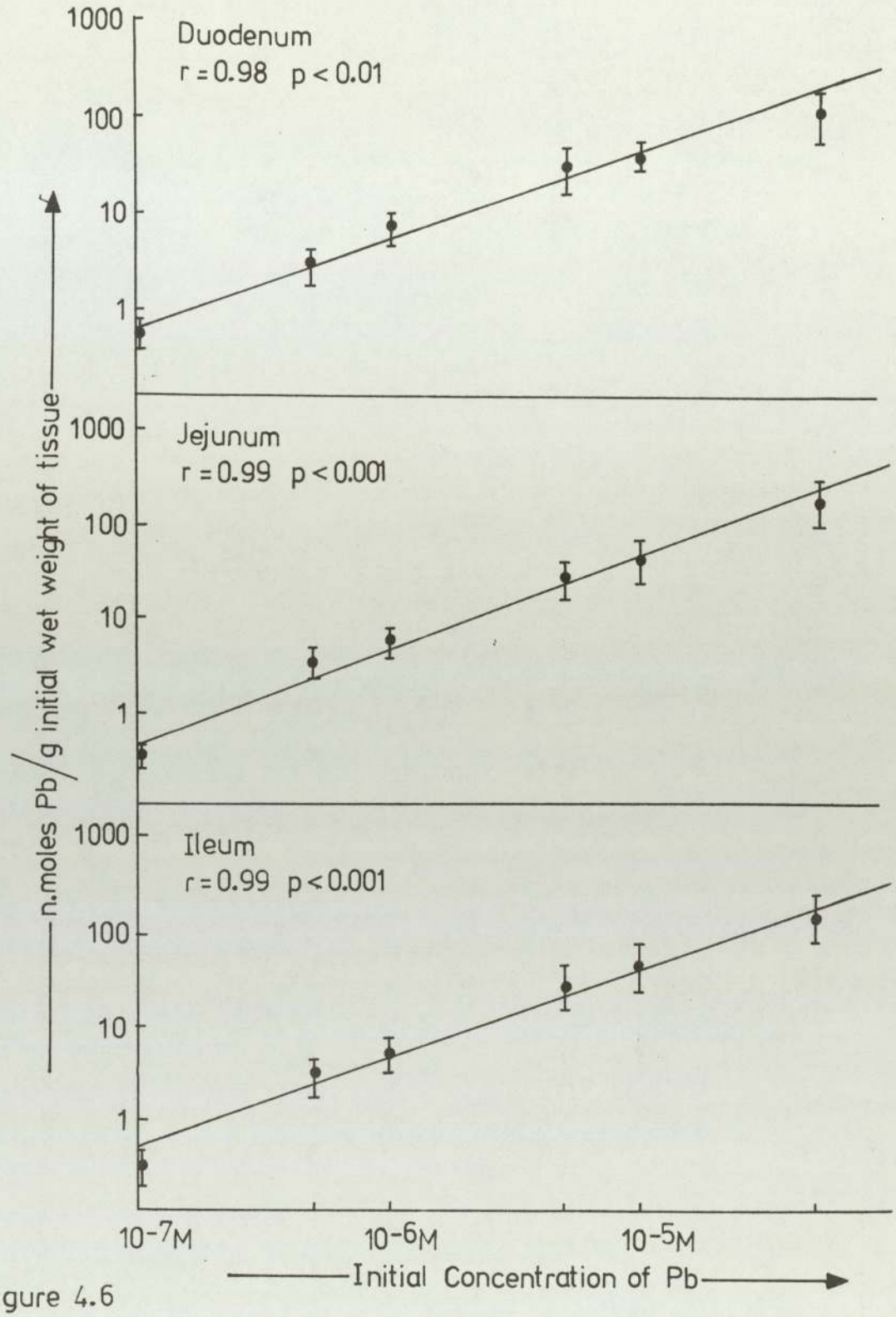


Figure 4.6

Uptake of lead by the intestinal tissue after 30 min incubation at 37°C in Krebs-Henseleit bicarbonate buffer containing 10^{-7} - 5 x 10^{-5} M-lead. Standard error of mean represented by vertical bars.

and mercury by the intestinal tissue but only a slow rate of transport to the serosal compartment. It would appear that the passage of certain trace metals across the intestinal wall may be dependent upon two separate rate-limiting steps. The first is the uptake and binding of the metal at cellular surfaces, and the second, the transport of the metal across the intestinal membrane and its subsequent accumulation in the serosal compartment. Therefore the transport of lead may depend upon:-

a) the affinity of lead for binding sites on the tissue

b) the number and availability of particular sites

c) the nature of the interaction between the lead cation and intestinal tissue.

4.3 The Nature of the Interaction Between Intestinal Tissue and Lead

The rapid massive binding of lead to the intestinal tissue and the slower entry of lead into the serosal compartment suggests that lead is strongly bound to the tissue by a surface adsorption process. To investigate the tenacity and possible nature of this binding, sacs prepared as described in Chapter 2.3 were initially incubated for 30 min in Krebs-Henseleit bicarbonate buffer containing 10^{-6} M-lead labelled with ^{203}Pb. After 30 min the sacs were transferred to either lead-free Krebs-Henseleit bicarbonate buffer, the same buffer containing 10^{-6} M-lead acetate or buffer containing 10^{-6} M-calcium trisodium diethylenetriminepentaacetic acid (DTPA) and incubated for a further 30 min. None of the solutions contained radioactive lead. During the second period of incubation 100 µl aliquots of the mucosal bathing solutions were removed at 5 min, 10 min, 20 min and 30 min intervals. Radioactive lead in these aliquots, as well as in the serosal fluid and tissue were assayed as in Chapter 2.5.

During the second incubation period no more than 35% of the lead originally bound to the tissue was removed. Approximately half was

removed during the first 5 min. More lead was removed from the duodenum than from the jejunum or ileum, irrespective of the incubation solution used (Fig. 4.7). For all three regions of the intestine a greater percentage of lead was removed by washing in leaded buffer than in the other two solutions, but the difference was not statistically significant.

If the interaction between the lead ions and the tissue is ionic, a rapid exchange between the lead in the tissue and that in the mucosal medium would be expected. However, the exchange was found to be slow and incomplete. The inability of 10^{-6} M-DTPA to remove the lead from the tissue by chelation indicates a tenacious binding of lead to the intestine. Both these facts are indicative of a covalent binding of lead to the tissue.

Recently Barton and Conrad (1978) suggested that an "acceptor" in the intestinal mucosa becomes saturated with lead, and will not accept more lead from the lumen of the gut, until the mucosal lead is transferred into the body of the animal. The same authors also report the isolation of two heat-stable intestinal protein fractions capable of binding lead (Barton, Conrad, Harrison and Nuby, 1978a and Barton, Conrad, Nuby and Harrison, 1978b).

However, the external surface of the mucosal epithelial cells is rich in phosphate ions, partially due to the hydrolysis of ATP (Koenig and Vial, 1970). Lead ions in the tissue could therefore be sequestered in a covalent form as lead dihydrogen phosphate. This interaction is analogous to that which occurs between erythrocytes and lead cations (Clarkson and Kench, 1958). A consequence of lead being sequestered as lead dihydrogen phosphate would be that lead would be less available for transport into the serosal compartment. Although it is often difficult to extrapolate from <u>in vitro</u> data to the normal physiological condition, it may be suggested that the

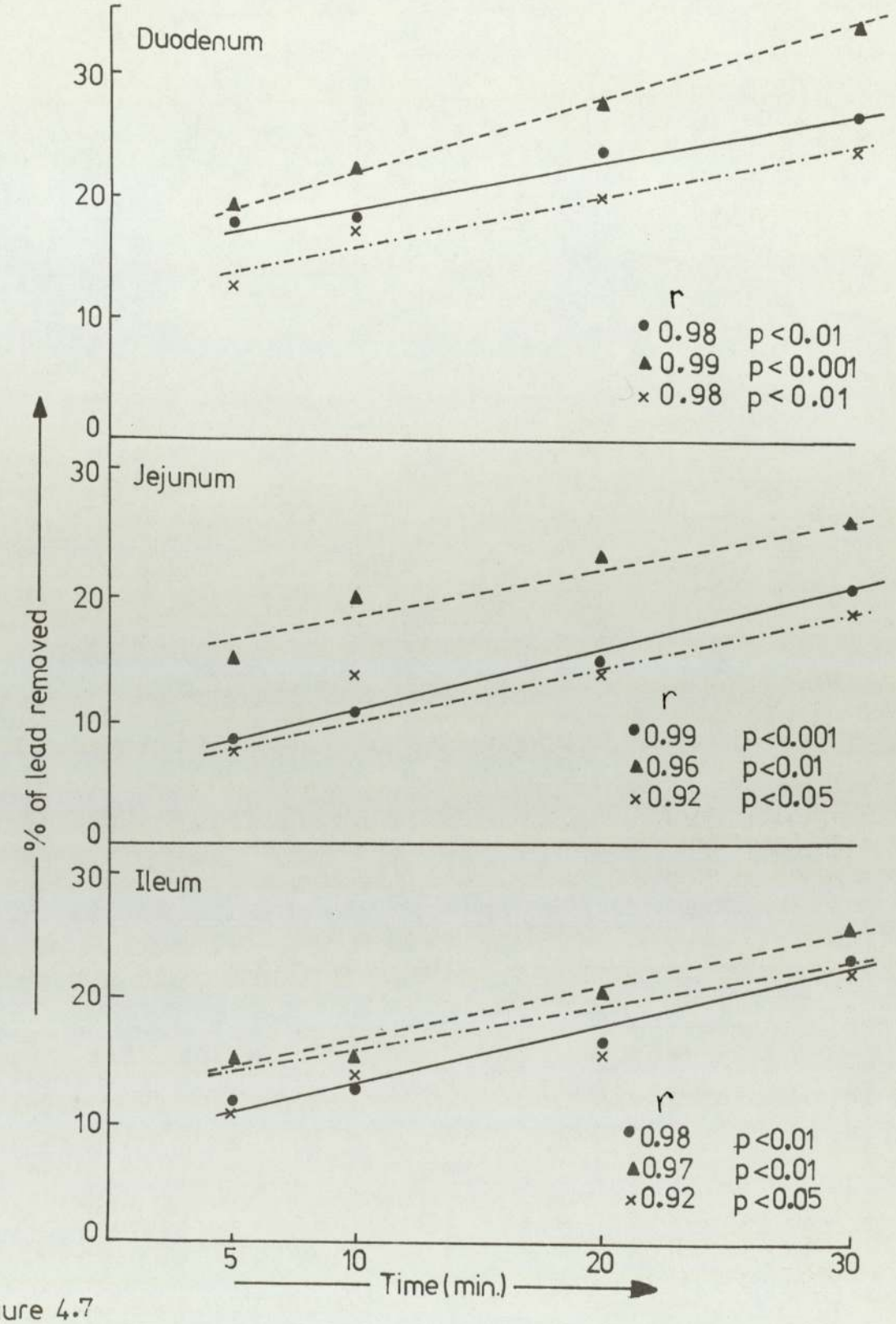


Figure 4.7

Effect of washing in Krebs-Henseleit bicarbonate buffer (●), buffer containing 10^{-6} M-lead (▲) or buffer containing 10^{-6} M - DTPA (x) on the removal of lead from everted sacs initially incubated for 30 min at 37°C in 10^{-6} M-lead.

tenacious binding of lead by the intestinal membrane may act as a protective mechanism against the absorption of a large proportion of the lead present in the normal diet.

4.4 The Effect of Initial Mucosal Volume on the Transport of Lead

The experimental system described in these investigations to measure the transport of lead across the intestine involves the incubation of approximately 0.5 g of tissue in 10 ml of Krebs-Henseleit bicarbonate buffer. The fluid to tissue ratio in the experimental design is far greater than in the normal physiological condition. Under the usual experimental conditions 10 - 15% of the lead is taken up, therefore the effect of tissue uptake of lead was investigated when the fluid-tissue ratio was reduced to values closer to the physiological condition.

Everted sacs, prepared as described in Chapter 2.3 were incubated for 30 min at 37°C in various volumes of Krebs-Henseleit bicarbonate buffer containing 10^{-6} M-lead labelled with ^{203}Pb. Tissue uptake and transport of lead into the serosal compartment was measured as described in Chapter 2.5.

When the mucosal volume was less than 5 ml there was a reduction in both the amount of lead taken up by the intestinal tissue and that transported into the serosal compartment (Table 4.1). Water movement was also depressed (Table 4.2). However, the percentage of lead taken up by the tissue and entering the serosal compartment was significantly increased ($p < 0.01$) (Table 4.3). It was not possible to measure the uptake and transport of lead using smaller mucosal volumes as the sac would not be completely covered by the bathing solution.

The approximate fluid to tissue ratio under normal physiological conditions is approximately 0.3 ml/g of wet tissue (Black, 1964; Biochemical Handbook, 1971). The lowest fluid/tissue value used in the present study was 5.0 ml/g tissue. By extrapolation it may be

INITIAL MUCOSAL VOLUME (ml)

	2.5	5.0	7.5	10.0 (Control)	20.0
SEROSAL (pmoles Pb/g initial wet weight of tissue)					
DUODENUM	14.9 ± 2.9*	15.4 ± 3.8*	17.3 ± 1.9*	23.1 ± 0.72	43.2 ± 5.8*
JEJUNUM	12.9 ± 1.4**	11.5 ± 1.9**	23.5 ± 6.3	25.0 ± 2.9	31.2 ± 1.9
ILEUM	8.7 ± 1.4*	12.9 ± 5.8*	31.2 ± 3.4	23.0 ± 1.9	36.1 ± 4.8
TISSUE (nmoles Pb/g initial wet weight of tissue)					
DUODENUM	0.8 ± 0.1*	0.7 ± 0.1*	0.8 ± 0.1*	1.3 ± 0.2	3.3 ± 0.8*
JEJUNUM	1.0 ± 0.1*	1.6 ± 0.2	1.7 ± 0.3	2.4 ± 0.6	3.2 ± 0.5
ILEUM	1.2 ± 0.2*	1.1 ± 0.1*	1.3 ± 0.3*	1.9 ± 0.1	4.3 ± 0.7*

Table 4.1

Effect of initial mucosal volume on serosal transport and tissue uptake of lead, after 30 min incubation at 37°C.

INITIAL MUCOSAL VOLUME (ml)

	2.5	5.0	7.5	10.0 (Control)	20.0
SEROSAL					
DUODENUM	-85 ± 10*	-70 ± 23*	-49 ± 48	33 ± 55	64 ± 21
JEJUNUM	178 ± 58*	203 ± 37*	429 ± 121	427 ± 60	345 ± 63
ILEUM	45 ± 13*	82 ± 9*	211 ± 46	290 ± 65	242 ± 56
TISSUE					
DUODENUM	295 ± 64	292 ± 53	373 ± 75	430 ± 48	522 ± 38
JEJUNUM	333 ± 42*	305 ± 26*	367 ± 38	507 ± 61	543 ± 55
ILEUM	78 ± 31*	157 ± 36*	237 ± 74	393 ± 85	389 ± 62
TOTAL					
DUODENUM	210 ± 58*	222 ± 39*	324 ± 58	463 ± 94	586 ± 37
JEJUNUM	512 ± 45**	508 ± 44**	795 ± 138	934 ± 70	888 ± 112
ILEUM	123 ± 35**	239 ± 37*	448 ± 115	683 ± 138	630 ± 91

Table 4.2

Effect of initial mucosal volume on water movement (mg H_2O/g initial wet weight) after 30 min incubation at 37°C.

	INITIAL MUCOSAL VOLUME (ml)				
	2.5	5.0	7.5	10.0 (Control)	20.0
SEROSAL (% x 10^{-1})					
DUODENUM	3.70 ± 0.76*	1.69 ± 0.37	1.28 ± 0.09	1.34 ± 0.45	1.33 ± 0.16
JEJUNUM	2.40 ± 0.02**	1.28 ± 0.28	1.15 ± 0.08	1.38 ± 0.13	0.80 ± 0.05**
ILEUM	1.67 ± 0.23**	0.76 ± 0.15	0.78 ± 0.16	0.72 ± 0.12	0.77 ± 0.12
TISSUE (%)					
DUODENUM	20.2 ± 3.5*	7.9 ± 1.0	5.8 ± 0.8	8.2 ± 1.1	9.8 ± 2.0
JEJUNUM	20.2 ± 3.0*	15.7 ± 1.7	11.0 ± 2.2	13.5 ± 1.2	8.0 ± 1.1*
ILEUM	22.6 ± 3.0**	10.4 ± 1.9	8.0 ± 1.7	9.2 ± 0.8	9.8 ± 1.3

Table 4.3

Effect of initial mucosal volume on serosal transport and tissue uptake of lead as a percentage of lead originally available in the incubation solution.

predicted that the percentage uptake of lead by the tissue would be even greater as the fluid to tissue ratio decreased.

4.5 The Effect of Glucose, Anoxia and Temperature on the Transport of Lead into the Serosal Compartment

Observations, previously reported in this Chapter indicate that lead is initially tenaciously bound to the tissue before being slowly transported to the serosal compartment probably by diffusion, linked to the concommitant movement of water. Kinetic analysis of the previous data precludes the presence of a saturable carrier-mediated system. However no data has been presented to indicate whether the movement of lead across the intestine is energy dependent. An energy dependent transport system would be less effective in the absence of an energy substrate e.g. glucose, under conditions of anoxia, or at reduced temperature. Consequently the transport of lead was measured under these conditions.

Variation of Glucose Concentration

Everted sacs, prepared as in Chapter 2.3 were incubated for 30 min at 37°C in Krebs-Henseleit bicarbonate buffer containing 10^{-6} M - lead labelled with ^{203}Pb, and glucose at initial concentrations of either 0, 10, 20, 30 or 40 mM. In a second experiment the sacs were incubated for 60 min under similar conditions in the presence of 0 or 20 mM glucose.

After 30 min and 60 min incubation there was no significant difference in the amount of lead taken up by the tissue, irrespective of the initial concentration of glucose (Tables 4.4, 4.5). The amount of lead that passed into the serosal compartment was decreased after 30 min incubation. After 60 min incubation the decrease in the duodenum and jejunum was statistically significant ($p < 0.01$) (Tables 4.4 and 4.5). For all regions of the intestine, the movement of water was significantly reduced in the absence of glucose ($p < 0.01$) (Tables 4.6, 4.7).

GLUCOSE CONCENTRATION (mM)

	0	10	20 (Control)	30	40
SEROSAL (pmoles Pb/g initial wet weight of tissue)					
DUODENUM	12.6 ± 1.4	25.5 ± 3.2	23.4 ± 9.3	28.0 ± 5.5	18.9 ± 4.9
JEJUNUM	14.3 ± 2.0	27.6 ± 5.0	20.3 ± 3.1	14.5 ± 2.8	23.2 ± 4.9
ILEUM	9.8 ± 0.67*	18.4 ± 2.1	15.0 ± 1.6	14.2 ± 3.1	18.0 ± 5.3
TISSUE (nmoles Pb/g initial wet weight)					
DUODENUM	1.58 ± 0.33	2.16 ± 0.19	2.10 ± 0.24	1.44 ± 0.29	1.54 ± 0.14*
JEJUNUM	3.46 ± 0.34	3.17 ± 0.63	3.06 ± 0.63	3.02 ± 0.38	3.02 ± 0.48
ILEUM	2.01 ± 0.43	2.40 ± 0.28	2.60 ± 0.38	1.92 ± 2.88	2.21 ± 2.40

Table 4.4

Effect of 0, 10, 20, 30, 40 mM glucose in incubation solution on transport of lead into the serosal compartment and uptake on to the tissue, after 30 min incubation at 37°C.

GLUCOSE CONCENTRATION (mM)	0	20 (Control)
SEROSAL (pmoles Pb/g initial wet weight of tissue)		
DUODENUM	25.9 ± 2.9*	34.7 ± 2.4
JEJUNUM	21.2 ± 3.3*	39.4 ± 4.8
ILEUM	25.9 ± 5.1	27.8 ± 4.4
TISSUE (nmoles Pb/g initial wet weight of tissue)		
DUODENUM	2.11 ± 0.14*	3.14 ± 0.14
JEJUNUM	2.42 ± 0.43	2.43 ± 0.15
ILEUM	2.18 ± 0.14*	2.97 ± 0.24

Table 4.5

Effect of 0 and 20 mM glucose in the incubation solution on the tissue uptake and serosal transport of lead after 60 min incubation at 37°C.

	GLUCOSE CONCENTRATION (mM)				
	0	10	20 (Control)	30	40
SEROSAL					
DUODENUM	-79 ± 32*	-28 ± 18*	36 ± 17	-74 ± 45*	34 ± 57
JEJUNUM	35 ± 26**	174 ± 49**	463 ± 52	170 ± 33**	263 ± 107
ILEUM	80 ± 30**	46 ± 34**	263 ± 33	145 ± 22*	43 ± 20**
TISSUE					
DUODENUM	291 ± 42*	365 ± 41	430 ± 39	400 ± 31	403 ± 51
JEJUNUM	372 ± 16**	441 ± 46	515 ± 34	483 ± 46	452 ± 51
ILEUM	330 ± 26	344 ± 49	364 ± 31	278 ± 52	375 ± 32
TOTAL					
DUODENUM	212 ± 14**	334 ± 42*	466 ± 42	327 ± 29*	438 ± 93
JEJUNUM	407 ± 19**	615 ± 82*	978 ± 75	653 ± 63*	715 ± 133
ILEUM	410 ± 33**	390 ± 70*	654 ± 45	423 ± 63*	418 ± 30**

Table 4.6

Effect of 0, 10, 20, 30, 40 mM glucose on water movement (mg H_2O/g initial wet weight of tissue) after 30 min incubation.

	GLUCOSE CONCENTRATION (mM)	
	0	20 (Control)
SEROSAL		
DUODENUM	-104 ± 59	59 ± 79
JEJUNUM	120 ± 87*	581 ± 152
ILEUM	528 ± 174	741 ± 151
TISSUE		
DUODENUM	439 ± 66*	651 ± 8
JEJUNUM	488 ± 84*	699 ± 52
ILEUM	515 ± 50	685 ± 94
TOTAL		
DUODENUM	338 ± 50**	711 ± 78
JEJUNUM	609 ± 108*	1281 ± 193
ILEUM	1043 ± 172	1426 ± 170

Table 4.7

Effect of 0 and 20 mM glucose on water movement (mg H_2O/g initial wet weight) after 60 min incubation at 37°C.

Effect of Anoxia

Everted sacs, prepared as described in Chapter 2.3 were incubated for 30 min at 37°C in Krebs-Henseleit bicarbonate buffer containing 10^{-6} M-lead labelled with ^{203}Pb. To simulate conditions of anoxia, sacs were continuously gassed with a mixture of 95% Nitrogen/5% Carbon Dioxide.

Transport of lead ions into the serosal compartment was reduced, across all regions of the intestine, although only in the jejunum was this effect statistically significant ($p < 0.001$). Tissue uptake of lead was also reduced, again the observation was only statistically significant in the jejunum ($p < 0.001$) (Table 4.8). Water transport into both the serosal compartment and the tissue was significantly depressed ($p < 0.001$) for all regions of the intestine (Table 4.9).

Effect of Temperature

Everted sacs prepared as described in Chapter 2.3 were incubated for 30 min in Krebs-Henseleit bicarbonate buffer, containing 10^{-6} M-lead labelled with ^{203}Pb, at 27°C, a temperature 10°C lower than the incubation temperature used throughout these investigations.

At an incubation temperature of 27°C the amount of lead transported into the serosal compartment was not consistently affected as indicated by the Q10 values (Table 4.10). Tissue uptake of lead was reduced but the effect was not statistically significant. Water movement across the everted sacs was significantly depressed ($p < 0.001$) (Table 4.11) and the relationship with lead transport was no longer maintained.

Discussion

Transport of lead into the serosal compartment was not generally influenced by alterations in the incubation temperature, anoxic conditions or changes in the initial glucose concentration of the bathing solution. The reductions in lead transport were probably the result

	ANOXIA	CONTROL
SEROSAL (pmoles Pb/g initial wet weight)		
DUODENUM	26.9 ± 3.4	30.8 ± 3.4
JEJUNUM	19.7 ± 2.4**	33.7 ± 3.8
ILEUM	25.4 ± 5.3	25.9 ± 4.3
TISSUE (nmoles Pb/g initial wet weight)		
DUODENUM	2.3 ± 0.3	2.6 ± 0.3
JEJUNUM	2.4 ± 0.2**	3.9 ± 0.6
ILEUM	2.7 ± 0.3	3.3 ± 0.3

Table 4.8

Effect of anoxia on the serosal transport and the tissue uptake of lead after 30 min incubation at 37°C.

	ANOXIA	CONTROL
SEROSAL		
DUODENUM	-62 ± 21	-6 ± 24
JEJUNUM	45 ± 17**	512 ± 79
ILEUM	14 ± 6**	282 ± 55
TISSUE		
DUODENUM	312 ± 29*	423 ± 26
JEJUNUM	387 ± 25*	531 ± 52
ILEUM	129 ± 13**	446 ± 45
TOTAL		
DUODENUM	232 ± 33*	417 ± 39
JEJUNUM	431 ± 35**	1040 ± 117
ILEUM	144 ± 13**	727 ± 88

Table 4.9

Effect of anoxia on water movement (mg H_2O/g initial wet weight) after 30 min incubation at 37°C.

INCUBATION TEMPERATURE

	27°C	37°C (Control)	Q10
SEROSAL (pmoles Pb/g initial wet weight of tissue)			
DUODENUM	16.3 ± 2.4	23.4 ± 3.8	1.4
JEJUNUM	22.1 ± 5.8	20.3 ± 3.1	0.9
ILEUM	22.0 ± 5.3	15.0 ± 1.6	0.7
TISSUE (nmoles Pb/g initial wet weight of tissue)			
DUODENUM	1.71 ± 0.10**	3.14 ± 0.19	1.8
JEJUNUM	2.63 ± 0.14	3.31 ± 0.77	1.3
ILEUM	2.52 ± 0.34	2.96 ± 0.24	1.2

Table 4.10

Effect of incubation at 27°C for 30 min on serosal transport and tissue uptake of lead.

INCUBATION TEMPERATURE

	27°C	37°C (Control)
SEROSAL		
DUODENUM	-77 ± 27*	36 ± 17
JEJUNUM	50 ± 25**	463 ± 52
ILEUM	-44 ± 21**	263 ± 33
TISSUE		
DUODENUM	361 ± 18	430 ± 39
JEJUNUM	412 ± 97	515 ± 34
ILEUM	227 ± 24*	364 ± 31
TOTAL		
DUODENUM	284 ± 19**	466 ± 42
JEJUNUM	462 ± 89**	978 ± 75
ILEUM	183 ± 36**	654 ± 45

Table 4.11

Effect of incubation at 27°C for 30 min on water movement (mg H_2O/g initial wet weight of tissue).

of markedly diminished water movement. Reductions in tissue uptake may have been due to a diminished rate of ATP hydrolysis which would lead to a decrease in the amount of phosphate available for interaction with lead ions.

The data suggests that lead has no direct requirement for an energy-dependent or "active" transport system. Taken in conjunction with the absence of evidence for a saturable carrier-mediated movement of lead across the intestine, the observations therefore strongly indicate that lead is transported across the intestine by passive diffusion. The transport of lead may be only partially linked to the concommitant movement of water as marked reductions in water transport were not paralleled by similar reductions in lead transport.

CHAPTER 5

THE EFFECT OF HYDROGEN AND CALCIUM ION CONCENTRATION ON THE INTESTINAL TRANSPORT OF LEAD

5.1 Introduction

The data reported in Chapter 4 suggests that the lead cation is passively transported across the intestinal membrane. Further experimentation was carried out to elucidate the route by which these cations traversed the intestinal barrier. The transport of solutes across the epithelium of the gastrointestinal tract may proceed by one of two routes.

1. A transcellular route that involves movement across at least two membranes, arranged in series.
2. An extracellular route whereby solutes pass between the epithelial cells.

The lead cation could diffuse across the intestine by dissolving into the lipoidal membrane of the gastrointestinal tract but the charged nature of the inorganic species prevents ready penetration of the cell membrane. In the intestinal mucosal membrane, individual cells are loosely attached to each other, and much of the net transfer of ions and water is thought to occur through pathways which by-pass the cells (Frizzell and Schultz, 1972). The pathways are believed to be situated at the zonulae occludentes (or 'tight junctions'), the region considered responsible for adhesion between cells (Ussing, Erlij and Lassen, 1974).

Barry, Diamond and Wright (1971) have shown that changes in hydrogen and calcium ion concentration can alter the integrity of this route. Therefore if lead ions were to pass through the intestinal barrier by an extracellular route their transport may be affected by alterations in these parameters.

5.2 Influence of Hydrogen Ion Concentration on the Transport of Lead

Everted sacs, prepared as in Chapter 2.3 from the duodenum, jejunum and ileum were incubated for 30 min at 37°C in Krebs-Henseleit buffer containing 20 mM glucose and 10^{-6} M-lead labelled with ^{203}Pb.

The amount of lead taken up by the tissue and transported to the serosal compartment was measured after incubation at pH values from 2.4 to 10.40. The different pH values were obtained by appropriate additions of hydrochloric acid or sodium hydroxide to the Krebs-Henseleit bicarbonate buffer.

Under increasingly acidic conditions there was an increase in the amount of lead transported into the serosal compartment, compared to that transported at the control pH value of 7.4 (Fig 5.1). The increase became statistically significant at pH 2.4 ($p = 0.001$). There was no significant difference in the amount of lead transported over the pH range 5.4 to 10.4. A sinusoidal pattern of lead transport with change of pH is discernible (Fig. 5.1), but the results recorded at pH 5.4 and 8.4 may be artefacts. No difference in the trend of lead transport between the duodenum, jejunum or ileum was observed with change in pH (Fig. 5.1).

With decreased acidity up to a pH of approximately 3.4, the tissue uptake of lead was gradually increased, compared to control values ($p < 0.01$) (Fig. 5.2). Further decrease in acidity caused a decrease in the amount of lead taken up by the tissue. Uptake of lead was increased at pH 9.4. The trend exhibited in Figure 5.2 was observed for all regions of the intestine.

There was no direct correlation between water movement and lead transport to either the tissue or the serosal compartment. The maximum value for total water movement across the everted sacs occurred over the pH range 6.0 - 7.0. A decrease was observed either side of this range (Table 5.1).

5.3 The Effect of Calcium Concentration on Lead Transport

Everted sacs prepared as in Chapter 2.3 from the duodenum, jejunum and ileum were incubated at 37°C for 30 min in Krebs-Henseleit bicarbonate buffer containing 20 mM glucose and 10^{-6} M-lead labelled

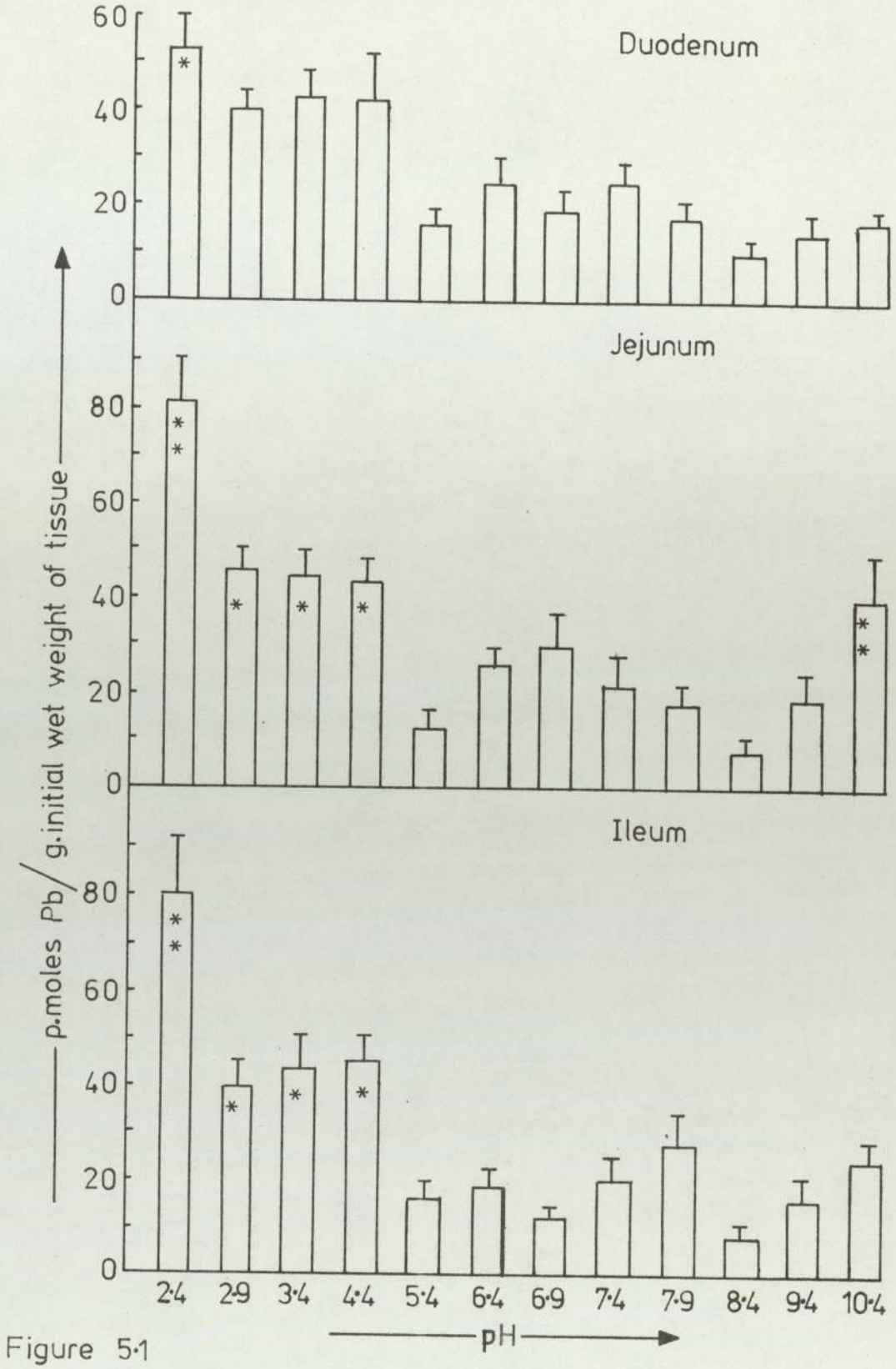


Figure 5·1

Effect of pH on the transport of 10^{-6} M-lead labelled with ^{203}Pb into the serosal compartment of the everted sac after incubation for 30 min at 37°C.
Standard error of mean represented by vertical bars.

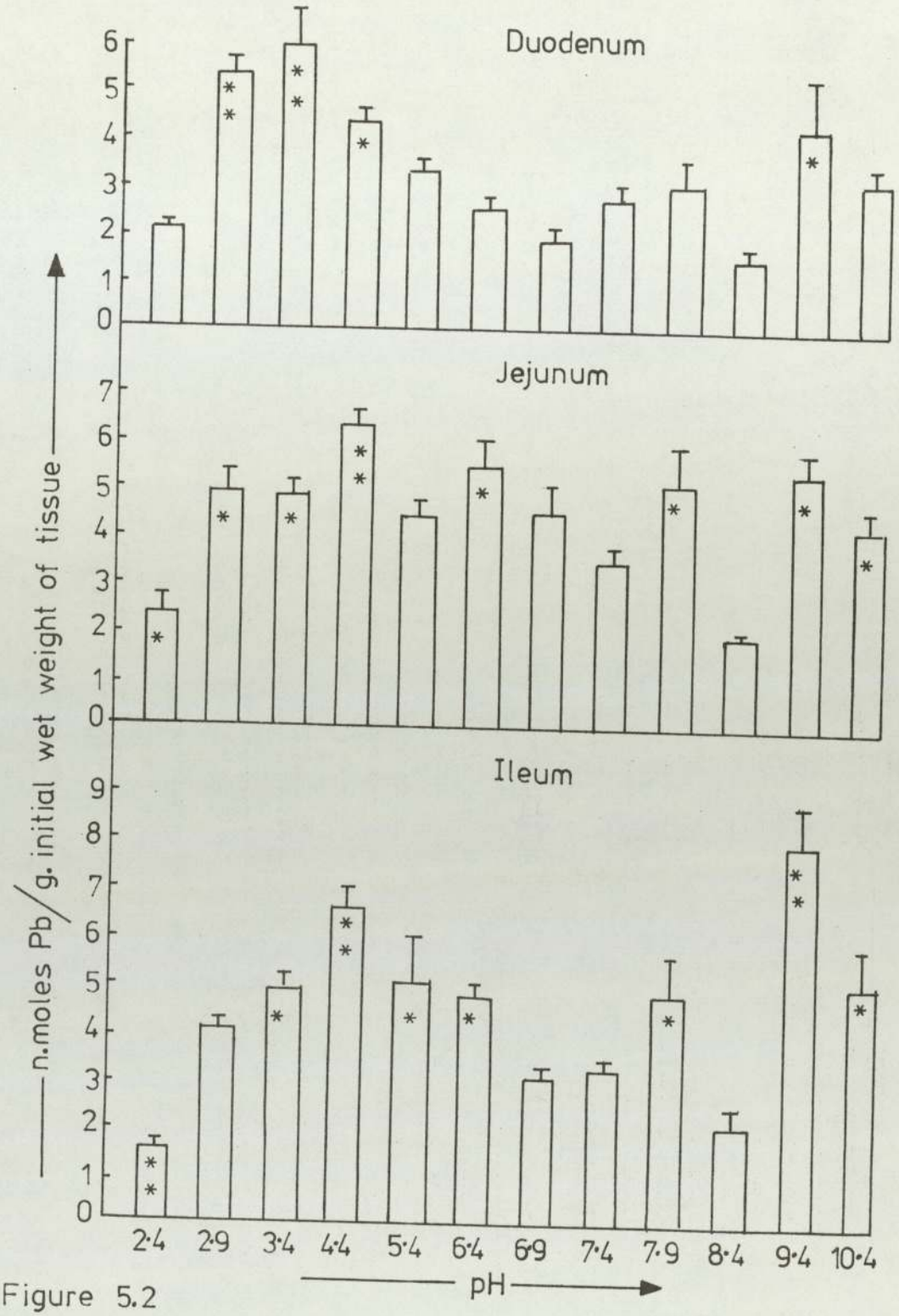


Figure 5.2

Effect of pH on the tissue uptake of 10^{-6} M-lead labelled with ^{203}Pb after incubation for 30 min at 37°C.
Standard error of mean represented by vertical bars.

pH SEROSAL	2.4	3.4	4.4	5.4	6.4	7.4 (Control)	8.4	9.4	10.4
DUODENUM	-129 ± 14**	-109 ± 15**	-93 ± 31*	-1 ± 66	35 ± 29	-7 ± 25	-10 ± 22	-54 ± 38	-165 ± 38*
JEJUNUM	-82 ± 43**	105 ± 51*	114 ± 17*	279 ± 59	499 ± 80	359 ± 75	232 ± 44	408 ± 40	92 ± 43*
ILEUM	-183 ± 78**	103 ± 27*	201 ± 15	215 ± 54	317 ± 52	241 ± 47	259 ± 53	182 ± 49	56 ± 13**
TISSUE									
DUODENUM	295 ± 13*	332 ± 26	324 ± 22	342 ± 24	550 ± 33	380 ± 26	483 ± 24	414 ± 30	303 ± 35
JEJUNUM	269 ± 34**	288 ± 37*	312 ± 24*	418 ± 44	662 ± 33	436 ± 38	509 ± 62	501 ± 53	320 ± 34*
ILEUM	191 ± 43*	150 ± 41*	288 ± 30	316 ± 33	460 ± 38	311 ± 37	366 ± 19	465 ± 54	154 ± 26*
TOTAL									
DUODENUM	169 ± 26**	222 ± 30*	232 ± 47*	340 ± 76	585 ± 49	373 ± 33	493 ± 13	315 ± 34	138 ± 52**
JEJUNUM	187 ± 49**	394 ± 65*	426 ± 16*	697 ± 63	1161 ± 72	795 ± 108	741 ± 71	908 ± 83	412 ± 64*
ILEUM	8 ± 118*	253 ± 48*	430 ± 33	531 ± 62	777 ± 60	531 ± 72	624 ± 47	647 ± 58	210 ± 30**

Table 5.1

Effect of pH on water transport (mg H_2O/g initial wet weight) across everted sacs after incubation for 30 min at 37°C in 10^{-6} M-lead labelled ^{203}Pb.

with ^{203}Pb. The calcium concentration (Ca^{2+}) was adjusted prior to experimentation by decreasing or increasing the amount of calcium chloride present and the solution molarity compensated by appropriate addition of mannitol or removal of sodium chloride. The amount of lead, taken up by the tissue and transported to the serosal compartment was measured as described previously.

The entry of lead into the serosal compartment of all sacs investigated was little affected by changes in the calcium concentration of the mucosal bathing medium (Fig. 5.3). However when calcium was absent from the mucosal bathing medium there was a statistically significant increase ($p < 0.01$) in the amount of lead transported to the serosal compartment. As the mucosal concentration of calcium increased from 0 mM to 4.8 mM there was a gradual increase in the amount of lead taken up by the tissue (Fig. 5.4). All regions of the intestine investigated exhibited the same gradual increase in tissue uptake of lead. Concommitant measurement of water movement displayed a similar rise in water transport figures as the calcium concentration increased to the control value of 2.4 mM (Ca^{2+}) (Table 5.2).

5.4 Discussion

The transport of solutes across the epithelium of the gastrointestinal tract may proceed by either a transcellular or an extracellular route (Schultz, Frizzell and Nellans, 1974). The extent to which a solute crosses an epithelium by the extracellular route (i.e. the permeability of the epithelium) is dependent upon both the nature of the solute and the arrangement of the zonula occludens and underlying lateral spaces between cells (Fromter and Diamond, 1972). Certain epithelia such as the urinary bladder possess junctions which are designated 'tight' as they display a high transmural resistance and a high resistance to passive ion permeation. Other epithelia, for example, the gall-bladder, are considered to possess 'leaky' junctions characterised by a low transmural resistance and relatively more

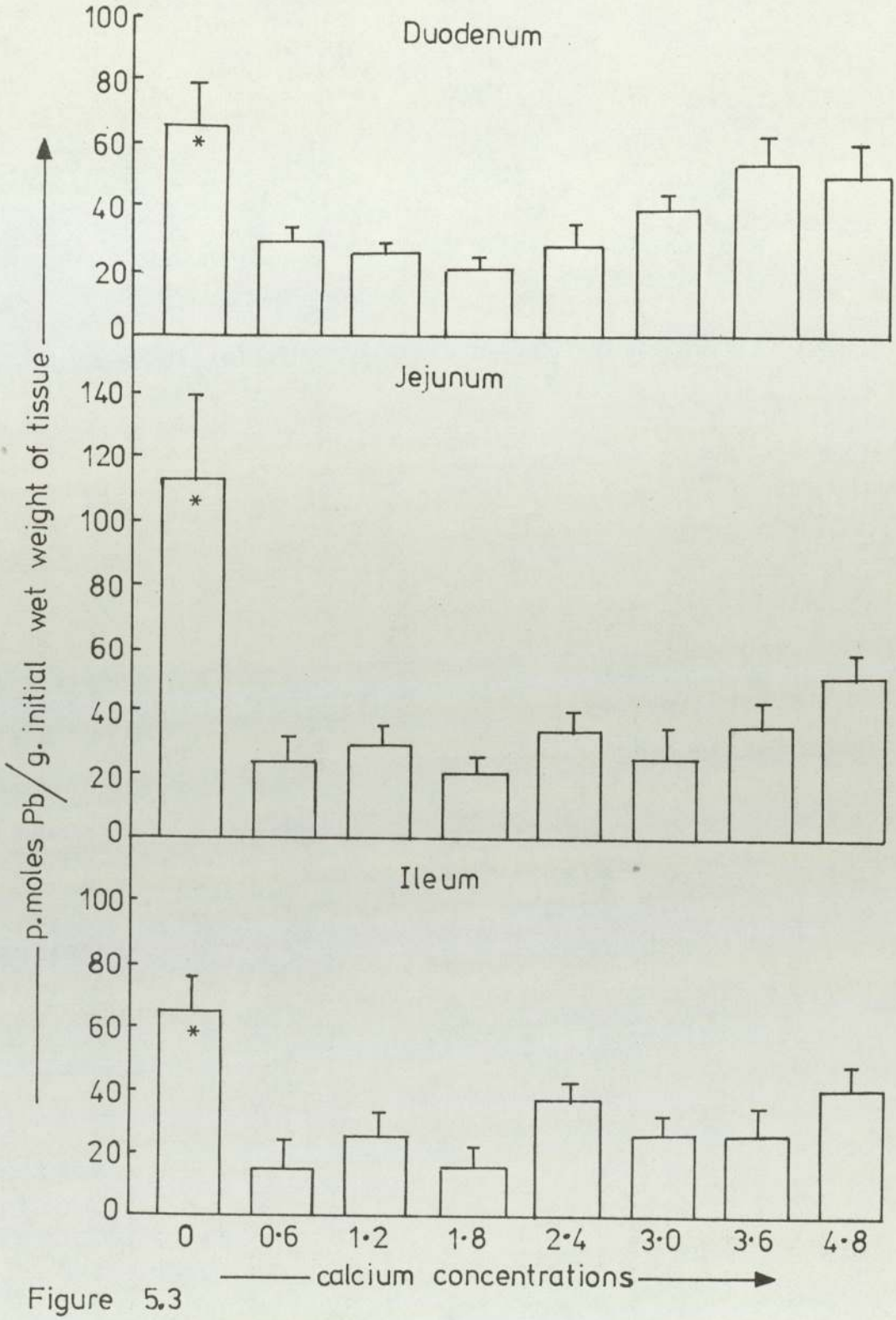


Figure 5.3

Effect of various concentrations of calcium on the transport of 10^{-6} M-lead labelled with ^{203}Pb into the serosal compartment of the everted sac after incubation for 30 min at 37°C.
Standard error of mean represented by vertical bars.

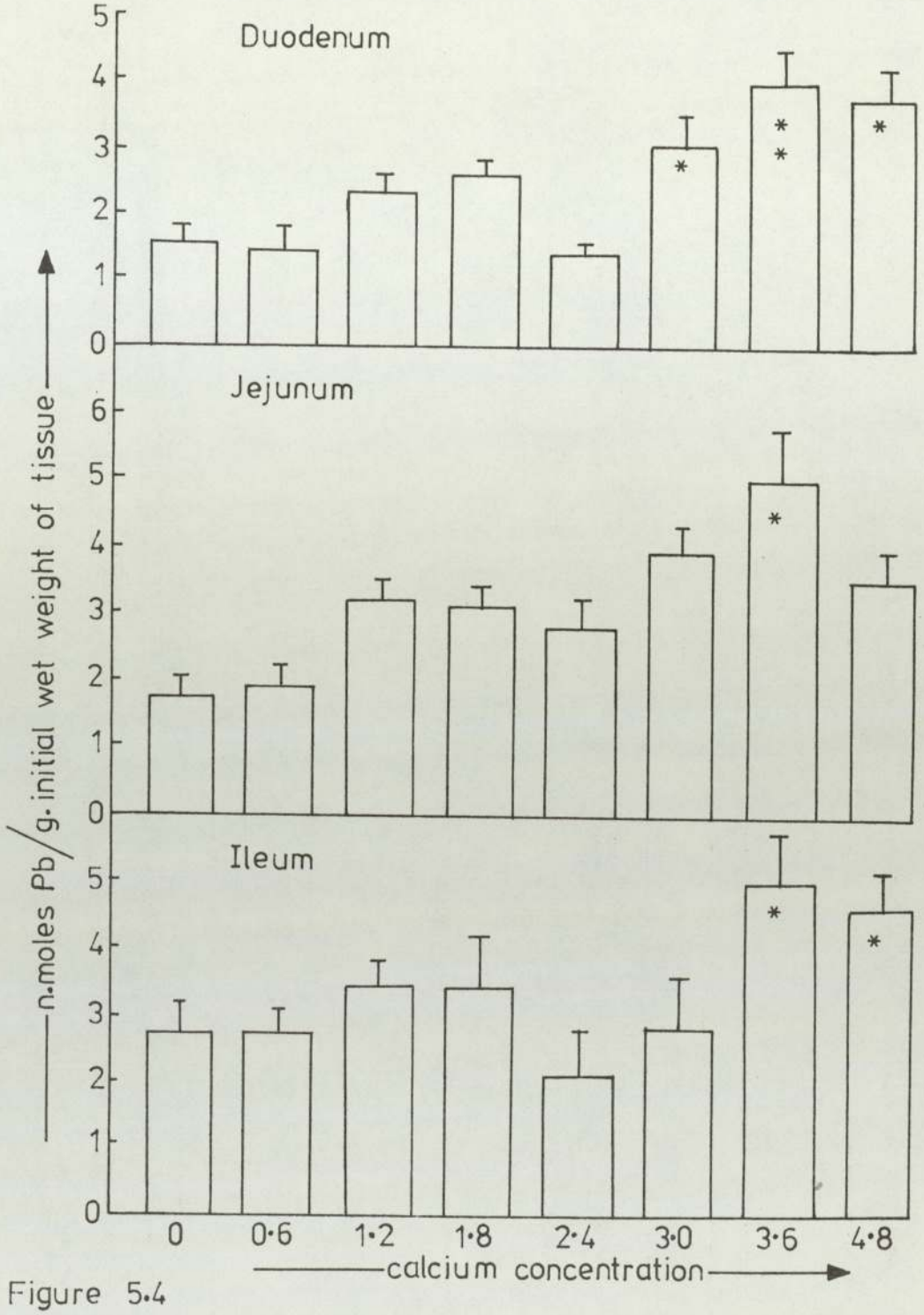


Figure 5.4

Effect of various concentrations of calcium on the tissue uptake of 10^{-6} M-lead labelled with ^{203}Pb into the serosal compartment of the everted sac after incubation for 30 min at 37°C. Standard error of mean represented by vertical bars.

CALCIUM CONCENTRATION mM	0	0.6	1.2	1.8	2.4 (Control)	3.0	3.6	4.8
SEROSAL								
DUODENUM	-107 ± 8*	-59 ± 19*	-126 ± 51*	-49 ± 26	66 ± 44	-41 ± 32	50 ± 24	6 ± 129
JEJUNUM	105 ± 65*	252 ± 43*	203 ± 44*	225 ± 46*	460 ± 92	409 ± 71	607 ± 36	458 ± 80
ILEUM	16 ± 29**	120 ± 52*	73 ± 27**	120 ± 24*	334 ± 59	225 ± 29	304 ± 28	324 ± 48
TISSUE								
DUODENUM	184 ± 28**	286 ± 25**	382 ± 64	378 ± 35	463 ± 36	437 ± 23	399 ± 35	370 ± 49
JEJUNUM	248 ± 70*	363 ± 35	473 ± 32	428 ± 51	480 ± 83	538 ± 44	534 ± 43	408 ± 39
ILEUM	216 ± 32*	169 ± 33*	261 ± 26*	264 ± 49*	445 ± 70	385 ± 28	373 ± 29	285 ± 48
TOTAL								
DUODENUM	77 ± 25**	228 ± 19**	256 ± 17*	329 ± 54*	529 ± 70	396 ± 29	449 ± 24	376 ± 77
JEJUNUM	353 ± 88**	584 ± 58*	673 ± 69*	653 ± 87*	940 ± 107	947 ± 103	1142 ± 77	866 ± 104
ILEUM	232 ± 41**	290 ± 41*	334 ± 33*	384 ± 46*	779 ± 115	609 ± 42	677 ± 56	609 ± 89

Table 5.2

Effect of various concentrations of calcium on water transport (mg H_2O/g initial wet weight) across everted sacs after incubation for 30 min at 37°C in 10^{-6} M-lead labelled with ^{203}Pb.

permeable junctional complexes, (Ussing, Erlij and Lassen, 1974).

In low resistance epithelia, of which the intestine is one example, 'leaky' tight junctions are formed by a number of areas of contact between adjacent cells, interrupted by stretches of separation between the two membranes that leave open spaces. Also the membranes of neighbouring cells are not in close apposition for the entire depth of the zonula occludens, there being fewer points of contact between membranes. In freeze cleavage studies of a number of epithelia, a branching network of threads or chains of small globular subunits has been found within the regions of the tight junctions (Claude and Goodenough, 1972; Stahelin, 1973). It has also been observed that the complexity of this network can be correlated with the relative leakiness of the epithelia, the leakier epithelia having a much less extensive network than the 'tight' epithelia. It is possible that the subunits are in some way connected with the formation of close membrane appositions and that as the number of subunits increases, so the tightness of the seal between cells increases.

Barry, Diamond and Wright, (1971) liken the zonula occludens to a channel lined with dipolar groups, orientated so that the anionic groups are directed into the channels. They predict that the association of hydrogen ions or polyvalent cations, such as calcium, with the anionic groups will neutralise the environment of the channel and therefore increase anion mobility whilst depressing cation mobility.

Over the range of calcium concentrations 0.6 mM to 4.8 mM, the transport of the lead cation into the serosal compartment was unaffected (Fig. 5.3). However in the absence of calcium from the mucosal bathing medium the transport of lead was markedly enhanced, possibly as a result of the greater cationic mobility via the extracellular route. Several workers have proposed that a concentration of at least 0.25 mM calcium is required to maintain adhesion between cells

(Wright and Diamond, 1968; Malenkov and Melikyants, 1977). Therefore calcium may have a role in the maintenance of the integrity of the zonula occludens possibly by binding with anionic groups across the tight junctions, (Fig. 5.5) consequently its removal may cause the tight junctions to open slightly.

Based on electrophysiological evidence a number of workers have suggested that an increase in hydrogen ion concentration will render the channels for extracellular transport more anion specific (Wright and Diamond, 1968; Barry, Diamond and Wright, 1971; Frizzell and Schultz, 1972). As a consequence they predict a decrease in the transport of monovalent cations and water. Table 5.1 shows a fall in water transport with increase in hydrogen ion concentration but lead transport into the serosal compartment is significantly increased. An alternative explanation is that excess hydrogen ions in the bathing medium could replace the calcium ions binding across the anionic groups (Fig. 5.5) and alter the integrity of the zonula occludens, causing it to open and permit more lead to pass into the serosal compartment via the extracellular route. This again offers support for the suggestion that the lead cation crosses the intestinal barrier via the zonulae occludentes between the epithelial cells.

Increase in hydrogen ion concentration to pH 3.4 increased the tissue uptake of lead (Fig. 5.2) ($p < 0.01$). This may be a result of the effect of hydrogen ions on either the acid microclimate or a stimulation of the intestinal Mg^{2+} dependent ATP-ase thus producing more phosphate ions from the hydrolysis of ATP which would interact with lead. However neither explanation is entirely satisfactory as

1. the optimum pH of intestinal ATP-ase ranges between 5-6 and further decrease in pH reduces the activity of the enzyme (Kesavan and Noronha, 1978).
2. further increase in hydrogen ion concentration is

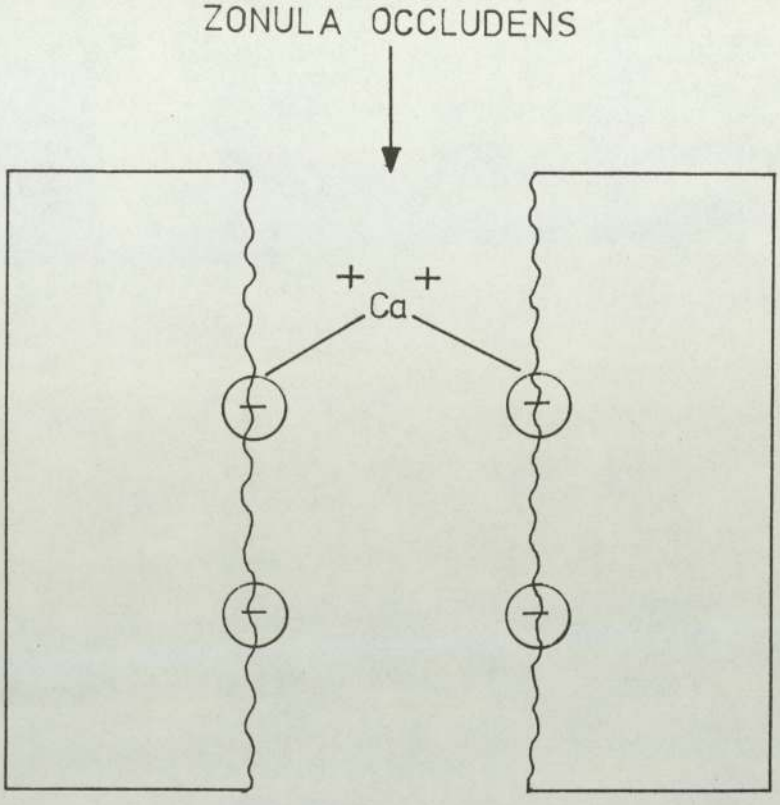


Figure 5.5

The possible role of calcium in the maintenance of the integrity of the zonula occludens by binding with anionic groups present.

> unlikely to influence the microclimate until the pH of the mucosal bathing medium is equal to or less than a proposed microclimate pH of 3.5 (Blair and Matty, 1974; Lei, Lucas and Blair, 1977).

After this point the membrane becomes more positively charged (Wright and Barry, 1968) which should result (as shown in Figure 5.2) in a decrease in lead uptake by tissue at hydrogen ion concentrations above pH 3.4.

Several authors have reported that calcium influences the intestinal uptake of lead, (Six and Goyer, 1970; Quarterman and Morrison, 1975; Barltrop and Khoo, 1976; Meredith, Moore and Goldberg, 1977). Their observations generally suggest that low dietary calcium will increase intestinal absorption of lead and that high dietary calcium will decrease the absorption of lead. However the intestinal absorption of lead was assessed in these studies from measurements of the whole body burden of lead and therefore does not differentiate between tissue uptake and that which has been transported across the intestinal barrier. Furthermore it is difficult to compare long term dietary experiments with short term <u>in vitro</u> incubation experiments where the effect of endogenous calcium also makes the interpretation of experiments difficult.

In a recent publication, Barton, Conrad, Harrison and Nuby (1978) suggested that the reported increase in the total body burden of lead, for calcium deficient rats, is due to a decrease in the urinary excretion of lead rather than an increase in gastrointestinal absorption. (The results reported in Figure 5.3 partially support their hypothesis). Barton and Conrad did not investigate the transport of lead in the absence of calcium.

The same authors reported that high levels of calcium inhibited the amount of lead taken up by the intestinal tissue and suggest that

there is competition between lead and calcium for mucosal binding proteins. However Figure 5.4 indicates that increased concentration of calcium results in a slight increase in the tissue uptake of lead.

CHAPTER 6

THE EFFECT OF THE SYNTHETIC CHELATING AGENT, DIETHYLENETRIAMINEPENTAACETIC ACID (DTPA) AND BILE SALTS ON THE INTESTINAL ABSORPTION OF LEAD

6.1 The Effect of DTPA on the Intestinal Absorption of Lead

Ionic lead is unlikely to pass directly across the predominantly lipoidal gastrointestinal barrier. The most likely route for the passage of the lead cation is via an extracellular pathway between the mucosal cells (as previously discussed in Chapter 5). However it is possible that a number of compounds, ingested as part of the diet or secreted into the intestine are capable of interacting with lead to form lipid soluble complexes which in turn, may pass across the epithelium by the intracellular route. Garber and Wei (1974) reported that orally administered d-penicillamine, nitriloacetic acid and citric acid, increased the absorption of lead, whilst Jugo, Maljkovic and Kostial (1975) have observed that both orally and intraperitoneally administered calcium ethylenediaminetetraacetic acid increased the gastrointestinal absorption of lead. However, both these studies were performed in vivo, a situation in which the influence of the chelating agents administered may have been modified by the presence of other dietary components or intestinal secretions. The aim of the present investigation was to measure the effect of a selected synthetic chelating agent, calcium trisodium diethylenetriaminepentaacetic acid (DTPA) on the uptake and transfer of lead by everted sacs, in the absence of interference from other putative chelating agents.

Effect of DTPA on the Intestinal Absorption of Lead

Everted sacs prepared as in Chapter 2.3 were incubated for 30 min at 37°C in Krebs-Henseleit bicarbonate buffer containing 10^{-6} M-lead labelled with ^{203}Pb. The uptake and transport of lead was measured, as in Chapter 2.5, in the presence of 10^{-6}M, 10^{-5}M, 10^{-4}M and 10^{-3}M - DTPA.

Over the range of concentrations of DTPA investigated there was an increase in the amount of lead transported from the mucosal bathing medium into the serosal compartment (Fig. 6.1) which became statisti-

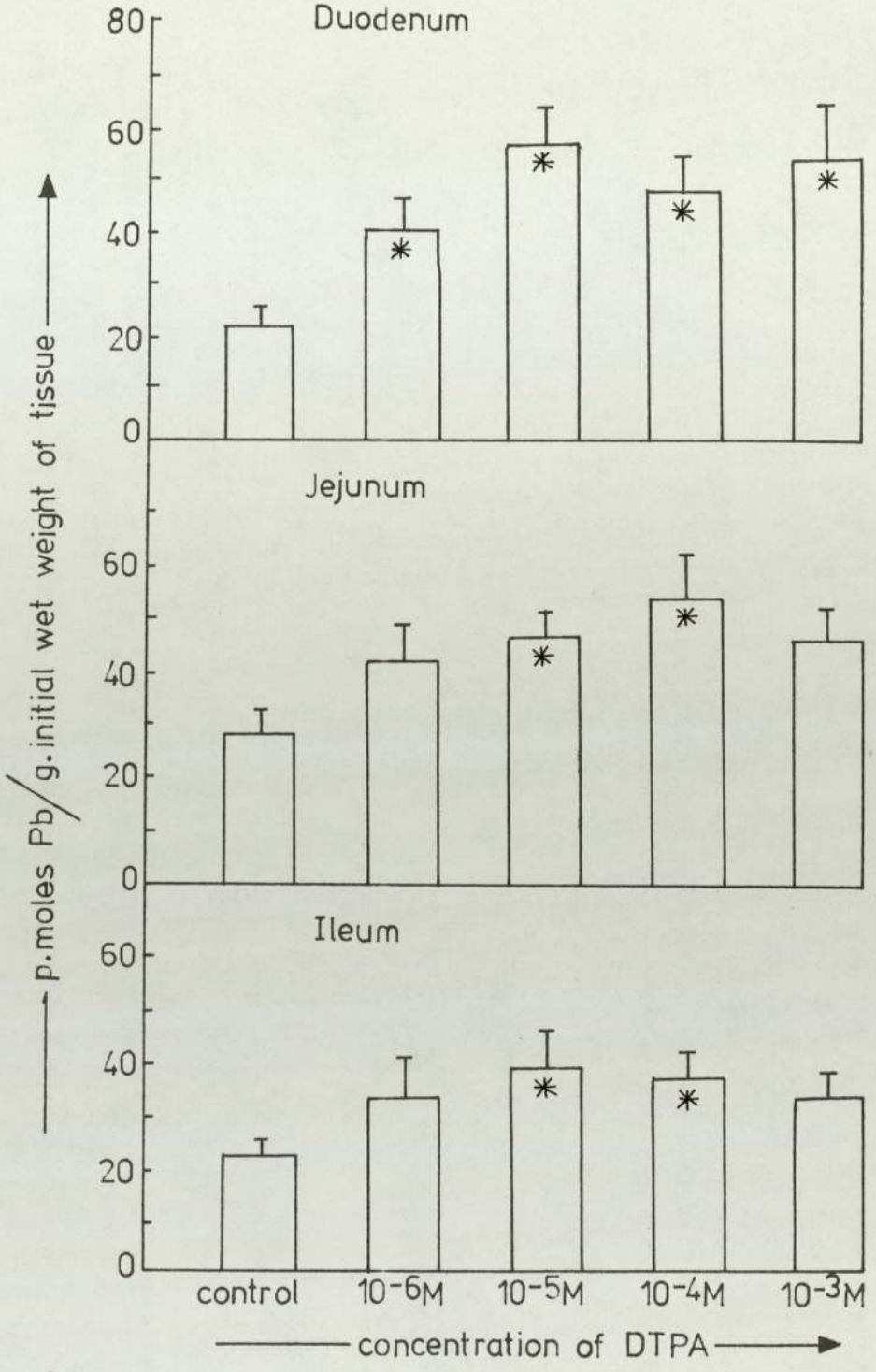


Figure 6·1

The effect of calcium trisodium diethylenetriaminepentaacetic acid (DTPA) on the transport of 10^{-6}M-lead labelled with ^{203}Pb into the serosal compartment of everted sacs after incubation for 30 min at 37°C.
Standard error of means represented by vertical bars.

cally significant at a concentration of 10^{-5} M - DTPA ($p < 0.01$). The enhancement of lead transport was more marked across the duodenum than across other regions of the intestine.

Tissue uptake of lead in the duodenum was largely unaffected in the presence of DTPA. However jejunal and ileal tissue uptake of lead was reduced, the reduction became statistically significant ($p < 0.001$) at a concentration of 10^{-3} M-DTPA (Fig. 6.2). Over the concentration range 10^{-6} M to 10^{-3} M-DTPA there was no effect on water transport across any region of the intestine (Table 6.1) and the relationship between lead transport and water transport into the serosal compartment appeared to be unaffected.

Discussion

The extent to which the presence of DTPA will influence the transfer of lead across the gastrointestinal tract depends upon at least two factors. One is the number of lead-DTPA complexes (Fig. 6.3) formed and the other is the ability of the chelate-metal complex itself to cross the gastrointestinal barrier. Lead interacts with DTPA with a 1:1 ratio (Chaberek and Martell, 1964). The extent of the interaction is related to the equilibrium constant for lead-DTPA, which is high compared to that of other lead-ligand interactions (Table 6.2).

DTPA chelates lead to form a neutral species, and as a result the usual interaction between lead cations and the predominantly negatively charged lipoidal membrane is reduced (Fig. 6.2) as shown by a decrease in tissue uptake of lead. However the transport of lead into the serosal compartment, as the more lipid-soluble DTPA complex, will be enhanced (Fig. 6.1). A number of workers have demonstrated that generally, polyaminopolycarboxylic acids, such as DTPA are poorly absorbed by the intestine (Foreman and Trujillo, 1954; Stevens, Rossoff, Weiner and Spencer, 1962) which would account for the

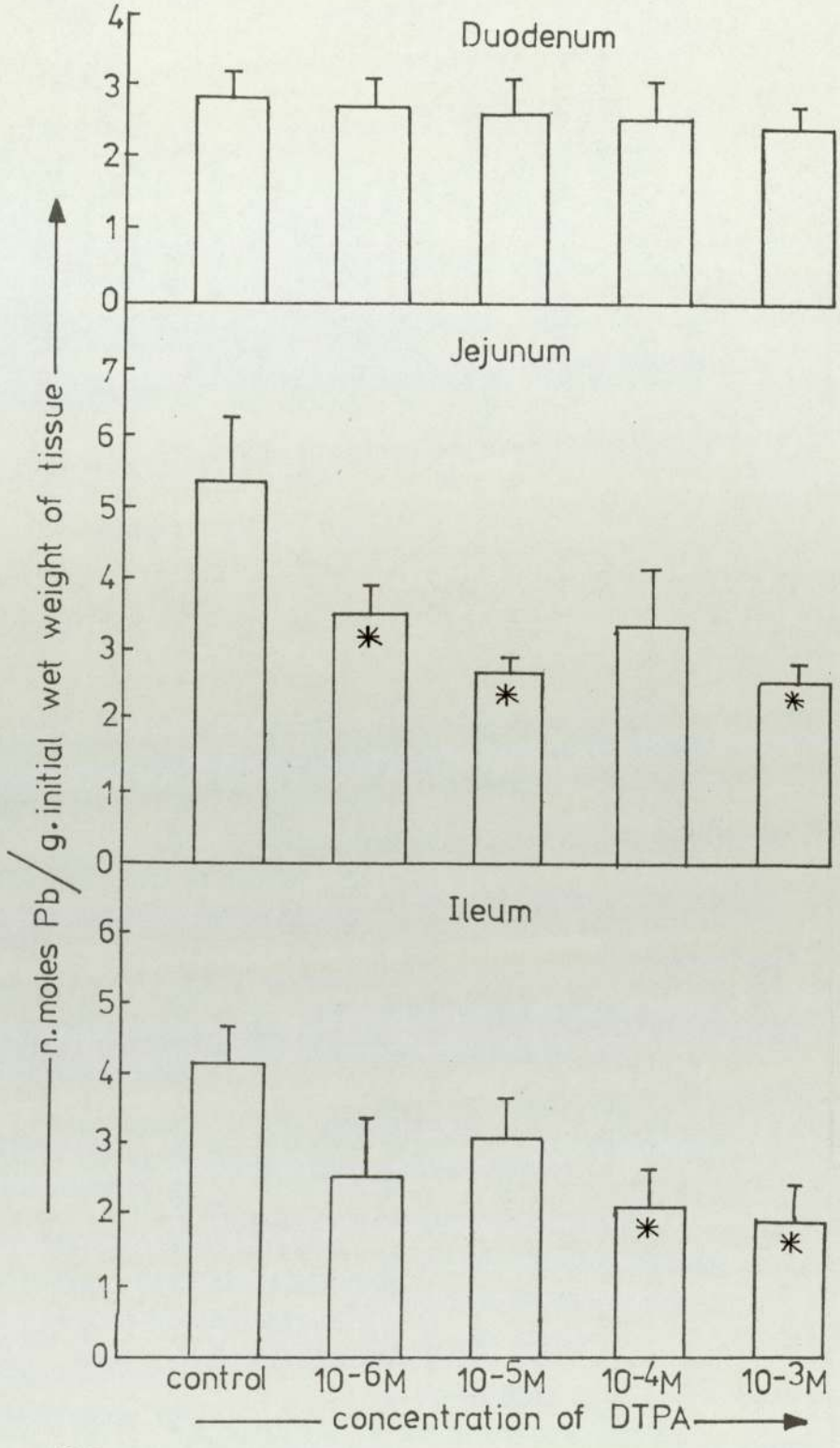
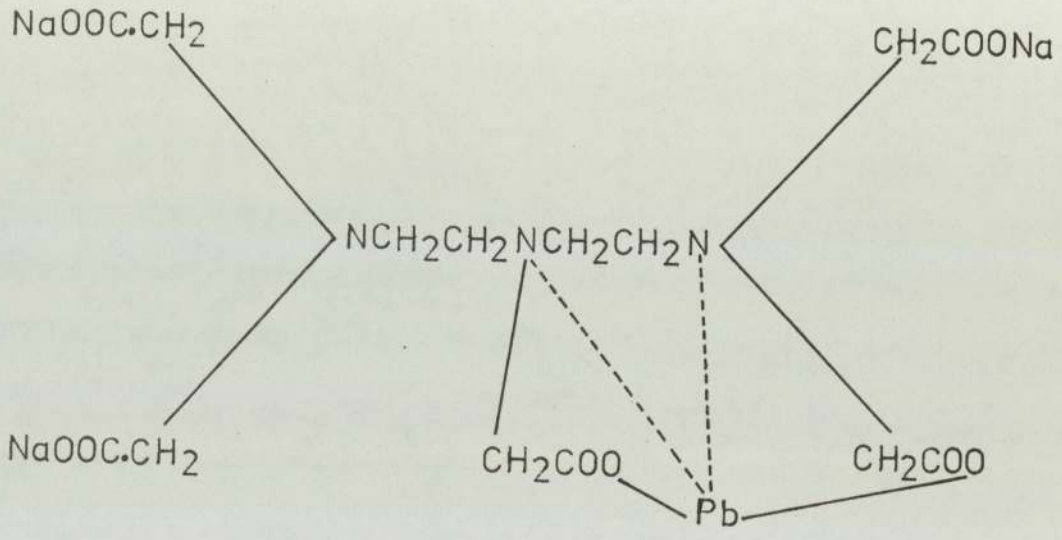


Figure 6.2

The effect of calcium trisodium diethylenetriaminepentaacetic acid (DTPA) on the tissue uptake of 10^{-6} M-lead labelled with ^{203}Pb after incubation for 30 min at 37°C.
Standard error of means represented by vertical bars.



Pb - DTPA complex

Figure 6.3

Structural formula of lead-DTPA complex.

	CONCENTRATION DTPA				
	0 (Control)	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
SEROSAL					
DUODENUM	-67 ± 21	-97 ± 76	-50 ± 12	-106 ± 32	-66 ± 39
JEJUNUM	382 ± 58	526 ± 51	440 ± 65	369 ± 74	474 ± 69
ILEUM	173 ± 25	247 ± 18	276 ± 63	243 ± 60	127 ± 21
TISSUE					
DUODENUM	374 ± 41	549 ± 77	478 ± 30	439 ± 28	442 ± 34
JEJUNUM	472 ± 92	487 ± 61	592 ± 31	482 ± 24	597 ± 73
ILEUM	261 ± 36	360 ± 32	395 ± 59	286 ± 30	309 ± 56
TOTAL					
DUODENUM	307 ± 53	452 ± 37	428 ± 29	334 ± 37	376 ± 66
JEJUNUM	853 ± 125	1012 ± 70	1034 ± 80	848 ± 68	1072 ± 123
ILEUM	434 ± 55	610 ± 49	671 ± 94	528 ± 81	436 ± 72

Table 6.1

Effect of DTPA on water movement (mgH_2O/g initial wet weight of tissue) across everted sacs after incubation for 30 min at 37°C in 10^{-6} M-lead labelled with ^{203}Pb.

	Log_{10} K
Lead - DTPA	18.6
" - EDTA	17.9
" - Nitriloacetic acid	11.5
" - Citrate	11.4
" - Phosphate	6.4
" - d-Penicillamine	4.23
" - Acetate	2.2

Table 6.2

Stability constants (log_{10} K) for a number of lead-ligand complexes. The higher the stability constant, the greater the affinity of the ligand for lead.

observation that above a ten fold increase in the concentration of DTPA, there is no further increase in the amount of lead traversing the intestinal barrier. It would appear that the intestinal absorption of the DTPA-lead complex is a limiting factor (Fig. 6.1).

It is often difficult to extrapolate from in vitro experimentation to the normal physiological condition, however, it may be stated that the presence of chelating agents in the intestinal lumen increases the transport of lead across the epithelial sheet. The data presented (Fig. 6.1) confirms the observations of various authors (Rieders, 1960; Byers, 1959; Kehoe, 1955; Chisolm, 1968; Jugo, Maljkovic and Kostial, 1975; Garber and Wei, 1974) who have indicated by indirect measurement in vivo that compounds capable of chelating lead may also promote its transfer across the gastrointestinal barrier.

Synthetic chelating agents, as a result of their high affinity for lead are useful in the therapy of acute overexposure to lead. However there must be some concern as regards the use of such agents as a prophylactic therapy in the treatment of chronic lead exposure, due to their ability to promote the intestinal absorption of lead.

6.2 The Effect of Bile Salts on the Absorption of Lead

Blaxter and Cowie (1946) and Klassen and Shoeman (1974) have shown that bile is an important excretory pathway for lead in a number of species including the rat. Other research by Castellino, Lamana and Grieco (1966) has revealed that the greatest percentage of parenterally administered lead, subsequently excreted in the faeces, was associated with bile. The observations of these authors suggest therefore that bile or some component of bile is capable of chelating lead.

Bile is a concentrated micellar solution composed mainly of bile salts and lecithin. Bile acids are C-24 carboxylic acids with a steroid nucleus containing hydroxyl groups and are secreted from the

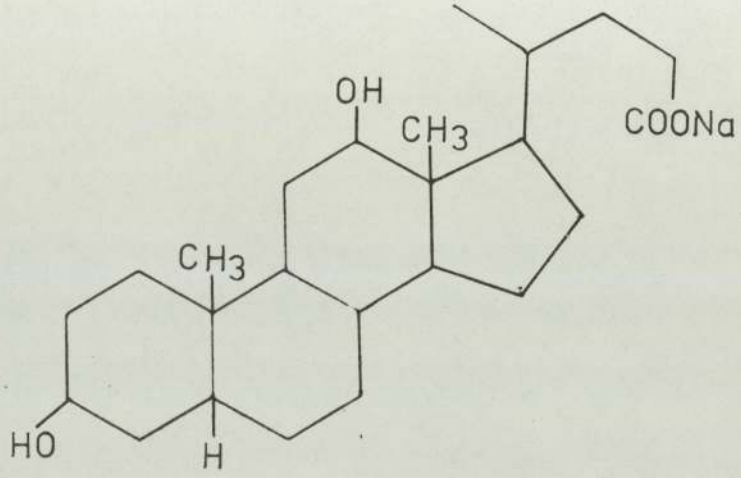
liver as glycine or taurine conjugates termed bile salts. There are several groups of bile salts, the proportion of which varies from species to species (Haslewood, 1968). Bile enters the intestinal tract midway down the duodenum (proximal to the section of the duodenum selected for investigation) and is consequently available for interaction with lead present in the lumen of the intestine. Few authors have investigated the influence of bile on the intestinal absorption of lead. Accordingly experiments were carried out to establish whether the presence of bile acids in the incubation medium influenced the transport of lead into the serosal compartment.

Everted sacs prepared as in Chapter 2.3 were incubated for 30 min at 37°C in Krebs-Henseleit bicarbonate buffer containing 10^{-6} M-lead labelled with ^{203}Pb. The uptake and transport of lead was measured as in Chapter 2.5, in the presence of 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M and 10^{-3} M unconjugated bile acid (as sodium deoxycholate) (Fig. 6.4).

At concentrations of 10^{-7} M and 10^{-6} M-sodium deoxycholate, there was no consistent effect on the transport of lead into the serosal compartment. However at higher concentrations there was a gradual decrease in lead transport across all regions of the intestine investigated (Fig. 6.5).

Tissue uptake of lead was markedly enhanced in the presence of sodium deoxycholate at initial mucosal bathing medium concentrations of up to 10^{-4} M. Above this concentration there was a statistically significant ($p < 0.001$) in the amount of lead taken up by the tissue, to approximately 50% of control (Fig. 6.6). This trend was observed across all regions of the small intestine.

Water transport across all sacs was largely unaffected in the presence of sodium deoxycholate except at a concentration of 10^{-3} M where there was a statistically significant ($p < 0.001$) decrease in water transport across all sacs investigated (Table 6.3).



Sodium Deoxycholate

Figure 6.4

Structural formula of sodium deoxycholate.

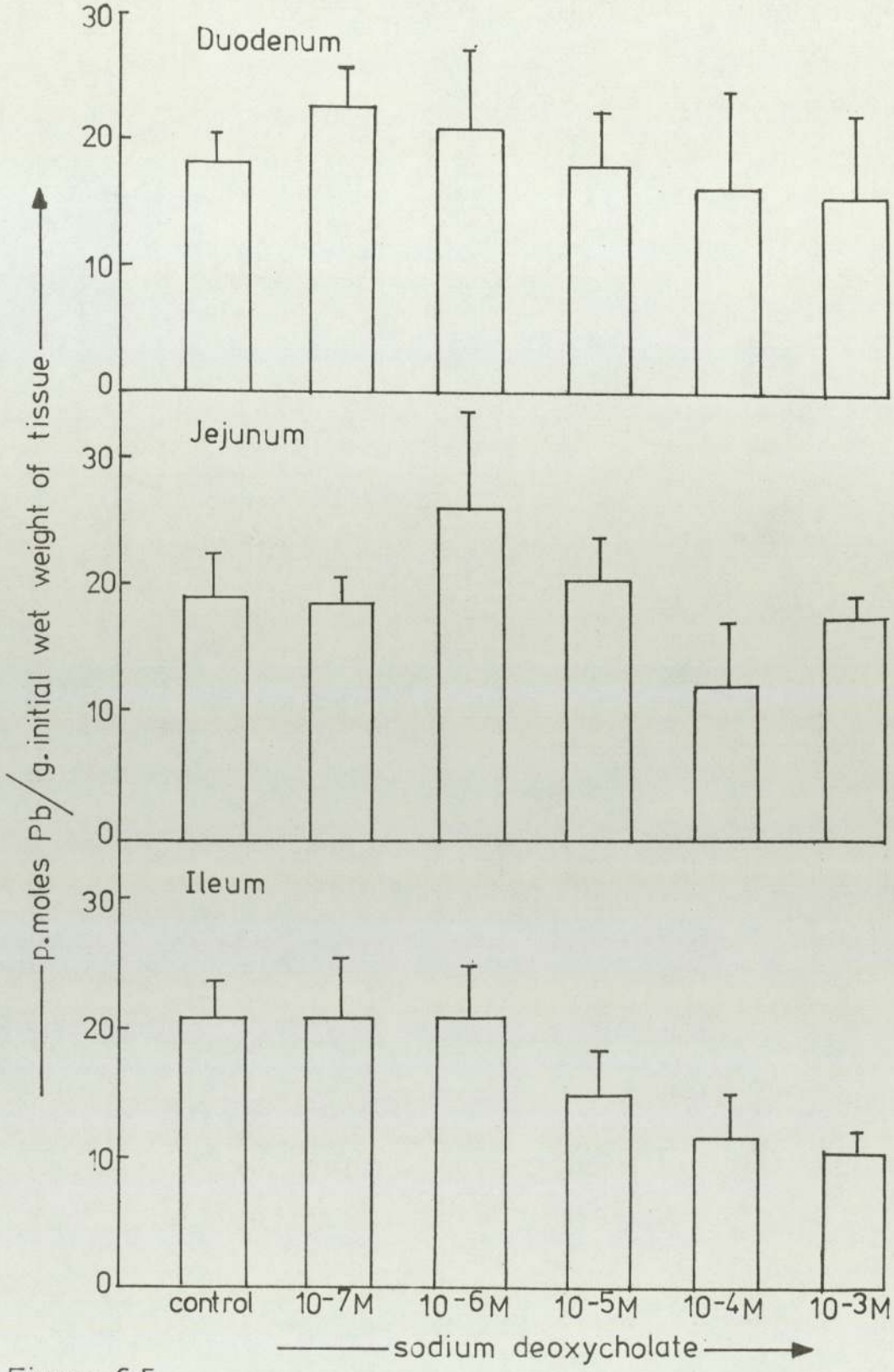


Figure 6.5

The effect of sodium deoxycholate on the transport of 10^{-6} M-lead labelled with ^{203}Pb into the serosal compartment of everted sacs after incubation for 30 min at 37°C.
Standard error of means represented by vertical bars.

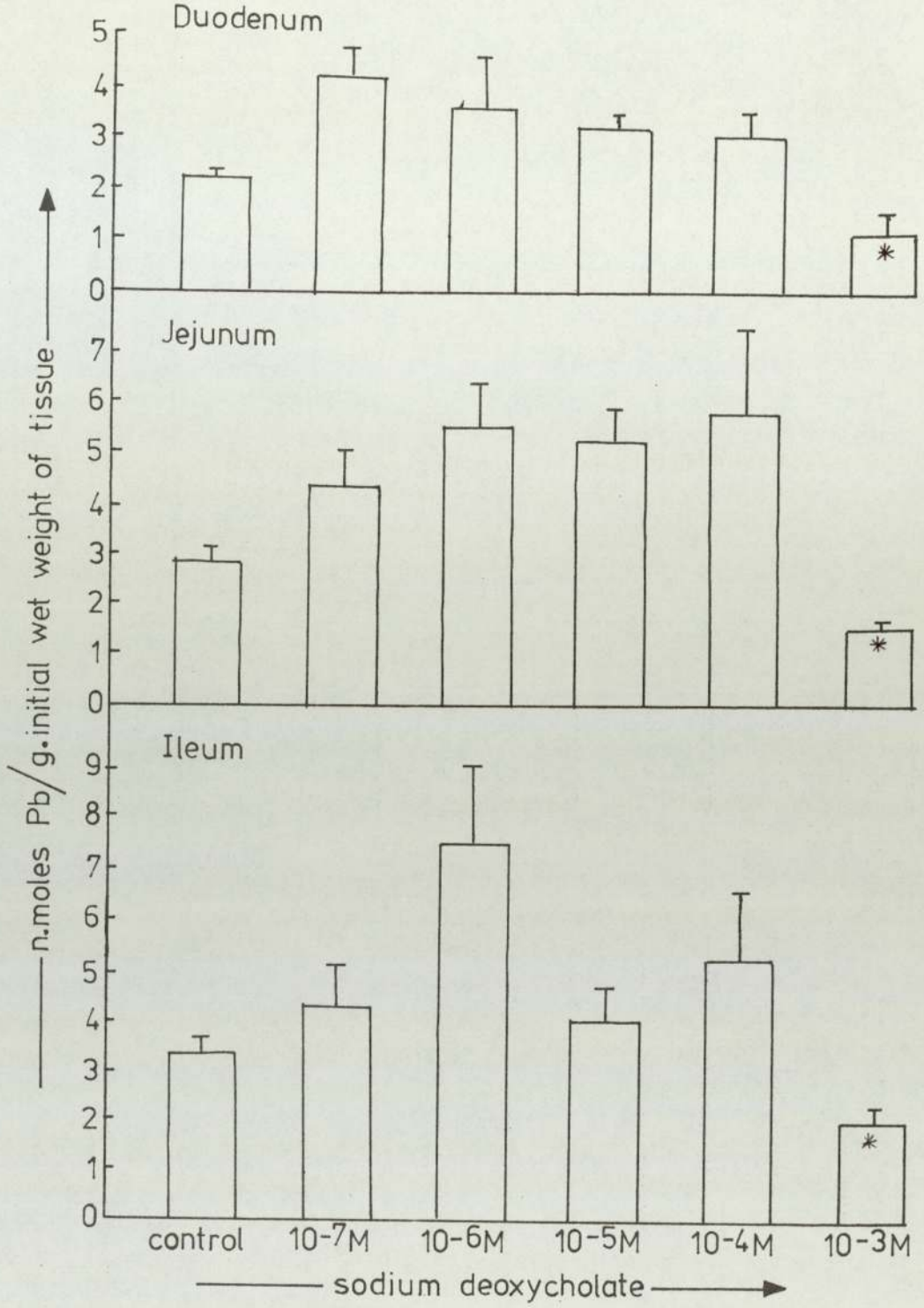


Figure 6.6

The effect of sodium deoxycholate on the tissue uptake of 10^{-6} M-lead labelled with ^{203}Pb after incubation for 30 min at 37°C. Standard error of mean represented by vertical bars.

	CONCENTRATION SODIUM DEOXYCHOLATE					
	0 (Control)	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
SEROSAL						
DUODENUM	-47 ± 24	16 ± 27	-38 ± 22	-135 ± 17	-40 ± 42	-81 ± 14
JEJUNUM	288 ± 66	351 ± 64	386 ± 89	396 ± 58	267 ± 112	33 ± 25*
ILEUM	261 ± 60	231 ± 51	229 ± 42	218 ± 47	124 ± 27	20 ± 13*
TISSUE						
DUODENUM	394 ± 31	386 ± 61	364 ± 18	466 ± 38	379 ± 56	198 ± 24**
JEJUNUM	406 ± 30	389 ± 44	465 ± 55	516 ± 32	486 ± 59	228 ± 26*
ILEUM	405 ± 40	300 ± 25	249 ± 24	305 ± 70	282 ± 40	134 ± 32**
TOTAL						
DUODENUM	347 ± 37	402 ± 64	325 ± 30	331 ± 46	339 ± 82	117 ± 19**
JEJUNUM	694 ± 82	740 ± 94	851 ± 117	914 ± 80	753 ± 93	262 ± 30**
ILEUM	666 ± 72	532 ± 64	477 ± 39	523 ± 62	406 ± 51	154 ± 20**

Table 6.3

Effect of sodium deoxycholate on water movement (mg H_2O/g initial wet weight of tissue) across everted sacs after incubation for 30 min at 37°C in 10^{-6} M-lead labelled with ^{203}Pb.

Discussion

Various authors have suggested that bile influences the movement of lead across the gastrointestinal barrier. Barltrop and Khoo (1975) reported that a high fat diet increased the absorption of lead and suggested that fatty acids, bile and lead interacted in the intestinal lumen to form a complex which was subsequently transferred across the intestine.

Prevention of bile entering the gastrointestinal lumen, by cannulation (Cikrt and Tichy, 1975) or ligation (Barton and Conrad, 1978) of the bile duct results in a decrease in the absorption of lead, which suggests that the transport of lead may depend upon the amount of bile present in the lumen of the intestine. However the authors provide no explanation either for the manner in which bile may influence the passage of lead across the intestine or the extent to which lead interacts with bile.

The presence of sodium deoxycholate failed to increase the amount of lead transferred from the mucosal bathing medium into the serosal compartment (Fig. 6.5). Indeed at higher concentrations there was a trend of a reduction in the amount of lead transferred across the intestine. Nevertheless, up to a concentration of 10^{-5} M-sodium deoxycholate, tissue uptake of lead was enhanced (Fig. 6.6) ($p < 0.01$). Forth and Rummell (1975) have suggested that the interaction between a metal and bile results in the formation of a more soluble moiety. It is possible that a micellar bile-lead complex is capable of being readily adsorbed on to the intestinal tissue.

At a concentration of 10^{-3} M-sodium deoxycholate both tissue uptake of lead and water were markedly reduced (Fig. 6.6, Table 6.3). The inhibitory influence of sodium deoxycholate is thought to be due to its action at the mucosal epithelium. With reference to studies of the effect of bile salts on intestinal metabolism and transport

processes, Dietshky (1967) reported that unconjugated bile acids, especially deoxycholate destroyed the mucosal epithelium in in vitro preparations. Sladen and Harries (1972) have also observed damage to mucosal villous tips at a concentration of 5×10^{-3} M-deoxycholate, a slightly higher concentration than that used in the present study. Other authors have also commented on the disruption of the mucosa by bile salts (Davenport, 1968; Winborn, Guerrero and Hodge, 1976; Martin, Marriot and Kellaway, 1978). It would therefore appear that due to disruption of the mucosa, identified by a 50% reduction in water transport (Table 6.3), the tissue uptake of lead is also diminished (Fig. 6.6).

It has been suggested that bile promotes the intestinal absorption of lead, based on the indirect methods of Cikrt and Tichy (1975) and Barton and Conrad (1978). Therefore, some component of bile, other than the bile acids may be responsible for the reported enhancements of lead transport.

Bile acids in conjunction with calcium ions prevent the inhibition of pancreatic lipase - the enzyme responsible for the breakdown of dietary fat into free fatty acids. In the absence of bile (as in the experiments of Cikrt and Tichy (1975); Conrad and Barton (1978)), this breakdown process would be reduced. As a result the intestinal absorption of lead may also be reduced, suggesting that bile may merely exert an indirect influence on the intestinal absorption of lead.

CHAPTER 7

THE TRANSFER OF FOLATES ACROSS THE GASTRO INTESTINAL TRACT AND THE EFFECT OF LEAD ON THEIR TRANSPORT

7.1 The Effect of Folic Acid on Lead Transport

Folic acid and its derivatives are a group of water soluble vitamins present in our diet. The major monoglutamate forms of dietary folate are, 5-methyltetrahydrofolic acid (5-MeTHF) and 10-formyltetrahydrofolic acid. Like many other dietary constituents they may be potential chelating agents and could modify the transport of lead across the gastrointestinal tract by either

a) forming a lead-folate complex, the molecular size of which would make it less amenable for transport, or

b) binding to the lead cation thereby neutralising the charge, and allowing an enhanced passage of lead as a more lipid soluble lead-folate complex.

Lead could also influence the transport of these essential dietary compounds. Evidence presented in recent years suggests that the acid microclimate located at the brush border of the intestinal mucosa and maintained by a supply of slowly diffusing hydrogen ions produced by the breakdown of adenosine triphosphate (ATP) may be responsible for producing the folate neutral species form (Blair and Matty, 1974). In this form, folates are more easily transported across the gastrointestinal tract (Blair, Johnston and Matty, 1974; Blair, Matty and Razzaque, 1975). Therefore anything which might interfere with the production of hydrogen ions may affect folate transport. Several workers (Wapnir, Exeni, McVicar and Lifshitz, 1975; Nechay and Saunders, 1978) have demonstrated that low levels of lead inhibit sodium-potassium dependent ATP-ase resulting in a decreased hydrolysis of ATP and reduced production of hydrogen ions. Consequently the proportion of folate zwitterions formed would be reduced therefore decreasing the intestinal transport of folate species.

The effect of both 10^{-6} M and 5×10^{-5} M-folic acid and 5-MeTHF on the intestinal transport of 10^{-6} M-lead labelled with ^{203}Pb was

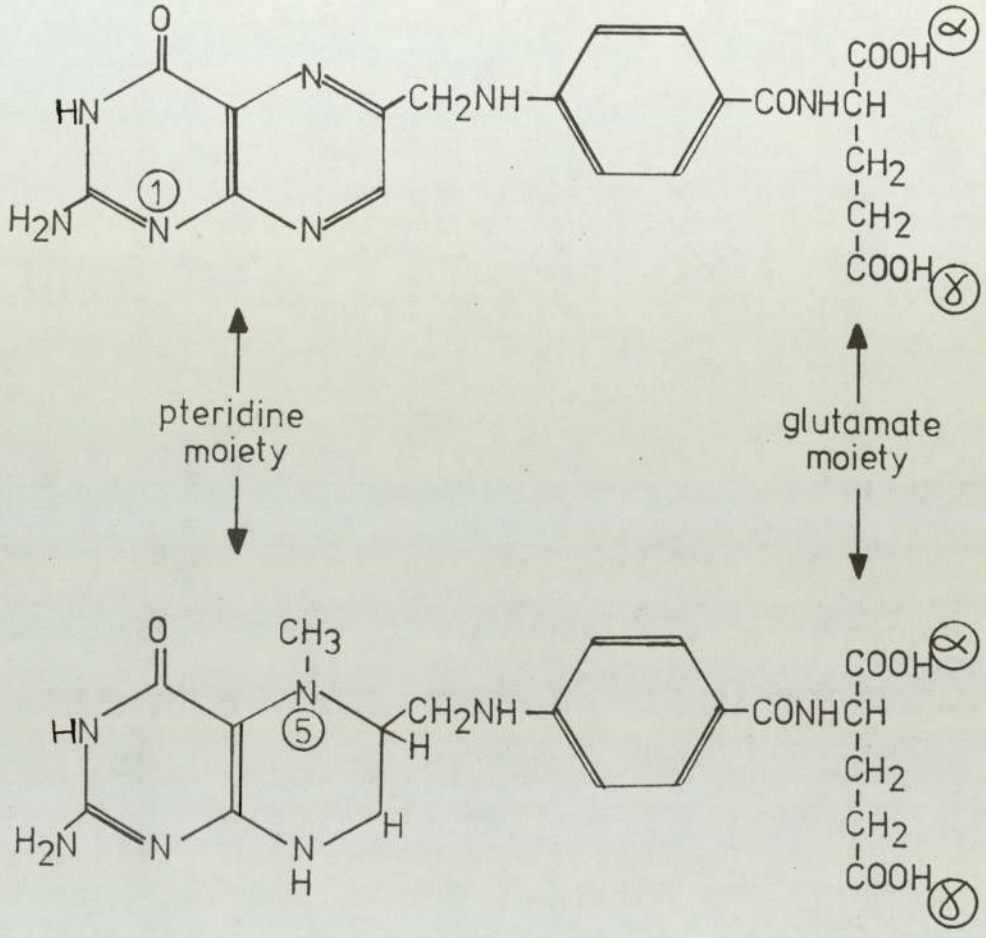
measured, using everted sacs, prepared as described in Chapter 2.3 and incubated for 30 min at 37°C in Krebs-Henseleit bicarbonate buffer. The effect of 10^{-6}M and 5 x 10^{-5} M-lead on the intestinal transport of 10^{-6} M 2-^{14}C-folic acid and 5-^{14}C-5-MeTHF was also measured. Radioactive folate was assayed as described in Chapter 2.5. Ascorbic acid (3 mg/ml) was added as antioxidant to all solutions of 5-MeTHF.

The presence of folic acid and 5-MeTHF at a concentration of 10^{-6} M, had no consistent effect on the transport of lead from the mucosal bathing medium into the serosal compartment (Tables 7.1, 7.2). However the presence of 5 x 10^{-5} M-folic acid and 5-MeTHF consistently increased the amount of lead transported across all regions of the intestine although this observation was not statistically significant. Neither folate species displayed any discernible influence on the tissue uptake of lead.

The presence of 10^{-6} M and 5 x 10^{-5} M-lead had no consistent effect on the transport of either folic acid or 5-MeTHF into the serosal compartment (Tables 7.3, 7.4). At the lower concentration of lead, tissue uptake of folic acid was also unaffected, but at the higher concentration of lead there was an increase in the tissue uptake of folic acid although this observation was not statistically significant (Table 7.3). In the presence of either concentration of lead, tissue uptake of 5-MeTHF was increased, the result was statistically significant ($p < 0.001$) at a concentration of 5 x 10^{-5} M-lead (Table 7.4). Water transport measurements for all these studies were within the normal range of control values.

The slight enhancement of the transfer of lead from the mucosal bathing medium into the serosal compartment in the presence of folic acid and 5-MeTHF suggests that a limited interaction may occur between lead cations and the α and γ carboxyl groups of both folic acid and 5-MeTHF (Fig. 7.1). (The dissociation constant for lead and the

Folic acid



5-methyltetrahydrofolic acid

Figure 7.1

Structural formulae of folic acid and 5-methyltetrahydrofolic acid.

	CONTROL	CONCENTRATION FOLIC ACID	
		10^{-6} M	5×10^{-5} M
SEROSAL (pmoles/g initial wet weight tissue)			
DUODENUM	25.5 ± 4.3	27.5 ± 3.3	30.3 ± 6.3
JEJUNUM	28.7 ± 5.2	29.3 ± 3.0	31.3 ± 6.0
ILEUM	24.7 ± 3.5	26.8 ± 1.0	35.2 ± 8.7
TISSUE (nmoles/g initial wet weight tissue)			
DUODENUM	2.2 ± 0.1	2.5 ± 0.1	2.1 ± 0.2
JEJUNUM	3.8 ± 0.4	4.3 ± 0.6	3.2 ± 0.5
ILEUM	4.3 ± 0.4	3.1 ± 0.5	2.9 ± 0.2

Table 7.1

Effect of 10^{-6} M and 5×10^{-5} M-folic acid on transport and uptake of 10^{-6} M-lead labelled with ^{203}Pb after 30 min incubation at 37°C.

	CONTROL	CONCENTRATION 5-METHYLTETRAHYDROFOLIC ACID	
		10^{-6} M	5×10^{-5} M
SEROSAL (pmoles Pb/g initial wet weight of tissue)			
DUODENUM	25.6 ± 4.3	23.0 ± 4.7	40.3 ± 7.2
JEJUNUM	28.6 ± 5.2	32.2 ± 6.4	30.4 ± 4.3
ILEUM	24.4 ± 2.8	23.2 ± 3.3	29.8 ± 2.1
TISSUE (nmoles Pb/g initial wet weight of tissue)			
DUODENUM	2.2 ± 0.1	1.9 ± 0.2	1.8 ± 0.2
JEJUNUM	3.8 ± 0.4	4.3 ± 0.5	4.1 ± 0.5
ILEUM	4.2 ± 0.4	3.8 ± 0.7	4.9 ± 0.9

Table 7.2

Effect of 10^{-6} M and 5×10^{-5} M-5-Methyltetrahydrofolic on transport and uptake of 10^{-6} M-lead labelled with ^{203}Pb after 30 min incubation at 37°C.

	CONTROL	CONCENTRATION LEAD	
		10^{-6} M	5×10^{-5} M
SEROSAL			
DUODENUM	0.13 ± 0.02	0.11 ± 0.01	0.12 ± 0.01
JEJUNUM	0.18 ± 0.03	0.21 ± 0.02	0.24 ± 0.04
ILEUM	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
TISSUE			
DUODENUM	0.86 ± 0.08	0.86 ± 0.05	0.96 ± 0.11
JEJUNUM	1.39 ± 0.16	1.35 ± 0.14	1.61 ± 0.12
ILEUM	0.20 ± 0.01	0.26 ± 0.04	0.24 ± 0.04

Table 7.3

Effect of 10^{-6} M and 5×10^{-5} M-lead on transport and uptake of 10^{-6} M ^{14}C labelled folic acid (nmoles Folic acid/g initial wet weight of tissue) after 30 min incubation at 37°C.

	CONTROL	CONCENTRATION LEAD	
		10^{-6} M	5×10^{-5} M
SEROSAL			
DUODENUM	0.20 ± 0.02	0.21 ± 0.02	0.22 ± 0.02
JEJUNUM	0.34 ± 0.05	0.40 ± 0.03	0.32 ± 0.03
ILEUM	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
TISSUE			
DUODENUM	0.84 ± 0.10	1.06 ± 0.10	1.51 ± 0.17**
JEJUNUM	1.26 ± 0.14	1.86 ± 0.10*	2.19 ± 0.28**
ILEUM	0.25 ± 0.01	0.29 ± 0.02	0.30 ± 0.03*

Table 7.4

Effect of 10^{-6} M and 5×10^{-5} M-lead on the transport and uptake of 10^{-6} M ^{14}C labelled 5-methyltetrahydrofolic acid (nmoles 5-MeTHF/g initial wet weight of tissue) after 30 min incubation.

folate species has not been published). The interaction would have the effect of neutralising the divalent lead cation thereby facilitating its passage across the lipoidal barrier.

Folic acid and 5-MeTHF transport into the serosal compartment was unaffected in the presence of lead suggesting that folate transport across the intestine was unaffected by:-

a) any possible interaction between lead and folate,

b) the large uptake of lead on to the tissue (as described in Chapter 4).

The result further suggests that the production of hydrogen ions and therefore the formation of the folate zwitterions is not reduced by lead.

The enhanced tissue uptake of folic acid and 5-MeTHF in the presence of lead (Tables 7.3, 7.4) may again be explained if the lead cation interacts with the α and γ carboxyl groups of the folates. The consequent neutralisation of negatively charged carboxyl groups would cause the positive charge, at the N1 position (folic acid) and the N5 position (5-MeTHF) to predominate (Fig. 7.1) which may permit an enhanced uptake of folate by the tissue $H_2PO_4^-$ ions. The greater enhancement of tissue uptake of 5-MeTHF as compared to folic acid (Tables 7.3, 7.4) may be due to the fact that within the existing acid microclimate of the glycocalyx the N5 position of 5-MeTHF is more readily protonated than the N1 position of the folic acid.

However, the same limited interaction between lead and folate will not influence the strong adherence of lead to the glycoprotein layer of mucosa, the tissue uptake of lead is therefore unaffected by folic acid or 5-MeTHF.

7.2 The Transfer of Folic Acid and 5-Methyltetrahydrofolic Acid Across Various Regions of the Intestine

At the physiological pH of the intestinal lumen both folic acid and 5-MeTHF exist in ionised forms, which are unlikely to be readily

transferred across the lipoidal barrier of the gastrointestinal tract. Under more acid conditions the proportion of folate species present as dianions would decrease while the more readily transported folate neutral species would increase (Fig. 7.2). It has been suggested that an acid microclimate, in existence at the surface of the brush border of the mucosa is responsible for the conversion of the folate molecules to their neutral forms, prior to movement across the intestine (Blair and Matty, 1974; Blair, Johnston and Matty, 1974; Blair, Matty and Razzaque, 1975).

Lucas and Blair (1978) using pH sensitive surface microelectrodes have demonstrated the presence of an acid microclimate, which declines slightly in acidity ($<$ 1 pH unit) in a proximal-distal direction. A possible consequence of the decline in surface ileal acidity would be a corresponding decrease in folate transport, as less neutral species would be present. Experiments were undertaken to observe whether the predicted decrease in folate transport across the ileum could be measured.

Everted sacs, prepared as in Chapter 2.3 from the duodenum and consecutive regions of the intestine from the mid-jejunum to the mid-ileum were incubated for 30 min at 37°C in Krebs-Henseleit bicarbonate buffer containing either 10^{-6} M 2-^{14}C-folic acid or 10^{-6} M 5-^{14}C-5-MeTHF (and 3 mg/ml ascorbic acid). The amount of folate transported was estimated as described in Chapter 2.5.

Across all the regions of the intestine investigated the amount of folic acid and 5-MeTHF transported into the serosal compartment was less than the amount taken up by the tissue (Figs. 7.3 and 7.4). In the more distal regions (Sacs VI, VII, VIII) of the intestine there was a marked decline in the total intestinal transport of both folic acid and 5-MeTHF. However, over this region total transport of 5-MeTHF was significantly greater than transport of folic acid (Fig. 7.5)

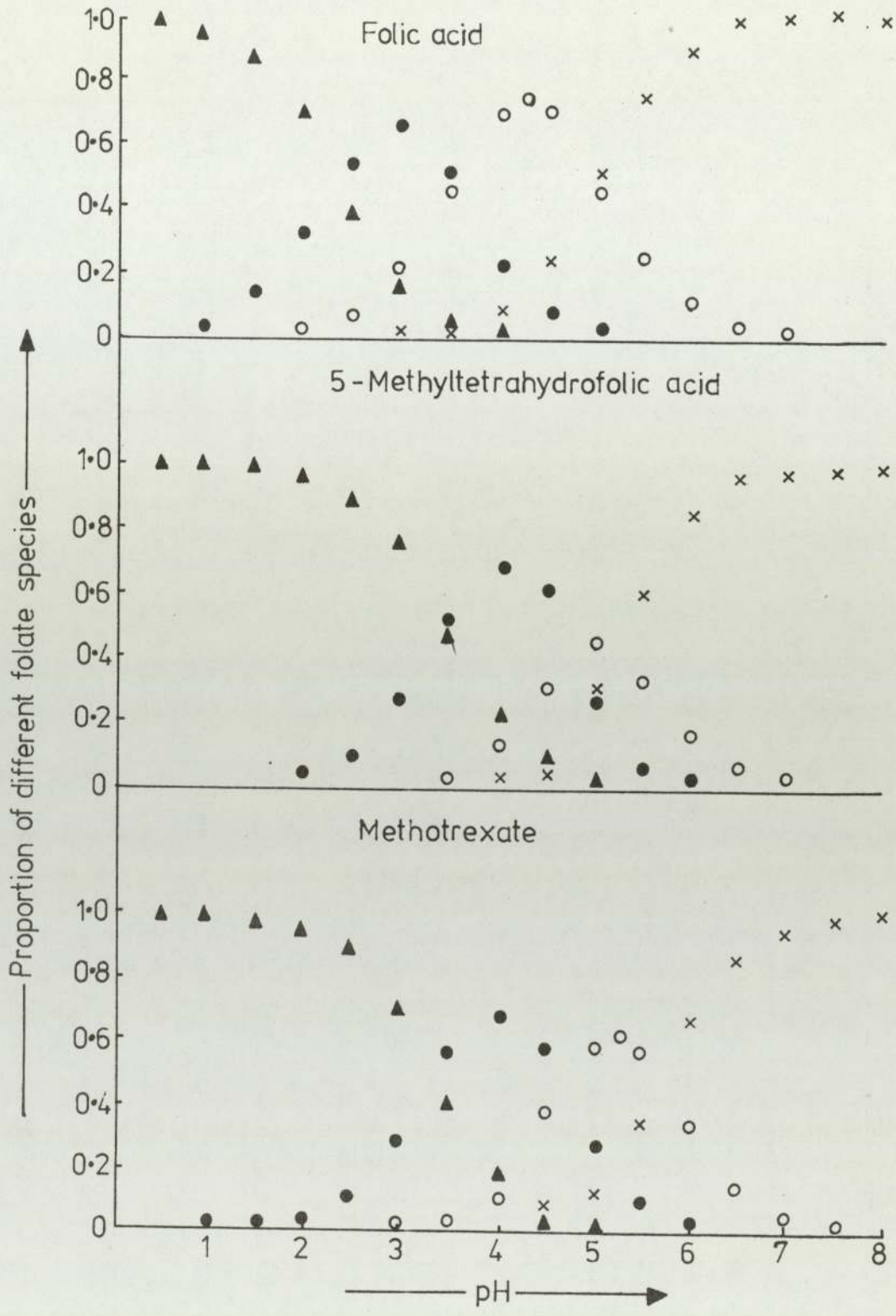


Figure 7·2

Percentage of folic acid and 5-methyltetrahydrofolic acid present as the dianion (x), monoanion (o), neutral species (●) and the cation (▲). Data were calculated by using pK values of 3.5 for the α carboxy group (Kallen and Jencks, 1966) and 5.0 and 4.8 for the γ carboxy groups of folic acid and 5-methyltetrahydrofolic acid respectively (Pohland, Flynn, Jones and Shive (1951); Kallen and Jencks, (1966), 5.2 for the N5 group of 5-methyltetrahydrofolic acid (Whiteley, Drais and Huennekens, (1969) and 2.35 for the N1 group of folic acid, Poe (1977)).

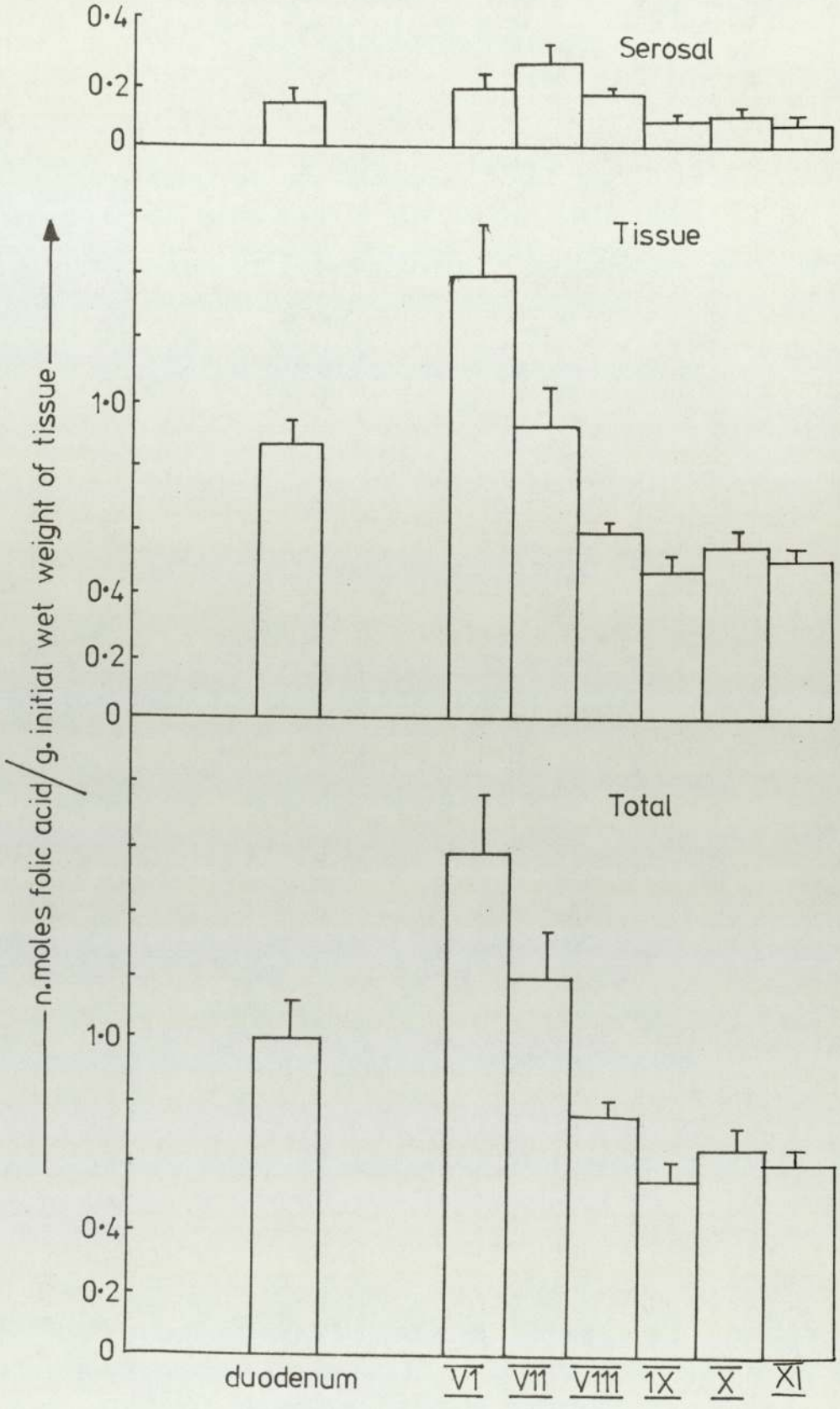


Figure 7.3

Transport of ^{14}C labelled 10^{-6} M-folic acid across everted sacs prepared from consecutive regions of the intestine, incubated for 30 min at 37°C.
Standard error of mean represented by vertical bars.

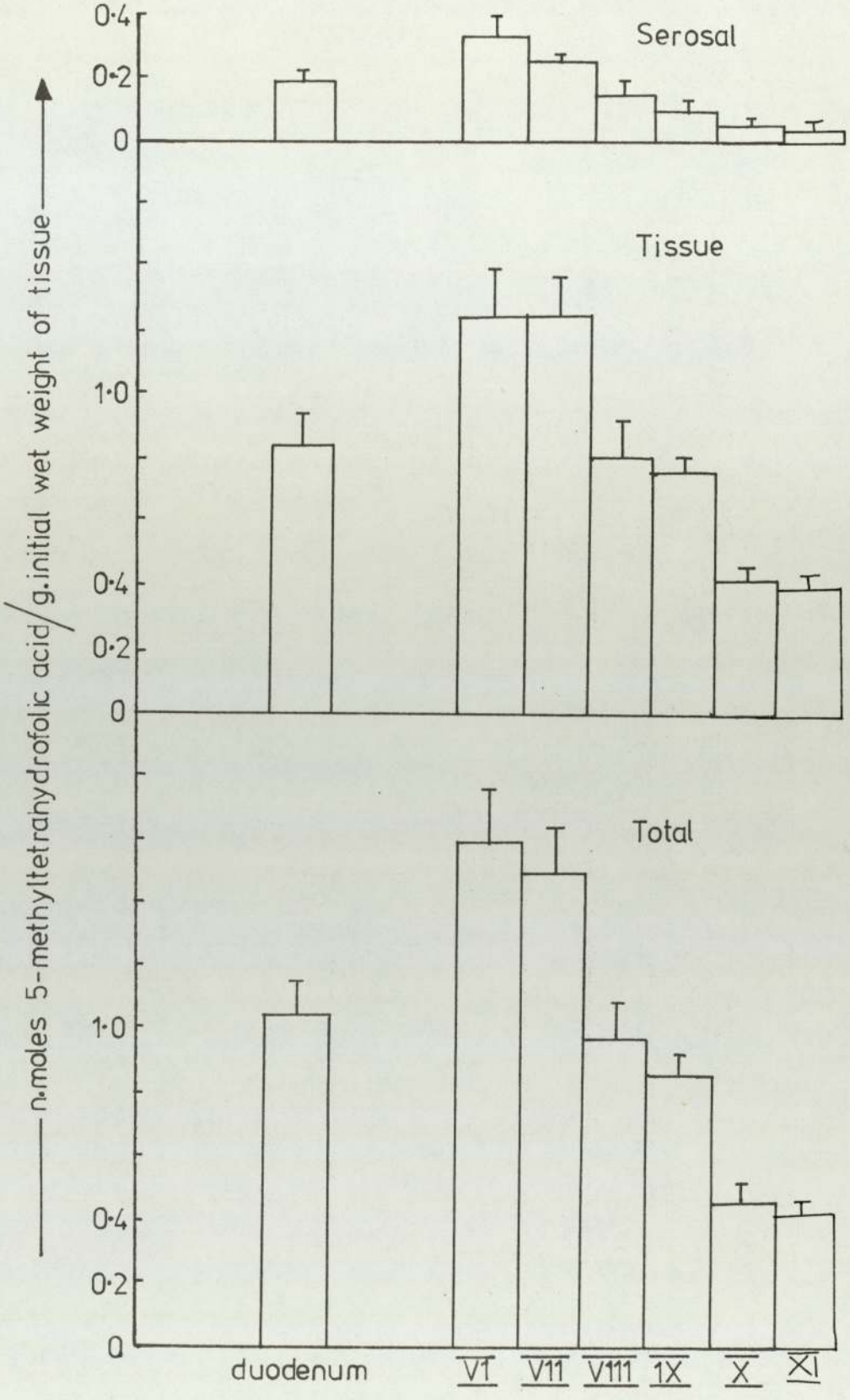


Figure 7.4

Transport of ^{14}C labelled 10^{-6} M-5-methyltetrahydrofolic acid across everted sacs prepared from consecutive regions of the intestine incubated for 30 min at 37°C.
Standard error of mean represented by vertical bars.

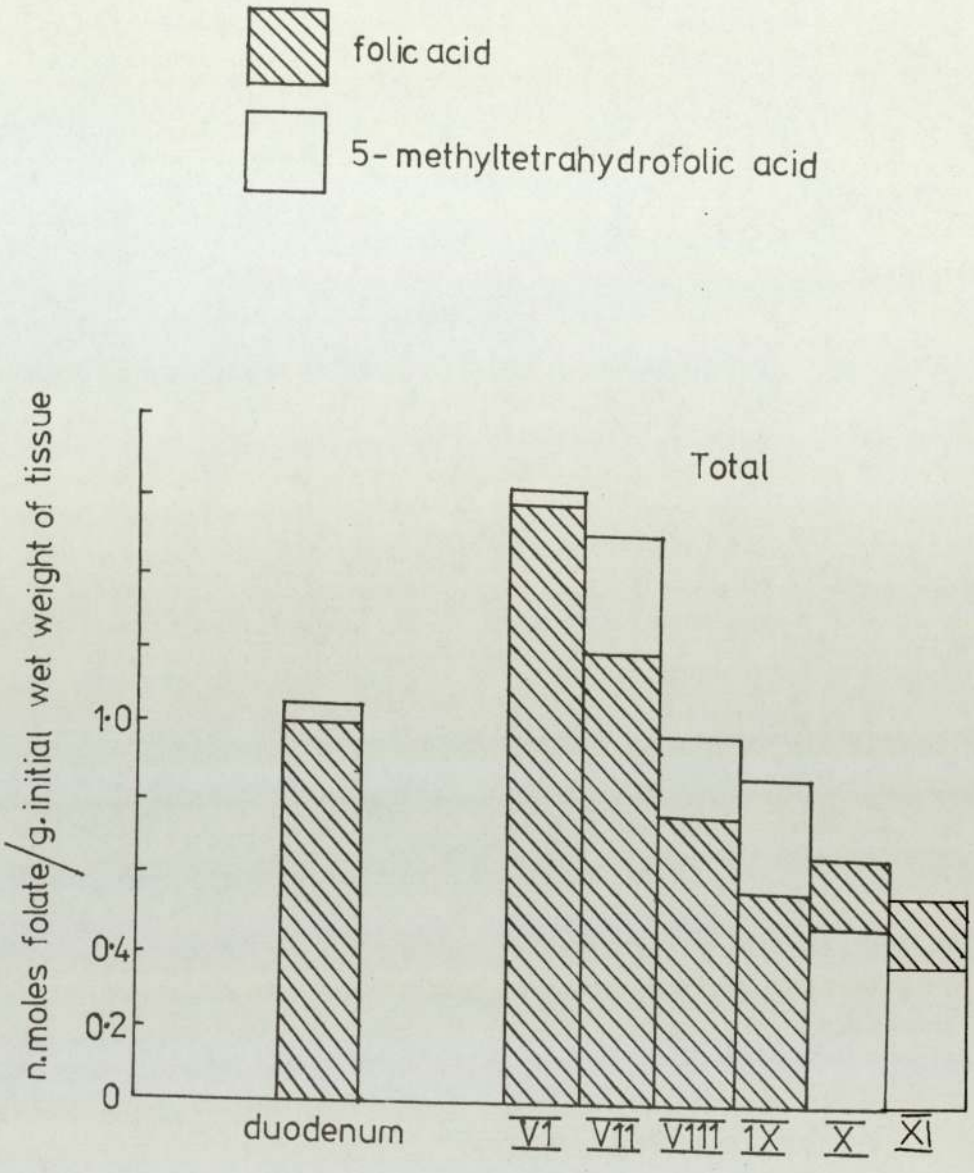


Figure 7.5

Comparison of the total transport of folic acid with that of 5-methyltetrahydrofolic acid across everted sacs prepared from consecutive regions of the intestine incubated for 30 min at 37°C.

($p = 0.01$). The difference was reflected by the threefold decline in total transport of folic acid as opposed to a twofold decline in the transport of 5-MeTHF. Equal amounts of folic acid and 5-MeTHF were transported across the mid-jejunum (Fig. 7.5). This was also the case across the duodenum although the amount transported was less, probably due to the lower water transport capacity of the duodenum.

Figures obtained for the total transport of folic acid (Fig. 7.3) across the mid-jejunum were in close agreement with those originally obtained by Smith, Matty and Blair (1970) and Blair, Johnston and Matty (1974). Measurement of the transport of 5-MeTHF into the serosal compartment gave figures similar to those obtained by Strum, Nixon, Bertino and Binder (1971) but these authors did not publish tissue uptake data. Measurements made by Blair, Matty and Razzaque (1975) for both serosal entry and tissue uptake of 5-MeTHF were less than the values presented in Figure 7.4. Water transport measurements made by these workers were also lower than the observations presented in Table 7.5.

Blair and Matty (1974) have postulated that the pH value of the acid microclimate in the mid-jejunum is approximately 3.5. At this pH, equal quantities of folic acid and 5-MeTHF occur in their readily transported neutral form (Fig. 7.2) therefore it would be anticipated that transport of both species would occur at similar rates. The reported observations (Fig. 7.5) offer good support for the theory as approximately equal amounts of both folic acid and 5-MeTHF are transported across the mid-jejunum and duodenum.

The values presented in Figure 7.3 indicate a decrease in the amount of folic acid transported across the more distal regions of the intestine. From the results of Lucas and Blair (1978) it may be predicted that the decline in acidity would be paralleled by a decline in the amount of folic acid transported, since with increase in pH, the

		10^{-6} M FOLIC ACID	10^{-6} M 5-MeTHF
SEROSAL			
DUODENUM	(D2)	19 ± 34	-39 ± 8
JEJUNUM	(V)	233 ± 38	203 ± 64
	(VI)	263 ± 74	233 ± 53
	(VII)	234 ± 41	226 ± 55
	(VIII)	171 ± 41	180 ± 35
	(IX)	324 ± 45	136 ± 30
	(X)	242 ± 73	109 ± 30
DISTAL ILEUM	(XI)	185 ± 33	139 ± 21
TISSUE			
DUODENUM	(D2)	483 ± 50	333 ± 26
JEJUNUM	(V)	515 ± 51	472 ± 20
	(VI)	444 ± 77	430 ± 34
	(VII)	347 ± 63	362 ± 49
	(VIII)	302 ± 50	321 ± 51
	(IX)	421 ± 84	402 ± 77
	(X)	390 ± 44	315 ± 66
DISTAL ILEUM	(XI)	384 ± 25	354 ± 35
TOTAL			
DUODENUM	(D2)	502 ± 76	296 ± 36
JEJUNUM	(V)	748 ± 72	675 ± 54
	(VI)	707 ± 111	662 ± 49
	(VII)	581 ± 79	588 ± 76
	(VIII)	473 ± 50	499 ± 46
	(IX)	745 ± 109	538 ± 85
	(X)	632 ± 114	420 ± 83
DISTAL ILEUM	(XI)	569 ± 32	493 ± 38

Table 7.5

Effect of sac position on water transport (mg H_2O/g initial wet weight of tissue) in the presence of folic acid and 5-methyltetrahydrofolic acid, after 30 min incubation.

proportion of neutral species present decreases (Fig. 7.2). Therefore these results (Fig. 7.3) are again in general agreement with the acid microclimate hypothesis.

The data presented in Figure 7.2 indicates that as pH increases from a value of 3.5, the proportion of 5-MeTHF neutral species increases, reaching a maximum value at pH 4.2 before declining with further decrease in acidity. The predicted increase in transport of 5-MeTHF in more distal and less acidic regions of the intestine (Sacs VI, VII, VIII) was not observed (Fig. 7.4). On the other hand Strum et al (1971) have reported that there was little difference in the amounts of 5-MeTHF transported across different regions of the intestine.

Significantly more 5-MeTHF than folic acid was transported across sacs VI, VII, VIII ($p < 0.01$). This observation, taken in conjunction with the data of Strum et al (1971) indicates the essential role of the acid microclimate in the transport of folates across the intestine.

Figure 7.2 shows that the proportions of neutral species at different pH values of methotrexate are similar to the proportions of 5-MeTHF neutral species at the same pH values. It would be of interest to further test the hypothesis by investigating the intestinal transport of methotrexate under the same experimental circumstances described here.

DISCUSSION

It has now been established that the majority of the body burden of lead, in adults not occupationally exposed to lead, results largely from the ingestion of inorganic compounds of the element rather than their inhalation (Hilburn, 1979). Therefore, a knowledge of the precise mechanism and route by which the lead cation crosses the epithelial membrane of the intestine is essential if one is to evaluate the dietary and physiological factors which influence lead absorption. In addition the development of a model to define the relationship between the intestinal absorption of lead and the body burden of lead must also take account of the fact that only 5-10% of the lead presented to the gastrointestinal tract enters the body of the normal adult (Kehoe, 1961). Throughout these studies, the in vitro everted sac preparation has been used to provide information concerning the movement of the lead cation across the small intestine. Consequently in developing a model, the interpretation of the observations reported must take into account, not only the complexity of the intestinal barrier, but also the differences between in vitro and normal physiological conditions.

A solute may cross the epithelial cell membrane by either passive or active transport. Active transport (movement across a membrane or a layer of cells against a chemical potential gradient) requires a source of energy to initiate and maintain the movement of solutes. If an energy supply is not utilised then the transmembrane movements are passive even though an interaction between the solute and membrane can occur.

The individual epithelial cells of the small intestine are polarised, in that brush border and basolateral facing membranes possess different properties. There are two theories to explain the active transcellular movements of solutes. In one model, the intracellular concentration of a solute is maintained below the external concentration by means of outwardly directed 'pumps' located

principally at the basolateral membranes of the cell. In this situation work is done in moving the solute out of the cell, across the membrane, whilst entry is passive via 'leaks'. In the other model, substances may be accumulated within the cell by an inwardly directed 'pump' located at the brush border membrane. Solutes are passively leaked from the cell at the basolateral membranes.

The movement of a solute across the intestinal epithelium to the blood or lymph system may proceed by either a transcellular or an extracellular route. The small intestine mucosal epithelium is composed of highly specialised cells joined tightly together in the terminal bar region to form a layer one cell thick. Between the cells there are potentially large intercellular spaces. Therefore, a solute that traverses the epithelium by a transcellular route, enters the cell at the brush border then passes out either directly, or via the intercellular spaces, to the basement membrane.

The intestinal mucosal cells, linked together at the terminal bar, rest on the basement membrane. The zonulae occludentes, where the individual mucosal cells are joined together, extend only for a short distance (less than 1 μm) immediately below the brush border. The remainder of the cell surface forms the 'output' membrane or base of the absorbing cell. Hence the intercellular spaces are long and narrow; limited at the luminal end by the tight junctions and at the distal end by the basement membrane. In all epithelia the intercellular spaces are specialised microenvironments within which local gradients of hydrostatic and osmotic pressure are established. These local gradients are of fundamental importance in determining fluid transport across epithelia. A constant feature of the small intestinal mucosa is that epithelia cells formed by mitosis in the crypts of Lieberkuhn move up the sides of the villi on the basolateral membrane to be desquamated at the tips of the villi, leaving gaps in the

cellular pallisade. Therefore extracellular transport may involve movement through the zonula occludens or through some defect in the cellular pallisade.

Water absorption is closely coupled to solute absorption. From isotonic solutions water movement follows solute movement. Under physiological conditions water is absorbed from solutions which are isotonic with plasma but under artificial conditions, water movement may occur against a gradient of water activity. Water absorption is produced by the local accumulation of solute in the intercellular spaces of the intestinal epithelium, making the fluid there hypertonic. As a result of this local hypertonicity water passes into the intercellular channel through the zonula occludens or the basolateral membranes of the cell. The net influx of water causes local hydrostatic pressure within the channel to rise. If hydraulic resistance at the basement membrane is lower than that at the zonula occludens, water and solute will move across the basement membrane. In the steady state, solute will continually emerge from the channel in a manner dependent on the length, radius and water permeability of the intercellular spaces (Parsons, 1975).

Under normal physiological conditions substances that have negotiated the epithelial barrier pass to the blood or lymphatic system for subsequent distribution. In the in vitro everted sac preparation substances absorbed at the mucosal side have to pass through submucosal and smooth muscle layers before reaching the serosal fluid. During in vitro experimentation it has been observed that the first fluid to gain access to the serosal compartment is blood stained which suggests movement of blood from the capillaries into the serosal compartment and also movement of absorbed substances (Smyth, 1963). Lee (1961) presents evidence that indicates that the lymphatic system is also a route for mucosal→serosal transfer.

However some authors have suggested that the muscle accumulates substances and that it acts as a barrier affecting kinetic measurements (McDougal, Little and Crane, 1960) and ion diffusion (Wright, 1966). Consequently although Smyth (1974) claims that there is little difference between normal physiological and *in vitro* routes of transport, experimental observations concerning the serosal entry of substances should be interpreted with caution. A difference between *in vivo* and *in vitro* observations does exist with regard to concentration gradients. *In vivo* substances enter the capillaries and are rapidly carried out so that no concentration build up occurs. In *in vitro* preparations, particularly the instance of the everted sac where the serosal volume is small, concentrations of absorbed substances may build up rapidly so that the transport process probably proceeds under less favourable conditions.

Kinetic studies demonstrated that lead ions are transported to the serosal space at approximately equal rates across all regions of the rat intestine and that there is no preferential site of absorption. Entry of lead into the serosal compartment increases linearly over the concentration range 10^{-7}M to 5×10^{-5}M-lead. The lack of evidence for saturation and the fact that changes in glucose concentration, temperature and anoxic conditions have little effect on lead transport, indicate passive movement of the cation into the serosal compartment.

During the incubation period from 10 min to 60 min the entry of lead and water into the serosal compartment are well correlated. However, during the first 10 min of incubation, lead appears almost immediately in the serosal fluid but no net movement of water occurs. One explanation for the effect may be that during the initial incubation period, entry of water into the serosal compartment is balanced by tissue uptake of water from the serosal compartment. The rapid serosal entry of lead implies that a proportion of the lead finds ready access

into the extracellular spaces and across the muscle layer since, if it were taken up and bound in the sub-epithelial cells, its appearance in the serosal fluid would be delayed. A detailed study of transport over the first 10 min of incubation could test this hypothesis. Fig. 4.2 shows a slowing of the rate of entry of lead into the serosal compartment during the initial 10 min of incubation. This would suggest the development of an adverse concentration gradient, a property which is more likely to be due to this in vitro technique rather than a feature of the transport mechanism. However the rate of entry remains nearly constant after approximately 10 min of incubation and indicates that serosal accumulation of lead achieves a steady state whereby lead transport is dependent upon the normal intercellular and extracellular gradients.

Failure to observe saturation kinetics and energy dependent transport negates the possibility of a specific membrane interaction for transcellular movement of lead. Also the small activation energy suggested, from the low Q_{10} values, for the transport of lead (Table 4.10) argues against transmembrane movement. The charge and solvation associated with the lead cation also prevents ready penetration of the lipoidal intestinal cell membrane at the brush border, and transepithelial movement is more likely to be via an extracellular route, into the intercellular spaces, assisted by flow of water through the zonulae occludentes. Decrease in mucosal calcium concentration and pH, increase the permeability of the epithelial barrier to lead, possibly due to changes in the size and charge associated with the zonula occludens.

Zonulae occludentes serve as a transepithelial route for a number of ions and as such they may well represent the "common pathway" postulated by Meredith et al (1977) for the intestinal transport of lead. This morphological feature of the epithelium may be likened to

an ion-exchange resin (Barry and Diamond, 1971). Therefore the rate at which lead permeates the membrane is dependent upon the charge associated with the membrane and the extent of the competition from other ions present.

In the experimental system, only a small percentage of the lead is transferred into the serosal compartment but a far greater proportion of lead is taken up by the intestinal tissue (10-15%). Smith, DeLuca, Tanaka and Mahaffey (1978), also using the everted sac preparation, have reported similar results for the tissue content of lead.

Studies using the everted sac do not reveal the precise location of lead in the tissue. Nevertheless the kinetic data does permit some speculation as to the localisation of lead. The kinetic data does indicate that there is a rapid uptake of lead by the intestinal tissue which is dependent on initial concentration of mucosal lead. The lead is not easily removed from the tissue either by washing or chelation and suggests a strong interaction. This may represent an uptake into the whole intestinal tissue from the epithelium to the peritoneum, whereby the lead is bound to the muscle cells as well as to the cells of the epithelium. Such a distribution of the lead would explain the difficulty of removing the element from the tissue by washing or chelation. However, a more likely localisation of the inorganic lead is within the epithelial region and/or at the mucosal surface of the intestinal epithelium.

The reasons for this are as follows:-

1. It is unlikely that lead is distributed throughout the tissue since the uptake of lead by the tissue reaches equilibration more rapidly than the tissue uptake of water.
2. Whilst a large proportion of lead available for transport is associated with the tissue, a relatively small percentage of the element gains access to the body. This suggests that the majority of the lead is present in the epithelial region.
3. Experimental data indicates that bile salts (sodium

deoxycholate) have little effect on tissue uptake of lead at low concentration. However at high concentrations when their critical micellar concentration is exceeded, bile salts behave as strongly surface active detergent agents and are capable of solubilising the membrane surface (Coleman, Iqbal, Godfrey and Billington, 1979). At such concentrations of bile salts the tissue uptake of lead is significantly reduced. The observation strongly implies that the interaction between lead and the intestinal tissue is a surface effect.

4. Conrad and Barton (1978) support this notion by suggesting from autoradiographic evidence that there is a dense accumulation of lead around the epithelium. No lead was detected in the sub-epithelial regions.

Nevertheless further confirmation of this theory is required and may be achieved in several ways.

1. Autoradiographic evidence to show the distribution of lead in the everted sac after various periods of incubation.
2. Measurement of the uptake of lead by:-
   a) Isolated mucosal cells.
   b) Isolated brush border fractions.
   c) Everted sacs after incubation for various periods of less than 10 min.
3. Attempts should also be made to characterise the species that interact with lead at the epithelial surface using techniques such as chromatographic separations.

As lead appears to be strongly bound to the surface of the epithelial cells it is possible to speculate as to the nature of the species with which lead interacts. These may be carboxylic groups, sulphydryl groups, hydroxyl or phosphate groups which are associated with various protein and lipid components of the cell membrane. Of these groups phosphate is the strongest candidate.

In animal cell membranes, the dominant lipids are glycerophospholipids although cell surface membranes also contain substantial proportions of sphingolipids and neutral lipids such as cholesterol. Glycerophospholipids are derivatives of glycerol which have a phosphate containing substituent. It is quite possible that lead may interact with these components of the cell membrane and that the interaction represents the proportion of lead which is extremely tenaciously bound to the tissue surface and may only be removed by the action of surface active agents or lost during the normal cycle of epithelial cell turnover. 'Free' phosphate is available also from the hydrolysis of ATP, and the action of alkaline phosphatase.

Clarkson and Kench (1958) have reported that 95% of the lead in whole blood is taken up by the erythrocyte. They suggest that the uptake of lead occurs by the combination of peptised lead phosphate sol particles, on the surface of the cell to form a precipitate or larger particulate form of lead. The cell surface, being largely dominated by phosphate groups, provides an ideal surface for such an aggregation. Once the coagulation of lead phosphate sol on the cell surface is complete its dispersion is slow. Clarkson and Kench (1958) also report that chelating agents only slowly remove the lead once attached to the cell. The interaction between lead and the intestinal tissue could be analogous, as similar kinetic data to that reported by Clarkson and Kench (1958) has been observed in the present studies.

An important implication of the lead-tissue interaction is that it may well represent an initial event in the control of the entry of the element into the body. When bound to the tissue the lead is not available for transport across the intestine. Consequently the uptake of lead on to the tissue, as much as 90% under normal physiological conditions, may explain why only 10% of an oral dose of lead is absorbed by an adult. It is therefore proposed that the tissue uptake of lead is an important intestinal process which serves to protect the animal from excessive absorption of lead.

A similar mechanism has been proposed for cadmium. Cadmium concentrates to a marked extent in the small intestinal wall, probably in the absorptive mucosal cells, thus a barrier is provided against massive entry of the element into the body. Due to the rapid turnover of intestinal mucosal cells most of the cadmium is subsequently lost from the body when the cells are sloughed from the tips of the villi (Spivey-Fox, 1974).

The <u>in vivo</u> uptake of lead is probably far greater than that which has been reported in these <u>in vitro</u> studies and may be explained as follows.

An equilibration may occur between the concentration of lead in the tissue and that in the mucosal solution. An equilibration constant (K) for the system may be defined as

$$K = \frac{x}{M} \Big/ \frac{100 - x}{V}$$

where x = percentage of lead attached to tissue

100 - x = percentage of lead remaining in the bathing medium

M = mass of tissue

V = volume of mucosal fluid

If $\bar{V}$ represents the ratio of volume of fluid (cm^3) to the mass of tissue (g) then

$$K = \frac{x\bar{V}}{100-x}$$

In the experimental system 10 ml of fluid were used to bathe approximately 0.5 g of tissue, a $\bar{V}$ value of 20. Under normal physiological conditions the ratio is much smaller and $\bar{V}$ approaches 0.3 (Luminal volume, 500 ml, Intestinal tissue weight, 1650 g (Black, 1964; Biochem. Handbook, 1971)). The equilibration relationship suggests that as the volume is reduced thereby lowering the value of $\bar{V}$, the percentage of lead bound to the tissue will increase. This was observed experimentally.

Across different regions of the intestine the following mean values of K were derived by substituting experimental observations into the above equation.

$$K_{\text{duodenum}} = 1.06 \pm 0.41$$
$$K_{\text{jejunum}} = 1.62 \pm 0.34$$
$$K_{\text{ileum}} = 1.70 \pm 0.49$$

These derived figures suggest that under normal physiological conditions the percentage of lead taken up by the intestinal tissue would always be greater than 70% in the duodenum and average between 80% and 90% in the jejunum and ileum.

Any conditions in which the interaction between lead and the tissue decreases will result in an increase in the amount of 'free' lead in the lumen. Consequently more lead would be available for transport across the intestinal barrier.

Although DTPA and bile salts reduce the amount of lead taken up by the tissue, the decrease is not reflected by a proportional increase in the amount of lead entering the serosal compartment. The failure to observe an increase in the serosal transport experimentally is probably due to the large fluid volume/tissue mass ratio of the *in vitro* experimental system.

Under in vitro experimental conditions

Lead Tissue Uptake	'Free' Lead in Mucosal Solution
13%	87%

Now, if the tissue uptake of lead is reduced by 33% then

Lead Tissue Uptake	'Free' Lead in Mucosal Solution
9%	91%

i.e. there is only a minimal increase in the amount of lead available in the mucosal solution.

However, under physiological conditions

Lead Tissue Uptake	'Free' Lead in the Gut Lumen
85%	15%

If the tissue uptake of lead is reduced by 33% then

Lead Tissue Uptake	'Free' Lead in the Gut Lumen
60%	40%

i.e. there is a greater than two-fold increase in the amount of lead available in the gut lumen.

Hence in the physiological condition, any decrease in the amount of lead taken up by the tissue may result in a marked increase in the amount of lead available for transport.

The simple model presented to explain the mechanism of lead transport can also be used to predict conditions in which the rate of absorption of lead may be increased. That is, any condition which a) reduces the interaction between lead and the intestinal tissue and / or b) affects cell to cell adhesion and renders the zonulae occludentes more permeable to lead.

Factors Affecting Intestinal Absorption of Lead

One factor which may affect the lead-tissue interaction is the pH of the luminal contents. The intestinal acid microclimate associated with the glycocalyx is responsible for the maintenance of a mucosal epithelium surface pH of 3.5 (Blair and Matty, 1974). The predominant

phosphate ion at this pH value is $H_2PO_4^-$. Therefore under normal conditions the adsorption of lead is probably the result of its sequestration as lead dihydrogen phosphate ($Pb(H_2PO_4)_2$). Above pH 3.5 the acid microclimate is little affected; however below pH 3.5 surface acidity equals luminal acidity (Lei, Lucas and Blair, 1977) and the phosphate species present gradually changes from $H_2PO_4^-$ to H_3PO_4 and $H_2PO_4^-$ with increase in acidity (pKa, H_3PO_4 = 2.1, pKa, $H_2PO_4^-$ = 7.2). For example, at pH 7.4, approximately 100% of the phosphate present is the $H_2PO_4^-$ species but at pH 2.4, 30% of the phosphate is the H_3PO_4 form and only 70% as the $H_2PO_4^-$ species, Therefore lead adsorption is reduced under very acidic conditions as less $H_2PO_4^-$ groups are available for interaction with lead.

Phosphate ions are formed in the glycocalyx by enzymatic hydrolysis of adenosine triphosphate. Any condition in which:-

a) the glycocalyx is lost or diminished,

b) the enzymatic activity of adenosine triphosphatase is decreased,

c) the supply of adenosine triphosphate is decreased,

will cause a decrease in the production of phosphate ions and a subsequent decrease in the amount of lead sequestered.

Therefore, any of the following conditions could result in a large proportion of lead being absorbed

a1) coeliac disease, Crohne's disease, (lack or diminution of the glycocalyx)

a2) high concentrations of sodium deoxycholate in the gut, (Blind loop syndrome)

a3) any molecule which exhibits detergent properties will solubilise the membrane and diminish the glycocalyx e.g. detergents

b1) deficiency of co-enzymes such as magnesium, sodium or potassium ions, (ATPase) or zinc (alkaline phosphatase)

b2) enzyme inhibitors (e.g. inhibition of adenosine triphosphatase, alkaline phosphatase)

c1) lack of glucose (starvation)

c2) glycolytic inhibitors e.g. fluoride ions.

c3) The presence of pharmacological or toxic agents, e.g. methotrexate, azides, propranolol.

The model also explains the influence of various dietary constituents on the absorption of lead. Dietary components may complex with lead, decrease the lead-tissue interaction and consequently increase the quantity of lead remaining in the lumen. However, whether or not there is an increase in the rate of lead transport will depend upon the size, charge and lipid solubility of the complex.

Any increase in the fluid volume to tissue mass ratio (i.e. increase in $\bar{V}$ values) will also decrease tissue uptake of lead. Consequently any conditions that increase the volume of the intestine, such as a large fluid or food intake, will also increase the amount of lead available for transport, provided the concentration of the lead in food or fluid is not decreased.

Conditions of extreme acidity or extreme calcium deficiency will also result in an increase in lead movement due to increased permeability of the zonulae occludentes. Any conditions in which the osmotic pressure, in either the underlying lateral spaces between the cells or the blood stream, is elevated may also result in an increase in the absorption of lead. Conditions in which this effect may occur are:-

a) cirrhosis of the liver; one symptom is an increase in Na^+ retention which leads to an increase in water movement across the intestinal tract and therefore increased concommitant movement of lead,

b) diabetes; where the osmotic pressure within the extracellular spaces is increased as a result of

elevated glucose levels. There may be an increase in water movement from the intestinal lumen with concommitant increase in the transport of lead.

Serum Levels of Lead

The simple model may also be used to demonstrate that the blood-lead level may be controlled by the concentration of lead in the gut lumen. The 'average' ingested intake of lead from food, water and other sources is approximately 200 μg per day. If 90% of the lead presented to the lumen is bound to the intestinal wall, the remaining 10% or 20 μg exists in free solution. Therefore, assuming that the fluid volume of the small intestine is 500 ml (Black, 1964), the luminal concentration of 'free' lead is 40 μg lt^{-1}.

The blood-lead level of the Birmingham adult not occupationally exposed to lead, is approximately 20 μg lead/100 ml blood (Hilburn, 1979). 90% of the lead is taken up by the erythrocytes (Clarkson and Kench, 1958). The erythrocytes comprise almost 50% of the total blood volume which means that the concentration of 'free' lead in the serum is 2 μg/50 ml or 40 μg lt^{-1}.

Therefore changes in the whole blood-lead value probably only reflect changes in the dietary intake of lead and is therefore not an accurate index of the body burden of lead.

The aims of this study, outlined in Chapter 1.10, have been achieved. However the inferences proposed require confirmation in humans and further work is needed to support the proposed model. The transepithelial route of lead transport should be further elucidated by electron microscope and autoradiographic studies to demonstrate the presence (or absence) of lead within the region of the zonulae occludentes. Sub-cellular preparations may reveal the proportion of lead that gains access to the cell and the pattern of distribution within the cell. The lead-tissue interaction requires further detailed

analysis by examining,

a) the effect of inhibition of adenosine triphosphatase and alkaline phosphatase,

b) the influence of bile and other molecules with detergent properties.

Nevertheless the simple model presented does suggest a physiological mechanism which indicates that the lead-tissue interaction governs the movement of lead across the gastrointestinal tract and also predicts conditions which will affect the absorption of lead.

APPENDIX 1

A1 The Limitations of the Isotope ^{210}Pb for Biological Experimentation

Three isotopes of lead, ^{203}Pb, ^{210}Pb and ^{212}Pb were considered for use as tracers in the study of the intestinal absorption of lead. Initially ^{210}Pb (supplied by the Radiochemical Centre, Amersham) was selected as the tracer for transport studies and was detected by means of β-scintillation techniques. The isotope was supplied in secular equilibrium with its daughter nuclides, bismuth (^{210}Bi) and polonium (^{210}Po) which can also be detected by β-scintillation counting. Transport studies in which ^{210}Pb was used as the tracer proved to be inconvenient and the results difficult to interpret.

^{210}Pb decays as illustrated in Figure A.1 which indicates that both γ and β emissions are produced and may be used for assessment of the activity present.

However the gastrointestinal membrane may be responsible for partial biological separation of the three nuclides involved, so that different proportions of the mixture would be present either side of the membrane. Therefore the detection of radioactive emissions from ^{210}Pb was examined to establish conditions where only the emissions resulting directly from ^{210}Pb were counted.

A2 Counting of ^{210}Pb

i) γ Counting

Despite the fact that γ rays are formed from ^{210}Bi* (0.047 MeV) the transitional state of ^{210}Bi* exists for only a short period of time (< 3 ns) and for practical counting purposes γ emissions may be regarded as being due to the disintegration of lead. However, only 4% of the total available disintegrations appear as γ radiation and therefore an effective counting efficiency of only 2-3% can be obtained. This figure is far too low for accurate counting unless the amount of activity is increased to very high levels.

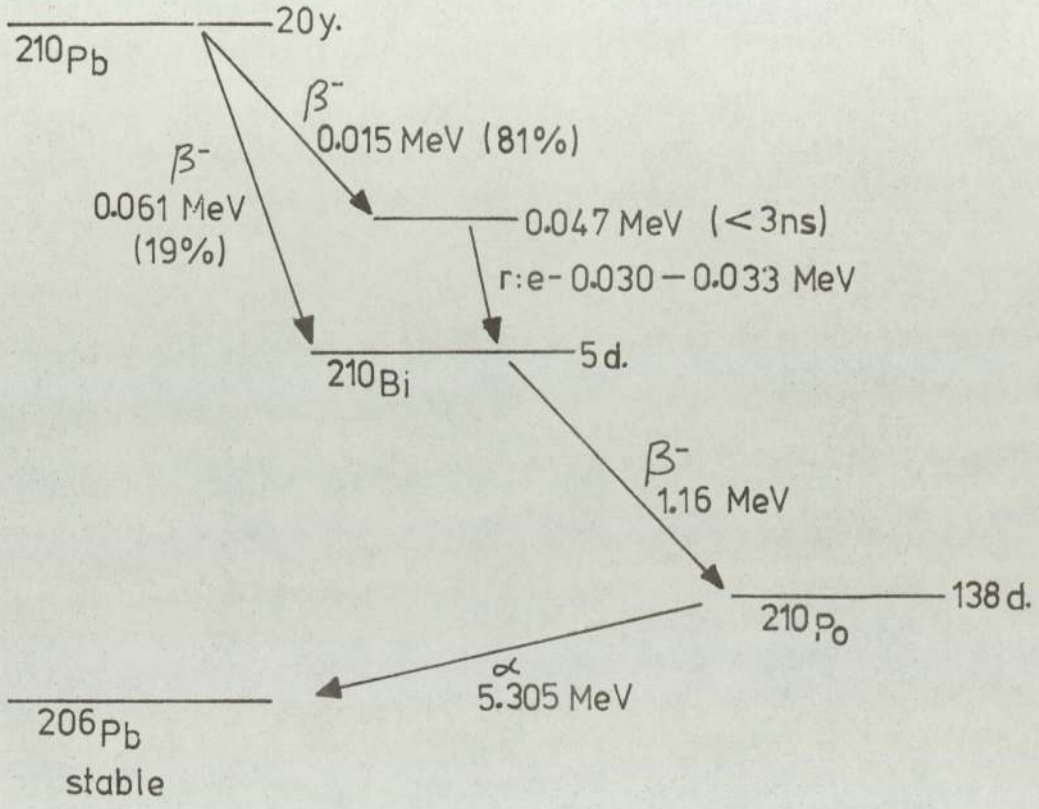


Figure A1

Decay scheme of ^{210}Pb.

ii) β Counting

To find the instrument settings that would permit only ^{210}Pb to be counted, a fluorescence spectrum of the isotopic mixture at equilibrium was plotted, using the β facility of a Nuclear Enterprises NE 8312 counter.

The spectrum produced (Fig. A2) is shown in conjunction with the spectra of

i) ^{3}H which has an end-point energy approximating to the 0.015 MeV end-point energy of ^{210}Pb.

ii) ^{14}C end-point energy approximating to 0.061 MeV of ^{210}Pb.

iii) ^{32}P end-point energy approximating to 1.15 MeV of ^{210}Bi.

iv) ^{63}Ni same end point energy as ^{210}Pb.

v) ^{125}I showing the effect of gamma emissions in a liquid scintillation system. γ energy of ^{125}I (0.035 MeV) is close to that of ^{210}Pb (0.047 MeV).

The spectra suggest that ^{210}Pb emissions can be isolated and counted from instrument settings 0-4.5 and that ^{210}Po appears as a higher energy peak. In the liquid scintillation system this peak is due to the mono energetic α particles and should be narrow. The peak was confirmed by comparison with a peak produced by the α emission of ^{241}Am. However the fluorescence spectrum of ^{210}Bi was smeared across the range of instrument settings and contributed to both ^{210}Pb and ^{210}Po peaks (Fig. A3). Therefore although the signals from ^{210}Pb emissions could be discriminated from those of ^{210}Po the presence of ^{210}Bi interfered with accurate counting of ^{210}Pb.

Separation of ^{210}Pb from its Daughter Nuclides

In an attempt to overcome the problem of interference from ^{210}Bi, ^{210}Pb was separated from its daughter nuclides by ion-exchange chromatography. The separation has been investigated by Nelson and

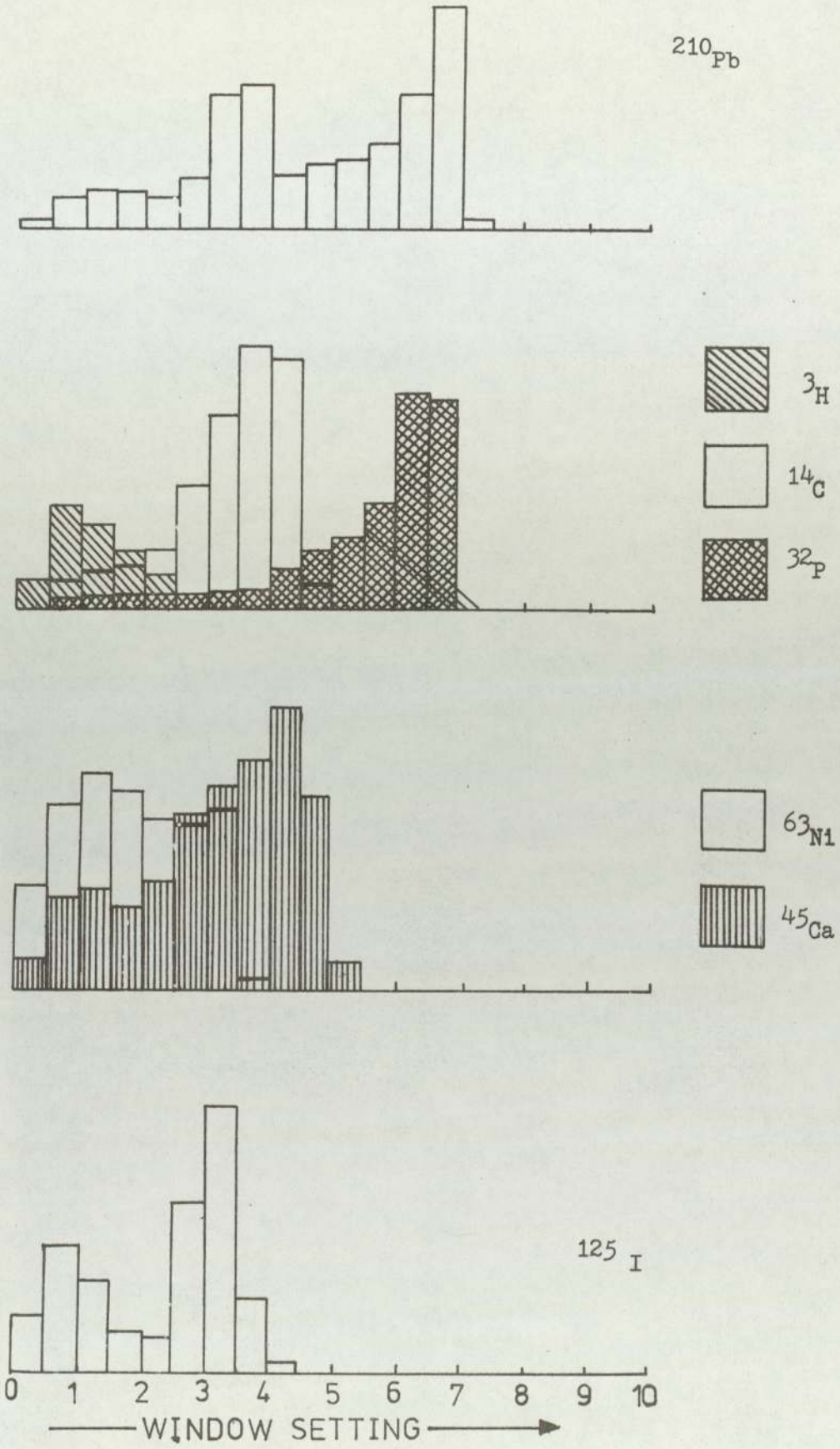


Figure A2

Fluorescence Spectra of nuclides with similar end-point energies to ^{210}Pb.

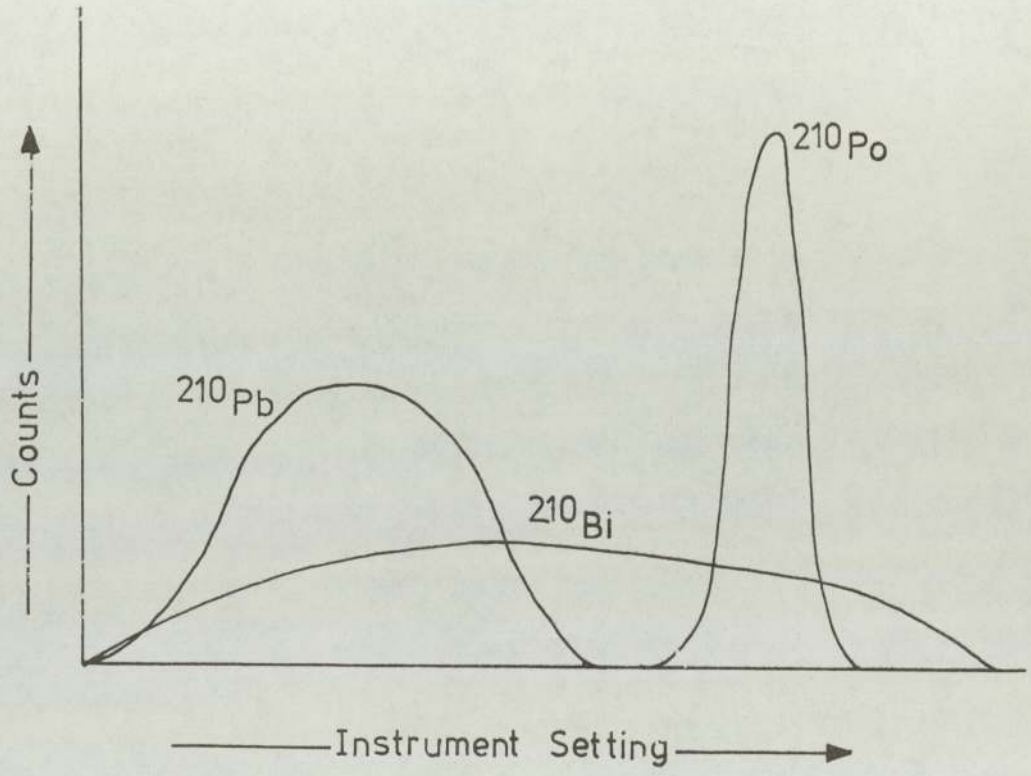


Figure A3

Fluorescence Spectra of ^{210}Pb, ^{210}Bi and ^{210}Po.

Kraus (1955) using a Dowex 1 resin (200-230 mesh). They found that lead was poorly adsorbed on to an anion-exchange resin in dilute hydrochloric acid, but increased with increased hydrochloric acid concentration. Adsorption was maximal in the presence of 1.5 M hydrocholoric acid. The adsorption of bismuth however was maximal at lower concentrations of hydrochloric acid (0.1 M) (Fig. A4).

Lead was removed with dilute hydrochloric acid bismuth, with concentrated hydrochloric acid, and polonium with concentrated nitric acid (Ishimori, 1955; Fairman and Sedlet, 1965).

From the methods indicated by these authors, a column, dimensions 0.7 cm (diam) 5 cm (depth) was prepared using Amberlite CG-400 (Cl form) 100-200 mesh primed with 1.5 M hydrochloric acid. The nuclide mixture was loaded in the same molarity of the acid. The column was eluted with 8 ml deionised water and a fluorescence spectrum prepared from the fraction collected. No counts were detected above an instrument setting of 4.5, indicating that neither ^{210}Bi or ^{210}Po were present. Therefore ^{210}Pb had been separated from its daughter products.

The radioactive decay of ^{210}Pb produces ^{210}Bi (detectable after only 7 h) at such a rate that after 28 d secular equilibrium is attained between ^{210}Pb/^{210}Bi and after 4 months the equilibrium between ^{210}Pb/^{210}Bi/^{210}Po is completely restored. In theory it is possible to separate ^{210}Pb from its daughter nuclides and use the separated product experimentally but the regrowth of the daughter products requires accurate correction of the counts obtained.

Although it was feasible to use ^{210}Pb as the tracer for transport studies, the problems of consistently accurate and appropriate quench correction contra-indicated its use. Finally as a result of a report from the Quality Control Unit, Radiochemical Centre, Amersham that stated that the α emitting nuclide ^{210}Po was volatile and capable of

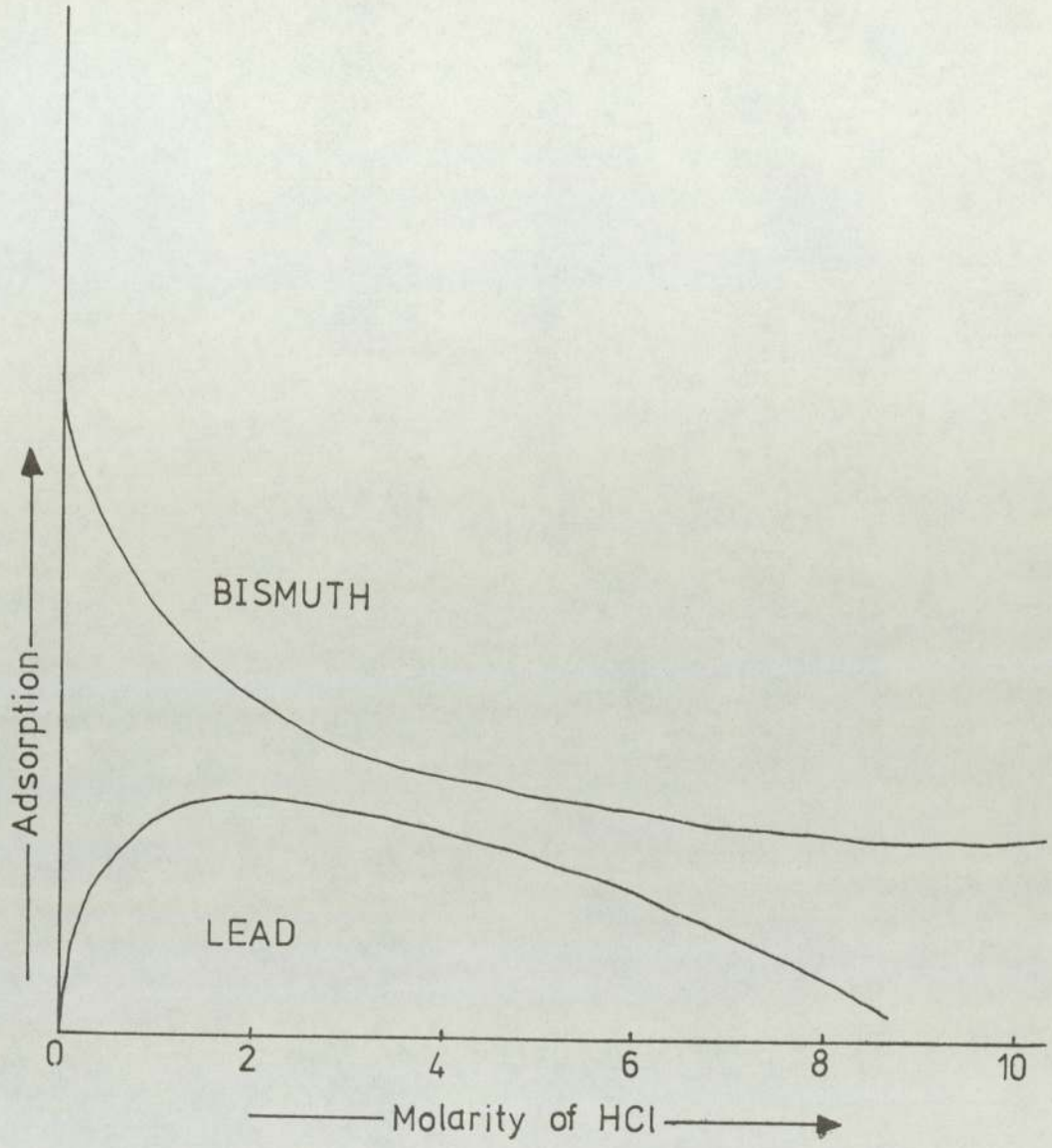


Figure A4

The effect of hydrochloric acid concentration on the adsorption of lead and bismuth on to Amberlite CG-400 Resin.

penetrating rubber gloves and face masks, the use of ^{210}Pb was discontinued. In its place the isotope ^{203}Pb was selected, which despite the disadvantage of a short half-life (52.4 h), possessed the advantage of a single 0.279 MeV γ emission, readily detected in both tissue and fluid samples.

^{203}Pb was therefore used as the only tracer isotope of lead throughout these studies of the gastrointestinal absorption of lead.

BIBLIOGRAPHY

Ahrén, C. and Haglund, U. (1973)
Mucosal lesions in the small intestine of the cat during low flow
Acta. Physiol. Scand. 88 541-550

Bamford, D.R. (1966)
Studies in vitro of the passage of serum proteins across the intestinal wall of young rats
Proc. Roy. Soc. (Series B) 166 30-45

Bamford, D.R. and Ingham, P.A. (1975)
Sugar absorption by foetal and neonatal rat intestine in vitro
J. Physiol. 248 335-348

Barltrop, D. (1969)
Environmental lead and its pediatric significance
Post-Grad. Med. J. 45 129-134

Barltrop, D. and Khoo, H.E. (1975)
The influence of nutritional factors on lead absorption
Post-Grad. Med. J. 51 795-800

Barltrop, D. and Khoo, H.E. (1976)
The influence of dietary minerals and fat on the absorption of lead
Sci. Tot. Environ. 6 265-273

Barry, B.A.; Matthews, J. and Smyth, D.H. (1961)
Transfer of glucose and fluid by different parts of the small intestine of the rat
J. Physiol. 157 279-288

Barry, P.H. and Diamond, J.M. (1971)
A theory of ion permeation through membranes with fixed neutral sites
J. Memb. Biol. 4 295-330

Barry, P.H.; Diamond, J.M. and Wright, E.M. (1971)
The mechanism of cation permeation in rabbit gall-bladder
J. Memb. Biol. 4 358-394

Barry, R.J.C.; Dikstein, S.; Matthews, J.; Smyth, D.H. and Wright, E.M. (1964)
Electrical potentials associated with intestinal sugar transfer
J. Physiol. 171 316-338

Barton, J.C.; Conrad, M.E.; Harrison, L. and Nuby, S. (1978a)
Effects of calcium on the absorption and retention of lead
J. Lab. Clin. Med. 91 366-376

Barton, J.C.; Conrad, M.E.; Nuby, S.; and Harrison, L. (1978b)
Effects of iron on the absorption and retention of lead
J. Lab. Clin. Med. 92 536-547

Biochemical Handbook (1971)
Ed. C. Long Section IV p. 675 E. & F.N. Spon. Ltd., London

Black, D.A.K. (1964)
The alimentary tract and body-fluid
Sci. Basis. Med. Ann. Rev. 291-307

Blair, J.A. and Matty, A.J. (1974)
Acid microclimate in intestinal absorption
Clinics in Gastroenterology 3 183-197

Blair, J.A.; Johnson, I.T. and Matty, A.J. (1974)
Absorption of folic acid by everted segments of rat jejunum
J. Physiol. 236 653-661

Blair, J.A.; Matty, A.J. and Razzaque, A. (1975)
Uptake of 5-methyltetrahydrofolic acid by the rat jejunum
J. Physiol. 250 221-230

Blair, J.A.; Johnson, I.T. and Matty, A.J. (1976)
Aspects of intestinal folate transport in the rat
J. Physiol. 256 197-208

Blake, K.C.H. (1976)
Absorption of ^{203}Pb from gastrointestinal tract of man
Environ. Res. 11 1-4

Blaxter, K.L. and Cowie, A.T. (1946)
Excretion of lead in the bile
Nature 157 588

Byers, R.K. (1959)
Lead poisoning. Review of the literature and Report on 45 cases
Pediatrics 23 585-603

Castellino, N.; Lamanna, P. and Grieco, B. (1966)
Biliary excretion of lead in the rat
Brit. J. Ind. Med. 23 237-239

Cerklewski, F.L. and Forbes, R.M. (1976)
Influence of dietary zinc on lead toxicity in the rat
J. Nutr. 106 689-696

Chaberek, S. and Martell, A.E. (1964)
Organic sequestering agents
John Wiley and Sons Inc. New York

Chisolm, J.J.Jr. (1968)
The use of chelating agents in the treatment of acute and chronic lead intoxication in childhood
J. Pediat. 73 1-38

Cikrt, M. (1970)
The uptake of Hg^{203}, Cu^{64}, Mn^{52} and Pb^{212} by the intestinal wall of the duodenal and ileal segment in vitro
Int. Z. Klin.Pharmakol. Ther. Toxikol. 3 351-357

Cikrt, M. and Tichý, M. (1975)
Role of bile in intestinal absorption of ^{203}Pb in rats
Experientia 31 1320-1321

Clark, S.L.Jr. (1959)
The ingestion of proteins and colloidal materials by columnar absorptive cells of the small intestine in suckling rats and mice
J. Biophys. Biochem. Cytol. 5 41-49

Clarkson, T.W. and Kench, J.E. (1958)
Uptake of lead by human erythrocytes in vitro
Biochem. J. 69 432-439

Claude, P. and Goodenough, D.A. (1973)
Fracture faces of zonulae occludentes from 'tight' and 'leaky' epithelia
J. Cell. Biol. 58 390-400

Coleman, R.; Iqbal, S.; Godfrey, P.P. and Billington, D. (1979)
Membranes and bile formation. Composition of several mammalian biles and their membrane damaging properties
Biochem. J. 178 201-208

Conrad, M.E. and Barton, J.C. (1978)
Factors affecting the absorption and excretion of lead in the rat
Gastroenterology 74 731-740

Davenport, H.W. (1968)
Destruction of the gastric mucosal barrier by detergents and urea
Gastroenterology 54 175-181

Davenport, H.W. (1970)
Effect of lysolecithin, digitonin and phospholipase A upon the dog's gastric mucosal barrier
Gastroenterology 59 505-509

Diamond, J.M. and Bossert, W.H. (1967)
Standing-gradient osmotic flow. A mechanism for coupling of water and solute transport in epithelia
J. Gen. Physiol. 50 2061-2083

Dietschy, J.M. (1967)
Effects of bile salts on intermediate metabolism of the intestinal mucosa
Fed. Proc. 26 1589-1598

Fairman, W.D. and Sedlet, J. (1968)
Direct determination of lead - 210 by liquid scintillation counting
Anal. Chem. 40 2004-2008

Fine, B.P.; Barth, A.; Sheffet, A. and Lavenhar, M.A. (1976)
Influence of magnesium on the intestinal absorption of lead
Environ. Res. 12 224-227

Fisher, R.B. and Parsons, D.S. (1949)
A preparation of surviving rat small intestine for the study of absorption
J. Physiol. 110 36-46

Forbes, G.B. and Reina, J.C. (1972)
Effect of age on gastrointestinal absorption (Fe, Sr, Pb) in the rat
J. Nutr. 102 647-652

Foreman, H. and Trujillo, T.T. (1954)
The metabolism of C^{14} labeled ethylenediaminetetraacetic acid in human beings
J. Lab. Clin. Med. 43 566-571

Forth, W. and Rummel, W. (1975)
Gastrointestinal absorption of heavy metals. In International Encyclopaedia of Pharmacology and Therapeutics
Section 39B 2 599-746

Frizzell, R.A. and Schultz, S.G. (1972)
Ionic conductances of extracellular shunt pathway in rabbit ileum. Influence of shunt on transmural sodium transport and electrical potential differences
J. Gen. Physiol. 59 318-346

Frömter, E. and Diamond, J. (1972)
Route of passive ion permeation in epithelia
Nature, [New Biol.] 235 9-13

Garber, B.T. and Wei, E. (1974)
Influence of dietary factors on the gastrointestinal absorption of lead
Toxicol. App. Pharmacol. 27 685-691

Gardner, M.L.G. (1978)
The absorptive viability of isolated intestine prepared from dead animals
Quart. J. Exp. Physiol. 63 93-95

Gerber, G.B. and Deroo, J. (1975)
Absorption of radioactive lead (^{210}Pb) by different parts of the intestine in young and adult rats
Environ. Physiol. Biochem. 5 314-318

Gibaldi, M. and Grundhofer, B. (1972)
Drug transport. VI. Functional integrity of the rat everted small intestine with respect to passive transfer
J. Pharm. Sci. 61 116-119

Goyer, R.A. (1971)
Lead Toxicity. A problem in environmental pathology
Am. J. Pathol. 64 167-179

Goyer, R.A. and Rhyne, B.C. (1973)
Pathological effects of lead
Int. Rev. Exptl. Pathol. 12 2-77

Gruden, N.; Stantić, M. and Buben, M. (1974)
Influence of lead on calcium and strontium transfer through the duodenal wall in rats
Environ. Res. 8 203-206

Gruden, N. and Stantić, M. (1975)
Transfer of lead through the rat's intestinal wall
Sci. Tot. Environ. 3 288-292

Haslewood, G.A.D. (1968)
Evolution and Bile Salts. In Handbook of Physiology
Section 6 5 2375-2390 Ed. C.F. Code Washington D.C.

Hilburn, M.E. (1977)
Environmental lead in perspective
British Assoc. Meeting University of Aston, Section B (Chem)

Hilburn, M.E. (1979)
Environmental lead in perspective
Chem. Soc. Rev. (In press)

Hoffman, W.S. (1937)
A rapid photoelectric method for the determination of glucose in blood and urine
J. Biol. Chem. 120 51-55

Hursh, J.B. and Suomela, J. (1968)
Absorption of ^{212}Pb from the gastrointestinal tract of man
Acta. Radiol. Ther. Phys. Biol. 7 108-120

Ishimori, T. (1955)
Separation of RaD, RaE and RaF by ion exchange
Bull. Chem. Soc. Japan 28 432-435

Jackson, M.J. and Cassidy, M.M. (1970)
Studies on intestinal fluid transport. II. The location of fluid accumulated in the wall of rat jejunum during incubation in vitro
Biochim. Biophys. Acta. 211 436-447

Jordana, R. and Ponz, F. (1969)
Effects of the X-irradiation in vitro on the O_2 uptake and on the utilization of glucose by the intestine
Rev. Esp. Fisiol. 25 129-135

Jugo, S.; Maljković, T and Kostial, K. (1975)
Influence of chelating agents on the gastrointestinal absorption of lead
Toxicol. App. Pharmacol. 34 259-263

Kallen, R.G. and Jencks, W.P. (1966)
The dissociation constants of tetrahydrofolic acid
J. Biol. Chem. 241 5845-5850

Kello, D. and Kostial, K. (1973)
The effect of milk diet on lead metabolism in rats
Environ. Res. 6 355-360

Kehoe, R.A. (1955)
Misuse of Edathamil calcium-disodium for prophylaxis of lead poisoning
J. Amer. Med. Assoc. 157 341-342

Kehoe, R.A. (1961)
The metabolism of lead in man, in health and disease. I. The normal metabolism of lead
J. Roy. Inst. Pub. Hlth. Hyg. 24 81-97

Kehoe, R.A. (1976)
Pharmacology and Toxicology of heavy metals
Pharmacol. Ther. Pt. A. 1 161-188

Kesavan, V. and Noronha, J.M. (1978)
An ATPase dependent, radiosensitive acidic microclimate essential for intestinal folate absorption
J. Physiol. 280 1-7

Klaassen, C.D. and Shoeman, D.W. (1974)
Biliary excretion of lead in rats, rabbits and dogs
Toxicol. App. Pharmac. 29 434-446

Koenig, C.S. and Juan D. Vial C. (1970)
A histochemical study of adenosine triphosphatase in the toad (Bufo Spinulosus) gastric mucosa
J. Histochem. Cytochem. 18 340-353

Kostial, K.; Šimonović, I. and Pišonić, M. (1971)
Lead absorption from the intestine in newborn rats
Nature 233 564

Krebs, H.A. and Henseleit, K. (1932)
Untersuchungen über die Harnstoffbildung in Tierkörper
Hoppe-Seyler's Z Physiol. Chem. 210 33-66

Lee, J.S. (1961)
Flows and pressures in lymphatic and blood vessels of intestine in water absorption
Am. J. Physiol. 200 979-983

Lei, F.H.; Lucas, M.L. and Blair, J.A. (1977)
The influence of pH, low sodium ion concentration and methotrexate on the jejunal-surface pH : A model for folic acid transfer
Biochem. Soc. Trans. 5 149-152

Levine, R.R.; McNary, W.F.; Kornguth, P.J. and LeBlanc, R. (1970)
Histological reevaluation of everted gut technique for studying intestinal absorption
Europ. J. Pharmacol. 9 211-219

Levin, R.J. and Syme, G. (1975)
Thyroid control of small intestinal oxygen consumption and the influence of sodium ions, oxygen tension, glucose and anaesthesia
J. Physiol. 245 271-287

Lucas, M.L. and Blair, J.A. (1978)
The magnitude and distribution of the acid microclimate in proximal jejunum and its relation to luminal acidification
Proc. Roy. Soc. Lond. Ser. B. 200 27-41

MAFF (1975)
Ministry of Agriculture, Fisheries and Food Survey of Lead in Food
H.M.S.O.

Malenkov, A.G. and Melikyants,A.G. (1977)
Ion permeability and strength of cell contacts. Ion permeability and mechanical properties of cell contacts in small intestine epithelium
J. Memb. Biol. 36 97-113

Martin, G.P.; Marriott, C. and Kellaway, I.W. (1978)
Direct effect of bile salts and phospholipids on the physical properties of mucus
Gut.19 103-107

McDougal, D.B.Jr.; Little, K.D. and Crane, R.K. (1960)
Studies on the mechanism of intestinal absorption of sugars. IV. Localisation of galactose concentrations within the intestinal wall during active transport in vitro
Biochim. Biophys. Acta. 45 483-489

Meredith, P.A.; Moore, M.R. and Goldberg, A. (1977)
The effect of calcium on lead absorption in rats
Biochem. J. 166 531-537

Monier-Williams, G.W. (1949)
Trace Elements in Food
Chapman and Hall

Moreno, J.H. and Diamond, J.M. (1975)
Nitrogenous cations as probes of permeation channels
J. Memb. Biol. 21 197-259

Nechay, B.R. and Saunders, J.P. (1978)
Inhibitory characteristics of lead chloride in sodium- and potassium-dependent adenosinetriphosphatase preparations derived from kidney, brain and heart of several species
J. Toxicol. Environ. Hlth. 4 147-159

Nelson, F. and Kraus, K.A. (1954)
Anion-exchange studies. XI. Lead (II) and Bismuth (III) in chloride and nitrate solutions
J. Amer. Chem. Soc. 76 5916-5920

Parsons, D.S. (1975)
In. Biological Membranes
Clarendon Press

Poe, M. (1977)
Acidic dissociation constants of folic acid, dihydrofolic acid and methotrexate
J. Biol. Chem. 252 3724-3728

Pohland, A.; Flynn, E.H.; Jones, R.G. and Shive, W. (1951)
A proposed structure for folinic acid - SF, a growth factor derived from pteroylglutamic acid
J. Amer. Chem. Soc. 73 3247-3252

Pollack, S.; George, J.N.; Reba, R.C.; Kaufman, R.M. and Crosby, W.H. (1965)
The absorption of nonferrous metals in iron deficiency
J. Clin. Invest. 44 1470-1473

Quarterman, J. and Morrison, J.N. (1975)
The effects of dietary calcium and phosphorus on the retention and excretion of lead in rats
Brit. J. Nutr. 34 351-362

Rabinowitz, M.B.; Wetherill, G.W. and Kopple, J.D. (1973)
Lead metabolism in the normal human. Stable isotope studies
Science 182 725-727

Rabinowitz, M.B.; Wetherill, G.W. and Kopple, J.D. (1976)
Kinetic analysis of lead metabolism in healthy humans
J. Clin. Invest. 58 260-270

Rieders, F. (1960)
Current concepts in the theory of lead poisoning. In Metal binding in Medicine (M.J. Seven, Ed.) pp 143-145
Lippincott, Philadelphia

Sahagian, B.M.; Harding-Barlow, I. and Perry, H.M.Jr. (1967)
Transmural movements of zinc, manganese, cadmium and mercury by rat small intestine
J. Nutr. 93 291-300

Salway, J.G. (1969)
The simultaneous determination of acetoacetate and glucose in capillary blood
Clin. Chim. Acta. 25 109-116

Schultz, S.G.; Frizzell, R.A and Nellans, H.N. (1974)
Ion transport by mammalian small intestine
Ann. Rev. Physiol. 36 51-91

Six, K.M. and Goyer, R.A. (1970)
Experimental enhancement of lead toxicity by low dietary calcium
J. Lab. Clin. Med. 76 933-942

Six, K.M. and Goyer, R.A. (1972)
The influence of iron deficiency on tissue content and toxicity of ingested lead in the rat
J. Lab. Clin. Med. 79 128-136

Sladen, G.E. and Harries, J.T. (1972)
Studies on the effects of unconjugated dihydroxy bile salts on rat small intestinal function in vivo
Biochim. Biophys. Acta. 288 443-456

Smith, C.M.; DeLuca, H.F.; Tanaka, Y. and Mahaffey, K.R. (1978)
Stimulation of lead absorption by Vitamin D administration
J. Nutr. 108 843-847

Smith, M.E.; Matty, A.J. and Blair, J.A. (1970)
The transport of pteroylglutamic acid across the small intestine of the rat
Biochim. Biophys. Acta. 219 37-46

Smyth, D.H (1963)
In.Recent Advances in Physiology
Churchill Ltd.

Smyth, D.H. (1974)
In. Intestinal Absorption. Biomembranes 4A
Plenum Press

Spivey-Fox, M.R. (1976)
In, Trace Elements in Human Health and Disease. Vol. 2. Essential and Toxic Elements
Academic Press

Staehelin, L.A. (1973)
Further observations on the fine structure of freeze-cleaved tight junctions
J. Cell. Sci. 13 763-786

Stevens, E.; Rosoff, B.; Weiner, M. and Spencer, H. (1962)
Metabolism of the chelating agent Diethylenetriaminepentaacetic Acid (C^{14} DTPA) in man
Proc. Soc. Exp. Biol. Med. 111 235-238

Strum, W.; Nixon, P.F., Bertino, J.B. and Binder, H.J. (1971)
Intestinal folate absorption I. 5-methyltetrahydrofolic acid
J. Clin. Invest. 50 1910-1916

Ussing, H.H.; Erlij, D. and Lassen, U. (1974)
Transport pathways in biological membranes
Ann. Rev. Physiol. 36 17-49

Waldron, H.A. and Stöfen, D. (1974)
Sub-clinical Lead Poisoning
Academic Press, London, New York

Wapnir, R.A.; Exeni, R.A.; McVicar, M. and Lifshitz, F. (1977)
Experimental lead poisoning and intestinal transport of glucose, amino acids and sodium
Pediat. Res. 11 153-157

Whiteley, J.M.; Drais, J.H. and Huennekens, F.M. (1969)
Synthesis and properties of 2-amino-4-hydroxy-5, 6-dimethyl-5,6,7,8-tetrahydropteridine and the 5,7-dimethyl isomer
Arch. Biochem. Biophys. 133 436-441

Wilson, T.H. and Wiseman, G. (1954)
The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface
J. Physiol. 123 116-125

Winborn, W.B.; Guerrero, D.L. and Hodge, E.E. (1976)
Scanning electron microscopic studies on the effects of bile on the gastric mucosa of the rat
Gastroenterology 70 966 (Abstract)

Wright, E.M. (1966)
The origin of the glucose dependent increase in the potential difference across the tortoise small intestine
J. Physiol. 185 486-500

Wright, E.M. and Diamond, J.M. (1968)
Effects of pH and polyvalent cations on the selective permeability of gall-bladder epithelium to monovalent ions
Biochim. Biophys. Acta. 163 57-74