ABSORPTION OF CHROMIUM ACROSS THE RAT SMALL INTESTINE

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Absorption of Chromium Across the Rat Small Intestine

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Summary

Chromium is an essential element in trace quantities, but toxic at high concentrations, particularly in the hexavalent form. In this study the transport of physiological non-toxic amounts of trivalent chromium across different regions of rat small intestine was investigated using the everted sac technique.

A very small percentage of chromium was transported to the serosal compartment, and a larger percentage of chromium was rapidly taken up by the intestinal tissue. There was no specific site of absorption for chromium in the rat small intestine. The amount of chromium both transported to the serosal compartment and taken up by the tissue was dependent upon the initial concentration of chromium and no evidence of saturation was observed. There was however a good correlation between water and chromium transport across the jejunum and ileum.

Changes in glucose concentration, temperature, or anoxic conditions appeared to have no affect on chromium transport to the serosal compartment. However transport was increased in the presence of decreased concentration of calcium ions and also in the presence of increased concentration of hydrogen ions. Bile salts and E.D.T.A. did not affect the serosal transport of chromium, however, the interaction of chromium with citric acid increased the amount of chromium transported to the serosal compartment.

There was strong and tenacious interaction between the intestinal tissue and chromium ions which displayed characteristics of covalent bonding. Variations in glucose concentration and pH, as well as anoxic condition and high concentration of bile salts, markedly influence the interaction of chromium with the intestinal tissue. Lowered luminal volumes resulted in an increase in the tissue-chromium interaction.

From the observations a model was developed to describe the transport mechanism for chromium, and factors which influence chromium absorption. Chromium crosses the intestinal epithelium by passive diffusion via the zonulae occludentes. Interaction of chromium with a dietary agent may increase the amount transported to the serosal compartment and result in the complex using an intracellular route.

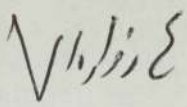
The tenacious binding of chromium to the intestinal tissue, possibly to phosphate groups, suggests that the intestinal barrier acts as a controlling factor for the amount of chromium entering the body pool, and also predicts other conditions that might affect the absorption of chromium.

Key Words

Chromium
Intestine
Adsorption

The research documented in this thesis was carried out between January, 1975 and October, 1978 in the Department of Chemistry in the University of Aston in Birmingham.

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



A. Zavareh'ee

To the memory of my father

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Compared with some metals such as iron, copper and lead which have been known and used by man for many centuries, chromium is a relatively new metal. It was discovered by Vanquelin in 1797, in the mineral, Crocoisite (lead chromate) $PbCrO_4$. It is only during the past three decades however that a considerable degree of interest has been created in the biological action of chromium. The discovery of the essential biochemical role of trivalent chromium in 1959 (86), has stimulated research into various other possible functional aspects of chromium including its nutritional role in animals, man, and plants. There has also been particular interest in the toxic effects of exposure to high concentrations of chromium in the industrial situation (9), (56).

1.2 The Chemistry of Chromium Compounds

1.2.a Valency State

The main source of the metal is chromite, or chrome ironstone, a ferrous chromite, $FeCr_2O_4$ (70). Chromium can be found in oxidation states from -2 to +6 but only the ground states 0, +2, +3 and +6 are common. Divalent chromium is unstable unless carefully protected from oxidation, which occurs easily in air. Therefore Cr^{2+} compounds are unlikely to occur in biological systems. The hexavalent form of chromium is almost always linked with oxygen and is a strong oxidizing agent, the only important ions of Cr^{6+} are chromates, CrO_4^{2-}, and dichromates, $Cr_2O_7^{2-}$. Both of these compounds are easily reduced to Cr^{3+} in acidic solutions.

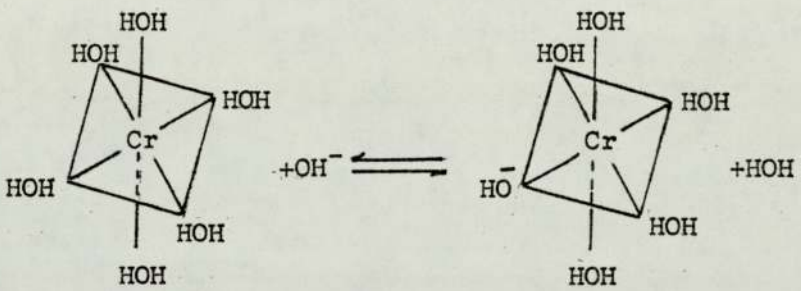
$$Cr_2O_7^{2-} + 14H^+ + 6e \longrightarrow 2Cr^{3+} + 7H_2, E = 1.33V$$

The difference in oxidation potential between Cr^{3+} and Cr^{6+} is so great that a reversible transition is unlikely under normal

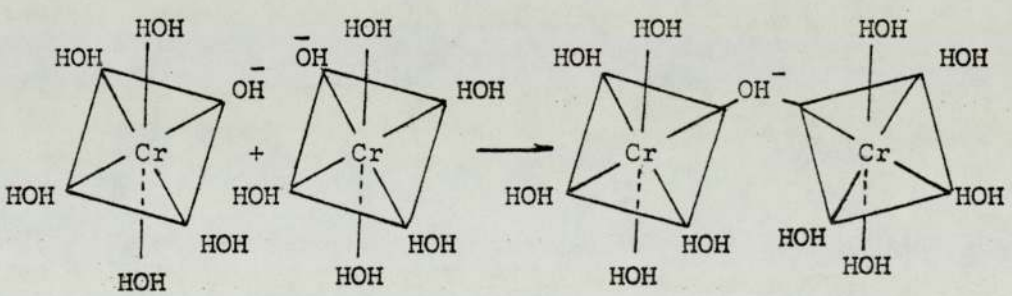
physiological conditions. It has also been reported that no hexavalent chromium could be found in human serum (32). Therefore on the basis of available data one can conclude that chromium is predominantly present in biological material in the trivalent form.

1.2.b Olation and Solubility

In solution there are six molecules of water in coordination with each chromium molecule. As the pH of the solution increases the coordinated water is hydrolysed.



This results in bridge formation between chromium ions through coordination of hydroxyl groups, rather than water.



The reaction becomes very complicated since more than two bridges are involved, and leads to the formation of polynucleate complexes; this process is called olation. The olation process will proceed when a stabilizing ligand is not present, and leads to the formation of large chromic hydroxide complexes of coloidal nature and of little or no biological activity. However the free Cr^{3+} as a hexaquo complex does not exist at physiological pH. The reaction

of Cr^{3+} with ligands successfully competes with hydroxyl ions for coordination, and protects chromium against precipitation at neutral pH by minimizing or preventing olation. There are a variety of small compounds in the blood and other biological fluids that can easily coordinate to chromium and make it possible for trivalent chromium to stay soluble in biological material; the following ligands maintain chromium in a diffusible form (77):

Pyrophosphate > Methionine > Serine > Glycine > Leucine > Lycine > Proline

1.3 Environmental Chromium

Chromium is a rare element, and its content in soil, water and air varies at different locations. These differences could well reflect themselves in the chromium content of plants and animals tissues (81). The existence of trace amounts of chromium in the soil is an important factor associated with the fertility of some soils (67). However the data available on the concentration of chromium in the soil are insufficient to describe any geographical distribution pattern. The concentration of chromium in the soil has been measured from traces to 250 ppm as Cr_2O_3 (67).

Both trivalent and hexavalent chromium have been shown to exist in sea water, but at concentrations below 1 ppb (37), (14), (76). Concentration of chromium in a sample of public drinking water does not always represent a true picture of the chromium content of water. This is shown by the wide variation in chromium content of products of different water works of one city (44). However drinking water does not contribute significantly to the chromium intake of man (67), (74).

Although there are problems in sampling and methods of analysis, it is unlikely that the concentration of airborne chromium found in the normal urban environment is an important biological factor to man. It is estimated that the normal intake of chromium from air is between 11 - 110 μg (92). A comparatively small intake plus the possibility that pulmonary chromium may reduce to trivalent form which is quite insoluble, and most probably does not interchange with the rest of the body stores (67), (2), explains the continuous accumulation of chromium in the lungs with age (81).

1.4 The Body Content of Chromium

No toxic effects have been found at the normal human body levels of chromium. However the work of a number of investigators indicates that there is an excessive risk of lung cancer involved through industrial exposure to high doses of hexavalent chromium (9), (33), (26), (91), (56). Lung cancer cases in the workers of the chromium industry were investigated in Germany as early as 1935, and recognised as an occupational hazard (9). It should be noted that the exposure time to chromium is an important factor as longer exposure can produce more definite damage (56). According to Langard and Norseth (56) who studied Norwegian workers, the exposure level which causes bronchial cancer is from 0.5 to 1.5 mg. Cr/m^3 over a working period of six to nine years. The toxicity of trivalent chromium has been reported only with comparatively high doses of parenteral administration (67).

The body content of chromium reported in the literature varies because the data were obtained by different methods of analyses (34), (53). Typical figures obtained by some Japanese investigators using the

flameless atomic absorption technique to measure chromium are shown in Table 1.1. The data indicates that the total body burden of chromium is more than 4 mg, with half of it in the muscle (53). The chromium concentration in the human body also varies in different geographical locations. Shroeder, et al (81) detected the following levels of chromium in liver samples:

Nigeria	0.47 ppm of ash
Hong Kong	0.6 " " "
Tokyo	3.4 " " "
Bombay-Delhi	4.5 " " "
Manila	4.8 " " "
Bangkok	5.5 " " "
Bern	5.8 " " "
Welkom	11.0 " " "

These values may be compared with an average figure of 1.6 ppm in the United States. The concentration of chromium in man also varies and is dependent upon a number of factors such as; a) the composition of the diet, b) the age of person, c) endocrine disturbances such as diabetes (45), and d) sex (53).

Organ or part	Sex	No	Average
Cerebrum	M	10	0.079
	F	9	0.030
Cerebellum	M	10	0.054
	F	10	0.045
Trachea	M	8	0.088
	F	5	0.093
Lung	M	14	0.38
	F	15	0.15
Heart	M	12	0.10
	F	11	0.073
Liver	M	14	0.078
	F	15	0.054
Pancreas	M	14	0.11
	F	14	0.090
Kidney	M	15	0.083
	F	15	0.070
Small Intestine	M	11	0.15
	F	12	0.084
Large Intestine	M	12	0.24
	F	13	0.093
Muscle	M	12	0.12
	F	9	0.077
Skin	M	10	0.075
	F	9	0.12
Blood	M	7	0.036
	F	8	0.052
Rib (bone)	M	6	0.020
	F	7	0.098

Table 1.1 Chromium concentration in Japanese human tissues expressed as micrograms per gram wet tissue.

(Data taken from Arch. Environ. Health 1975, ref. 53)

1.5 Biological Functions of Chromium

1.5.a Enzymes and Nucleic Acid

Phosphoglucomutase, which has an important role in glucose metabolism requires the presence of Mg^{+2} (0.003 M) for maximal activity, together with a second metal (e.g. Cr^{3+}, Fe^{3+}) Cr^{3+} is the most effective second metal and is the only metal that alone can maintain some enzyme activity in the absence of magnesium. The effective concentration of chromium is 500 ppb which is not much different from naturally occuring levels (90), (66).

Trypsin also contains one atom of chromium per enzyme molecule, but the chromium can be removed by dialysis, indicating that it is not firmly bound. The activity of the enzyme however after removing the chromium decreases to 5% of the original activity, but can be restored to normal by the addition of chromium (15). There may be other enzyme activities affected by chromium as there are reports of *in vitro* experiments in which it was shown that chromium increased the incorporation of acetate into cholesterol in rat liver (20). This of course may only be of pharmological interest rather than an essential biological effect, as the level of chromium required is almost toxic. Transition metals of the first series also stabilize the ordered structure of RNA (38). According to Wacker et al (98), (99) there is a very high concentration of chromium (260 to 1080 ppm) in beef liver fractions, which consists of 70% RNA and 30% protein. Although many other metals are also present, chromium is outstanding among the metals investigated in its bond stability.

1.5.b Glucose Tolerance Factor (G.T.F.)

The most well known and established biological role of chromium is its active incorporation into the Glucose Tolerance Factor (G.T.F.), first identified by Schwarz, K. and Mertz, W. (86). The glucose tolerance factor is a dietary agent required to maintain normal glucose tolerance in the rat (85), (86), (65), (35). G.T.F. may be considered a hypoglycemic agent effective in lowering plasma glucose levels, particularly if glucose is elevated above the normal fasting range (8). G.T.F. can be isolated from natural sources such as brewers' yeast or acid hydrolyzates of pork kidney powder; liver is also recognised as another potentially rich source.

G.T.F. is an organic water soluble compound, extractable with phenol and isobutanol, absorbable on charcoal and ion exchange resin; it is a heat-stable, low molecular weight complex (86), (8). However the molecular structure of G.T.F. has not yet been determined. The complex contains two nicotinic acid molecules per chromium atom and may contain cysteine, glycine, and possibly glutamic acid residues (8). In 1974 biologically active chromium complexes were prepared by Walter Mertz (69), which were similar to, but not identical with, naturally occuring G.T.F. complexes.

1.6 Chromium Deficiency

1.6.a Chromium Deficiency in Animals and Man

Chromium is the only element known to decline in most organs with age (94). Chromium deficiency in animals has been observed (64), (83). The term "low chromium state" or "chromium deficiency" is used to describe the result of sub-optimal supplementation of an organism with biologically available chromium. Since the chemical

methods to determine chromium, measure the total chromium concentration, the data obtained from chemical analyses are not always representative of biologically available chromium. Therefore a low chromium state can be described as an impairment of some function that can be prevented or cured by supplementation of the diet with physiological amounts of the element. Schroeder H.A. (82) suggests that the clinical manifestation of chromium deficiency are; a) diabetes mellitus, or at least, impaired glucose tolerance, b) hypercholesteremia, or c) a tendency to atherosclerosis. Newman, H.A.I. et al (72) reported that the group of people with coronary artery disease had significantly lower serum chromium concentration than did the group with normally patent arteries. However the hallmark of chromium deficiency is impaired glucose tolerance. In rats and squirrel monkeys maintained on chromium-deficient diets, restoration of a normal glucose tolerance was accomplished by oral administration of Cr^{3+} or Glucose Tolerance Factor (G.T.F.) (86), (22). The blood glucose levels also return to the normal range after administration of the glucose tolerance factor in genetically diabetic mice with hyperinsulinemia (27). There is also indirect evidence to support the view that chromium deficiency occurs in man. Maturity-Onset diabetics and elderly subjects have shown improvement of impaired glucose tolerance by simple addition of 150 µg of inorganic chromium III to their daily diets (40), (57).

1.6.b Chromium in the Diet

The daily intake of chromium has been reported as low as 5 µg/day and as high as 115 µg/day, with an average of 52 µg/day (57). Other investigators suggest much lower average daily diet content of 7.8 µg of

chromium (23), (81).

The highest concentration of chromium can be found in some spices, with lesser amounts in meats, vegetable and fruits; liver and kidney are also high in chromium content (81), (43). The chromium content of food stuffs gives only limited information on the dietary adequacy. The availability of chromium for absorption, and also how much is in the form of G.T.F. are important considerations. A correlation of chromium content and G.T.F. activity of various foods has been studied by Toepfer et al (95) in terms of the relative biological values for G.T.F. activity and the following data were obtained: brewers' yeast 44.88, black pepper 10.21, calf's liver 4.52, chicken leg muscle 1.89, haddock 1.86, patent flour 1.86, and skimmed milk 1.59. There is much work needed before a standard level of chromium in the diet can be recommended. However it has been suggested that 10 - 30 µg of G.T.F.-chromium per day is enough to meet man's daily requirement (68).

The drinking water content of chromium measured in the United States reveals that the water supplies provide only small amounts of chromium (0.43 µg/ml) (8). Therefore it appears not to play an important part in the daily intake of chromium.

1.7 Absorption, Transport, and Excretion of Chromium

Very few investigations have been carried out into the intestinal absorption of chromium. Donaldson and Barreras (30) investigated the intestinal absorption of $^{51}CrCl_3$ and $Na_2{}^{51}CrO_4$ in both human subjects and rats. They demonstrated a significant absorption of radioactive ^{51}Cr in their jejunal perfusion studies in humans and by faecal and urinary excretion tests in humans and rats. They also suggested that hexavalent chromium was better absorbed than trivalent chromium.

Mackenzie et al (62) also reported the absorption of hexavalent and trivalent chromium by the albino rats after stomach and intestinal administration, and showed more absorption of the chromite ion than trivalent chromium. Previous published data is shown in Table 1.2.

Author	Subject	Species	% Absorbed
Doisy et al	Human	Cr^{3+}	0.69%
Donaldson et al	Human & Rat	Cr^{3+}	Non-absorbable
Donaldson et al	Human & Rat	Cr^{6+}	0.50%
Mackenzie et al	Unfasted Albino Rat	Cr^{6+}	3%
Mackenzie et al	Fasted Albino Rat	Cr^{6+}	6%
Visek W.J. et al	Rat	Cr^{3+}	0.50%

Table 1.2 Absorption of chromium ions across gastro-intestinal tract in animals and man as reported by different investigators.

Reduction of hexavalent chromium ions ($^{51}CrO_4^{2-}$) to trivalent (Cr^{3+}) chromium ions by gastric juice has been observed (30), (62). Therefore the proportion of chromium absorbed is related to the acidity of the gastric juice and the presence of food. Absorption of chromium is not significantly different in elderly subjects than young normal adults (29), (28). The only group of persons studied that display an abnormal rate of chromium absorption are insulin-requiring diabetics, who in contrast to maturity-onset diabetics, absorbed two to four times more chromium in the first 24 hours following a single oral dose (8).

After absorption chromium appears in the plasma protein fractions (67), (97). At least two forms of chromium circulate in the plasma compartment. Trivalent chromium is bound to transferrin (siderophilin)

in the β-globulin fraction (49), (47), (80), (1) and the other form is thought to be bound to the glucose tolerance factor (G.T.F.). If the concentration of chromium saturates the binding sites of siderophilin, chromium is then transported by other plasma proteins. Chromium was found to accumulate with time in several organs such as bone, kidney, spleen, and liver (73). Most of the chromium from the body (approximatley 80%) is excreted via the urine, and a minor portion by intestinal secretion (23), (50). However the site of chromium excretion into the intestine is unknown.

1.8 Morphological Features of the Small Intestine

In order to understand the precise mechanism by which a cation crosses the intestinal barrier a knowledge of the unique morphological feature of the small intestine is essential.

The small intestine is a long tube which may be sub-divided into three regions (Duodenum, Jejunum, and Ileum). This digestive tube consists of an inner lining, the mucous membrane, composed of epithelium and connective tissue (lamina propria). The mucous membrane is surrounded by a thin muscular layer, (the muscularis mucosae) and loose connective tissue, (the submucosa). This is in turn surrounded by two or three layers of smooth muscle. The entire intestinal tube is surrounded by a sheet of connective tissue, known as the serosa.

In the small intestine the mucous membrane is highly folded (the mucosal folds of Kerkring) to form the intestinal villi, whose major function appears to be the presentation of a large absorptive area. Mucosal folds are prominent in man but absent in many smaller animals (19). Villi are strikingly longer in the jejunum than the ileum.

It has been observed under the light microscope that the surface of the absorptive cells of the villi consist of a specialised "brush border" of very small parallel rod-like projections, termed micro villi. Estimation has been made that a combination of these specialised structures increase the surface area of the small intestine by a factor of 600 (48).

The micro villi 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. It has been suggested that the existence of such an acid microclimate influences the transport of various ionised species (12).

Substances can travel across the intestinal tract by either an intracellular route which means the movement of molecules across two membranes in series, or an extracellular route, via tight junctions (Zonulae Occludentes) that lie immediately beneath the brush border region.

1.9 Aims of the Investigation

The few studies which have been published indicate only a small amount of chromium is absorbed (62). However these studies give little or no detail as to the precise mechanism or site by which the chromium cation passes across the intestinal barrier. Nor is any detailed information published as to which dietary factors affect chromium transport.

A person exposed to the normal or even industrial level of chromium is unlikely to ingest toxic quantities of the metal. On the other hand, as chromium is an essential element, what is more

important is to identify those conditions which would lead to relative deficiency in the body burden of chromium. Consequently, the knowledge of the precise mechanism and route by which the chromium cation passes across the intestinal membrane is required. Therefore the primary interest in this study was to measure various aspects of chromium transport, such as; a) the rate of transport of chromium to the serosal compartment and its uptake by the intestinal tissue, b) revealing any possible preferential site or sites of absorption, c) elucidating the mechanism of the transport of the chromium cation, d) identifying factors which influence the passage of chromium across the gastro-intestinal barrier and understanding the mechanism of their action, e) to investigate the relative importance of the gastro-intestinal tract as the first physiological barrier between the environment and the body.

Experiments aimed to investigate these points were designed and carried out in vitro, using the everted sac preparation of Wilson and Wiseman (101) at the normal dietary level of chromium.

CHAPTER 2

METHOD AND MATERIALS

VIABILITY OF THE EVERTED SAC PREPARATION

2.1 Animals

The animals used throughout these studies were male Wistar rats weighing between 200 and 230 grams (approximately 55 days old) (Figure 2.1). The rats were maintained on a Heygate 41B diet (Table 2.1) and water *ad libitum* until 24 hours prior to sacrifice whereupon they were starved of food but not water.

2.2 Physiological Buffer

In an *in vitro* experiment it is essential to use a solution that is isotonic to plasma and contains similar quantities of electrolytes found in plasma. One of the commonly used solutions is the Krebs-Henseleit bicarbonate buffer (55), the components of which are as below:-

1) 100 parts of 0.90% sodium chloride NaCl (154 mM)
2) 4 parts of 1.15% potassium chloride KCl(154 mM)
3) 3 parts of 1.22% calcium chloride $CaCl_2$(110 mM)
4) 1 part of 2.11% potassium dihydrogen ortophosphate KH_2PO_4 (154 mM)
5) 1 part of 3.82% magnesium sulphate $MgSO_4$, $7H_2O$ (154 mM)

The solution was buffered to pH 7.4 with;

6) 21 parts of 1.3% sodium bicarbonate $NaHCO_3$ (154 mM)

This buffer with the addition of 20 mM glucose was used throughout these studies and is referred to as Krebs-Henseleit bicarbonate buffer throughout the text.

2.3 Preparation of Everted Sacs

After sacrifice the abdomen of the rat was opened by a mid-line incision, and the intestine cut at the point of entry of the bile and

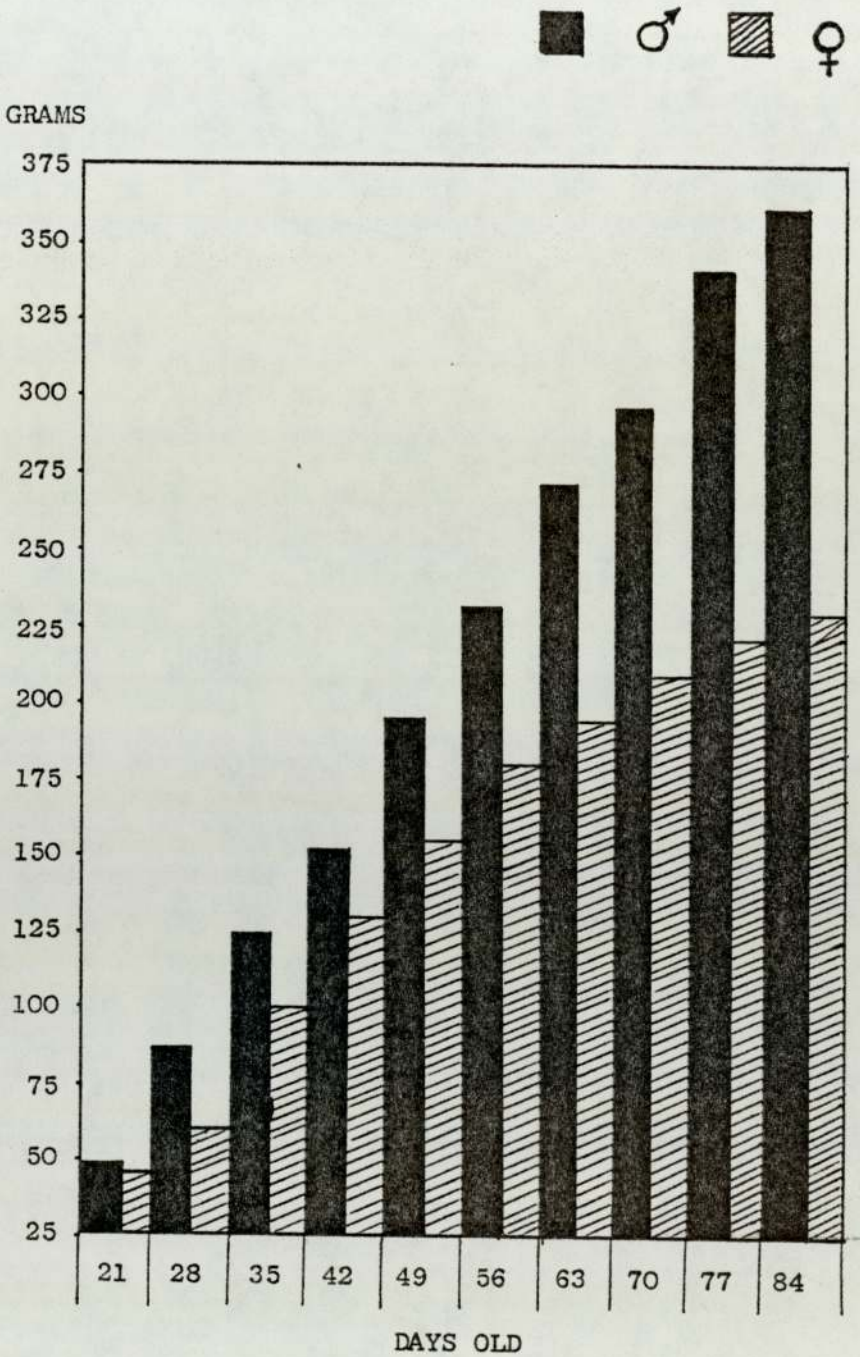


Figure 2.1 Increase in weight of Wistar rats. (Bantin and Kingman Ltd.)

	%
Crude protein	17.069
Crude oil	2.732
Crude fibre	4.352
Digestible oil	2.114
Digestible fibre	1.723
Arginine	0.801
Lysine	0.877
Methionine	0.309
Cystine	0.261
Tryptophan	0.192
Histidine	0.326
Leucine	1.089
Tyrosine	0.326
Isoleucine	0.710
Phenylalanine	0.674
Threonine	0.555
Valine	0.837
Glycine	0.981
Calcium	1.3
Phosphorus	0.72
Ca:P	1:0.6
Sodium	0.575
Chloride	0.154

Vitamins:		
B_{12}	14.09	µg/kg
E	19.25	mg/kg
Thiamine	6.201	"
Riboflavin	3.499	"
Niacin	55.1	"
Pantothenic acid	14.99	"
Choline	1311	"
Biotin	0.904	"
Folic acid	0.838	"
Pyridoxine	5.95	"
Inositol not less than	220	"
A	10,220	I.U/kg
D_3	2,556	"

Iron	65	mg/kg
Manganese	32	"
Copper	7	"
Iodine	4.18	"
Cobalt	0.89	"
Zinc	8.29	"

Table 2.1 Composition of Heygates Diet 41B.

pancreatic ducts, and above the duodenal-jejunal flexture. This section was selected as representative of the distal duodenum (D). The rest of the small intestine to the Ileo-Caecal junction was removed in one piece by severing at the caecum and manually freed of gut mesentery and fatty material. Both sections were immediately transferred to the Krebs-Henseleit bicarbonate buffer at 0°C and gassed continuously with 5% CO_2/95% O_2. The jejunal-ileal portion of the small intestine was divided into twelve sections of equal length (approximately 8.0 cm), and the fifth and eleventh sections chosen as representative of the mid-jejunum (J) and distal-ileum (I) respectively. The three selected sections were then everted with a glass rod of constant diameter (approximately 2 mm).

2.4 Water Transport Measurements

The everted section was placed in a petri-dish containing filter paper moistened with the incubation medium (Krebs-Henseleit bicarbonate buffer), and the section tied with a cotton ligature and a loop formed to enable the sac to be hung from a torsion balance. Each section was weighed on a torsion balance (Whites Instrument Company) to an accuracy of 5 mg (W_1). The sac was then filled using a blunt needle syringe with approximately 0.5 ml of Krebs-Henseleit bicarbonate buffer at 37°C (serosal solution) and a second ligature tied to enclose the serosal volume. The sac was then reweighed on the torsion balance (W_2), and transferred to a 25 ml conical flask containing 10 mls of Krebs-Henseleit bicarbonate buffer (mucosal solution) in a water bath thermostated at 37°C. The mucosal solution was continuously gassed with 5%

CO_2/95% O_2 and shaken at 60 oscillations per minute, for various periods of time. After incubation, each sac was placed on a moist filter paper, and lightly blotted, before reweighing (W_3). The serosal volume of the sac was collected by cutting the sac at one end and allowing the serosal contents to drain into a glass vial. The cut end of the sac was re-blotted, and the empty sac reweighed (W_4). From the four weighings it was possible to calculate:

i) The injected initial serosal volume $(W_2 - W_1)$

ii) The total water movement $(W_3 - W_2)$

iii) The uptake of water by the intestinal tissue $(W_4 - W_1)$

iv) The increase in serosal volume or transport of water into the serosal compartment $(W_3 - W_2) - (W_4 - W_1)$

2.5 Preparation of Cannulated Sacs for Transmural Potential Difference Measurements

An approximate 8 cm section of rat small intestine was everted as previously described (Section 2.3) and ligatured to a flared hollow glass rod. The lower end was tied with another ligature and a small weight attached to keep the sac vertical during the experiment. Using a syringe, Krebs-Henseleit bicarbonate buffer was injected into the sac, and the formation of air bubbles avoided, to ensure a continuous column of fluid. The filled sac was immersed in 70 ml of Krebs-Henseleit bicarbonate buffer and continuously gassed with 5% CO_2/95% O_2 (Figure 2.2). The preparation is similar to that described by Barry et al (6). Electrical contact between serosal and mucosal solution was maintained with two salt bridges connected to calomel half-cells. The salt bridges consisted of polythene tubing of constant diameter, filled with 3M KCl-Agar. The pairs of calomel half-cells were balanced with each other to

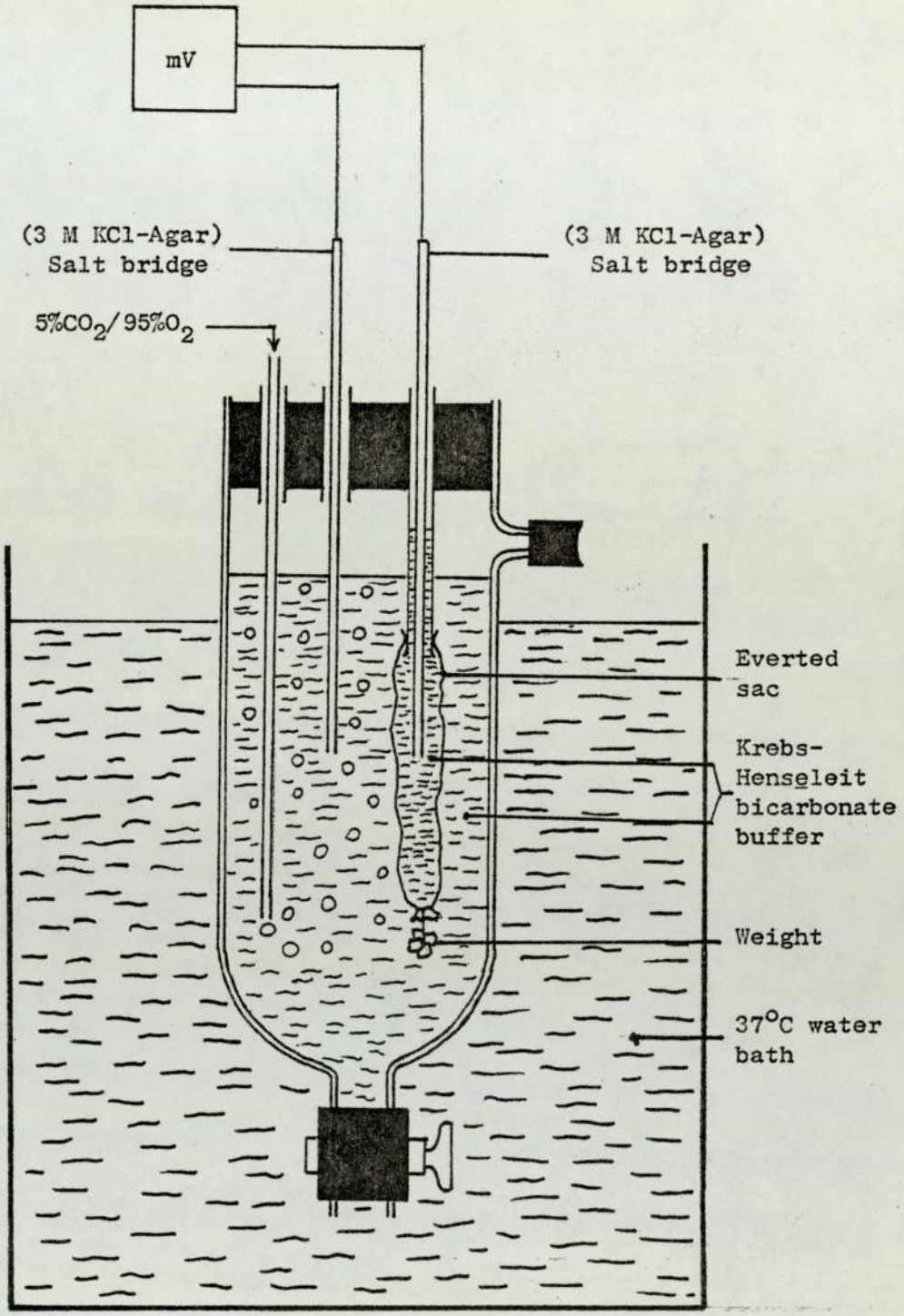


Figure 2.2 Apparatus for transmural potential difference measurement.

within 0.1 mV. Transmural potential difference readings were measured by connecting the half-cells to a Pye Unicam digital voltmeter. Readings were taken at ten minute intervals for an hour.

2.6 Assessment of the Viability of the Everted Sac Preparation

Throughout these studies an *in vitro* intestinal preparation has been used, therefore important physiological parameters such as blood flow, that affects gut function in the intact animal, are excluded. As a consequence the source of oxygen provided by the blood vascular system is not present, and the possibility of anoxic conditions exists. Reduced oxygen supply has been shown to reduce or abolish many transport processes in the proximal jejunum, (3), (31), (10). Therefore it is necessary to demonstrate that the intestinal tissue is viable after preparation and further that it is functional during the incubation period, under the artificial conditions provided (87). There are several different parameters commonly accepted as demonstrating viability, for example, oxygen consumption, transmural potential difference, glucose transport, potassium loss, histological criteria, and water transport measurements (39), (58). Therefore to assess the viability of the everted sacs, transmural potential difference and water transport was measured for different periods across the three different regions of the small intestine.

2.6.a Transmural Potential Difference

The ability of the rat small intestine to maintain a transmural potential difference of several millivolts has been shown by Barry et al (6). They demonstrated that when an ion is transported across a membrane

against an electrochemical gradient, a difference in electrical potential is generated across the membrane due to the change in chemical potential in the two compartments. It has been suggested that the potential is the result of uphill sodium movement, and is related to the transport and metabolism of hexose sugars (88), (7). It follows that the maintenance of the transmural potential difference indicates the existence of some other primary transport system in the epithelium and therefore provides a good indication of the functional viability of an in vitro preparation.

Using the cannulated everted sac preparation (Section 2.5), transmural potential difference measurements were determined for three different regions of the small intestine, and the results are illustrated in Figure 2.3. The mean figures for transmural potential difference of 6.18 $\pm$ 0.3 mV, 8.76 $\pm$ 0.4 mV, and 7.07 $\pm$ 0.4 mV over 60 minutes, for duodenum, jejunum, and ileum respectively were obtained. These mean values are in agreement with the findings of other investigators (60), (16), (75).

The potential difference across the rat small intestine was also measured in the presence of 5×10^{-5} M Cr^{3+} ($CrCl_3$, $6H_2O$), the highest concentration of chromium used throughout these studies, to investigate any possible effect of chromium ions on the viability of the preparation. The results (illustrated in Figure 2.4) indicated that the presence of 5×10^{-5} M Cr^{3+} had no significant affect on the transmural potential difference across the intestinal membrane. The mean figure for different sections of the small intestine are 5.5 $\pm$ 0.4 mV, 8.5 $\pm$ 0.4 mV and 6.9 $\pm$ 0.6 mV for duodenum, jejunum, and ileum respectively.

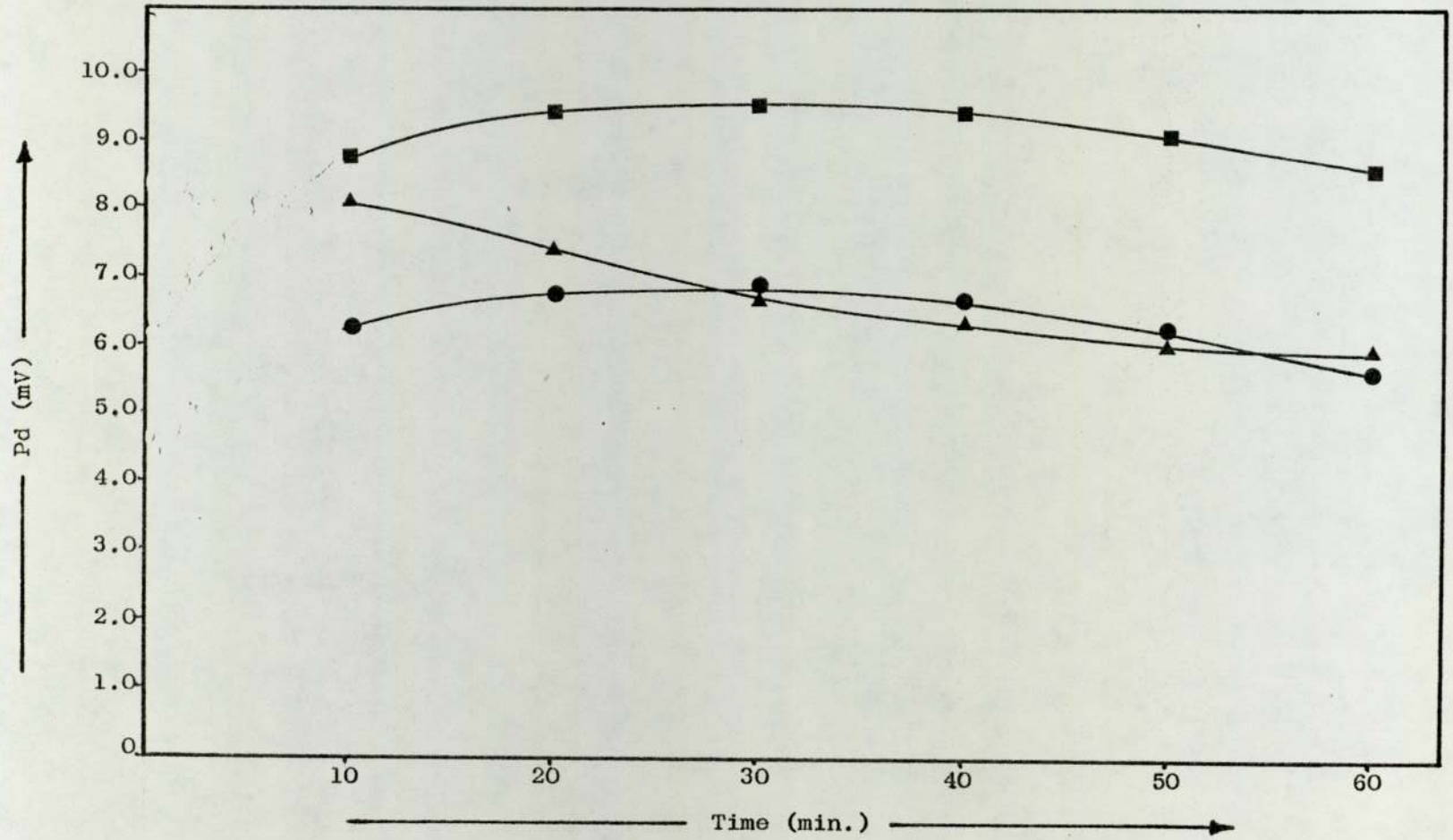


Figure 2.3 Potential difference across cannulated sacs prepared from different regions of the small intestine.
(●) Duodenum, (■) Jejunum, (▲) Ileum

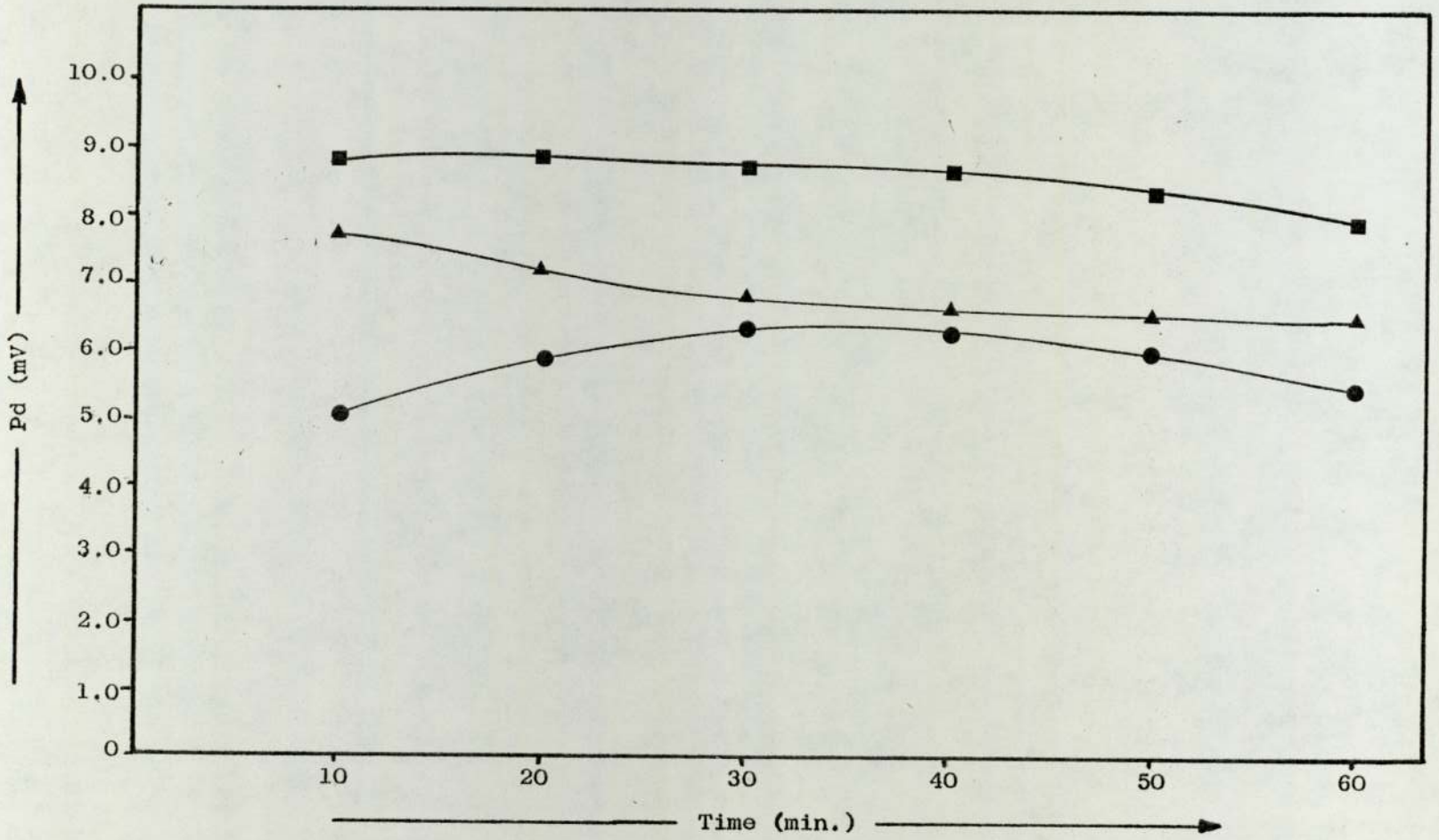


Figure 2.4 Potential difference across cannulated sacs prepared from different regions of the small intestine, in the presence of 5×10^{-5} M Cr^{3+} ($CrCl_3, 6H_2O$). (●) Duodenum, (■) Jejunum, (▲) Ileum

2.6.b Water Transport Criteria

Further investigation to ascertain the viability of the intestinal preparation was carried out by measuring water transport into and across the intestinal preparation. One of the features of a viable *in vitro* intestinal preparation is the transport of an isotonic fluid such as Krebs-Henseleit bicarbonate buffer across the mucosal surface. However the physiological mechanism of fluid uptake is not as yet fully resolved, but is generally thought to occur as a corollary of active transport of solutes at the mucosal surface. According to the "standing osmotic gradient" model (21), (24), a local osmotic gradient is set up within an epithelial compartment as a result of an active accumulation of solute. Water is then absorbed, because of the osmotic gradient, and subsequent increases in hydrostatic pressure become another force for water and solute movement into the serosal compartment. Transport of water results in an increase of the serosal volume (serosal transport), and also an increase in the wet weight of the tissue (tissue uptake). Fluid uptake by gut tissue has been suggested to be localised to the epithelial cells which expand longitudinally during absorption (52). However there seems to be little doubt that the fluid uptake by gut tissue occurs as a consequence of an energy dependent transport process, and it is known to be dependent upon the presence of metabolic substrates (52), (51).

Over the different incubation periods of 10, 20, 30, 45, and 60 minutes, water transport measurements were carried out as previously described in detail in Section 2.4. A linear uptake of water with tissue was observed (Table 2.2, Figures 2.5, 2.6 and 2.7).

	INCUBATION PERIOD (Min.)				
	10	20	30	45	60
SEROSAL					
DUODENUM	-40 (31)	61 (42)	165 (83)	208 (49)	221 (71)
JEJUNUM	51 (30)	127 (51)	154 (34)	454 (82)	513 (159)
ILEUM	78 (10)	75 (25)	112 (28)	283 (61)	360 (132)
TISSUE					
DUODENUM	240 (52)	288 (62)	536 (123)	545 (78)	629 (41)
JEJUNUM	169 (25)	208 (48)	351 (93)	444 (40)	511 (43)
ILEUM	118 (30)	216 (27)	351 (74)	354 (102)	363 (33)
TOTAL					
DUODENUM	200 (37)	349 (36)	701 (191)	753 (99)	850 (68)
JEJUNUM	220 (24)	335 (83)	505 (49)	898 (79)	1024 (162)
ILEUM	196 (26)	291 (38)	463 (76)	637 (110)	723 (154)

Table 2.2 Movement of water across and into the intestinal tissue in different regions of the small intestine and for different incubation periods at 37°C expressed as mg. water/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

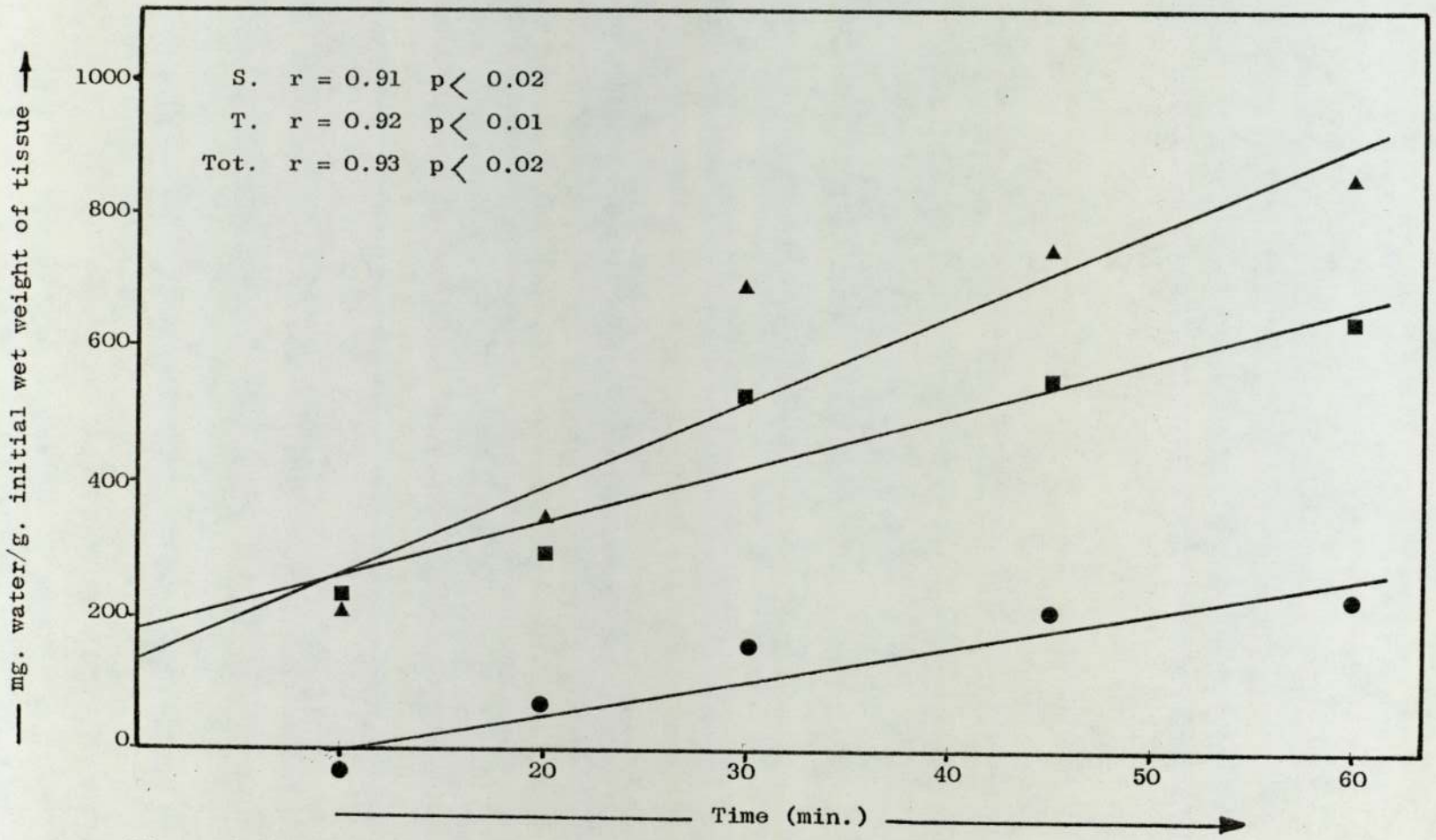


Figure 2.5 Water movement to the serosal compartment and into the intestinal tissue in the duodenal region at 37°C.
(●) Serosal, (■) Tissue, (▲) Total

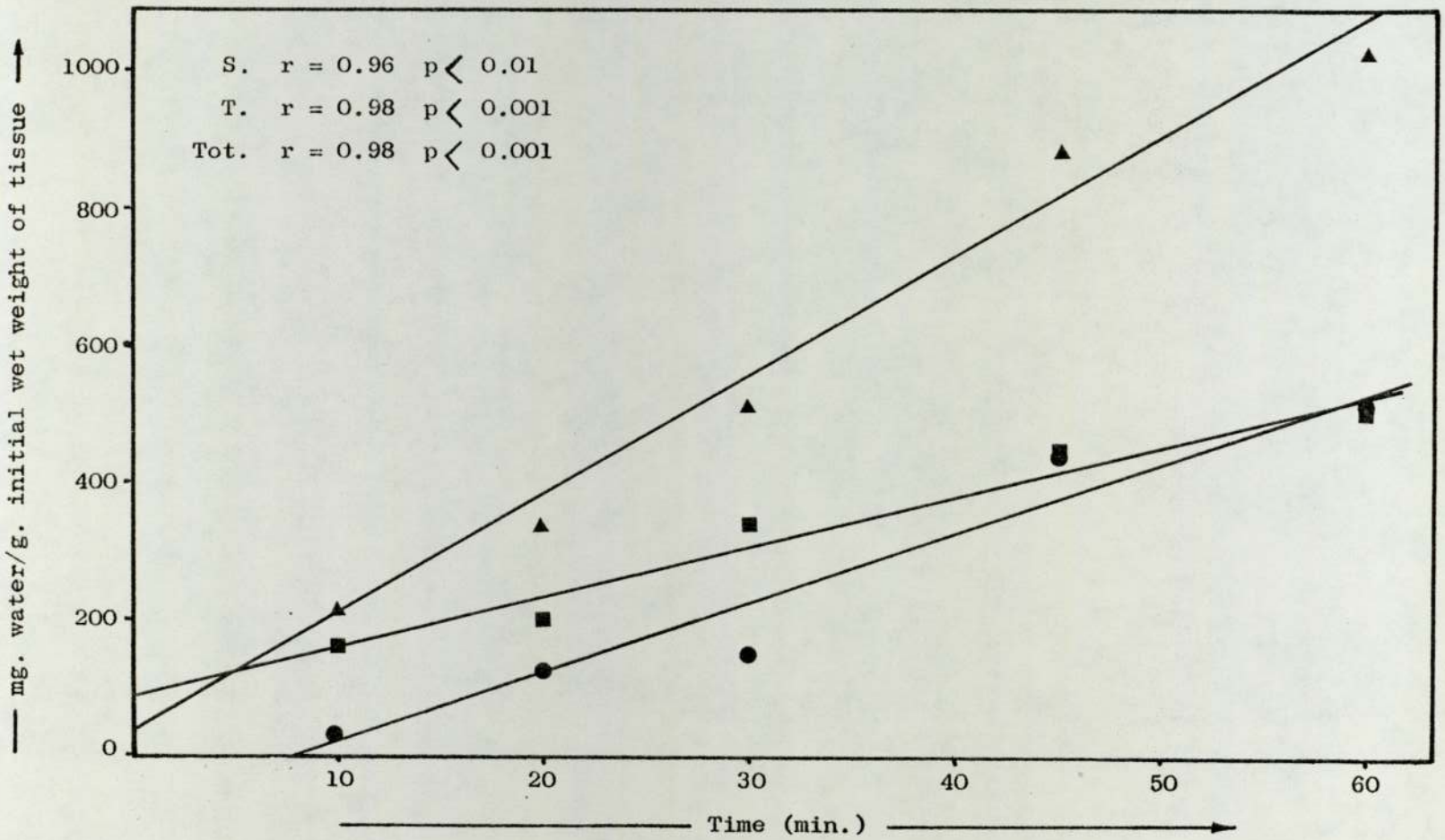


Figure 2.6 Water movement to the serosal compartment and into the intestinal tissue in the jejunal region at 37°C.
(●) Serosal, (■) Tissue, (▲) Total

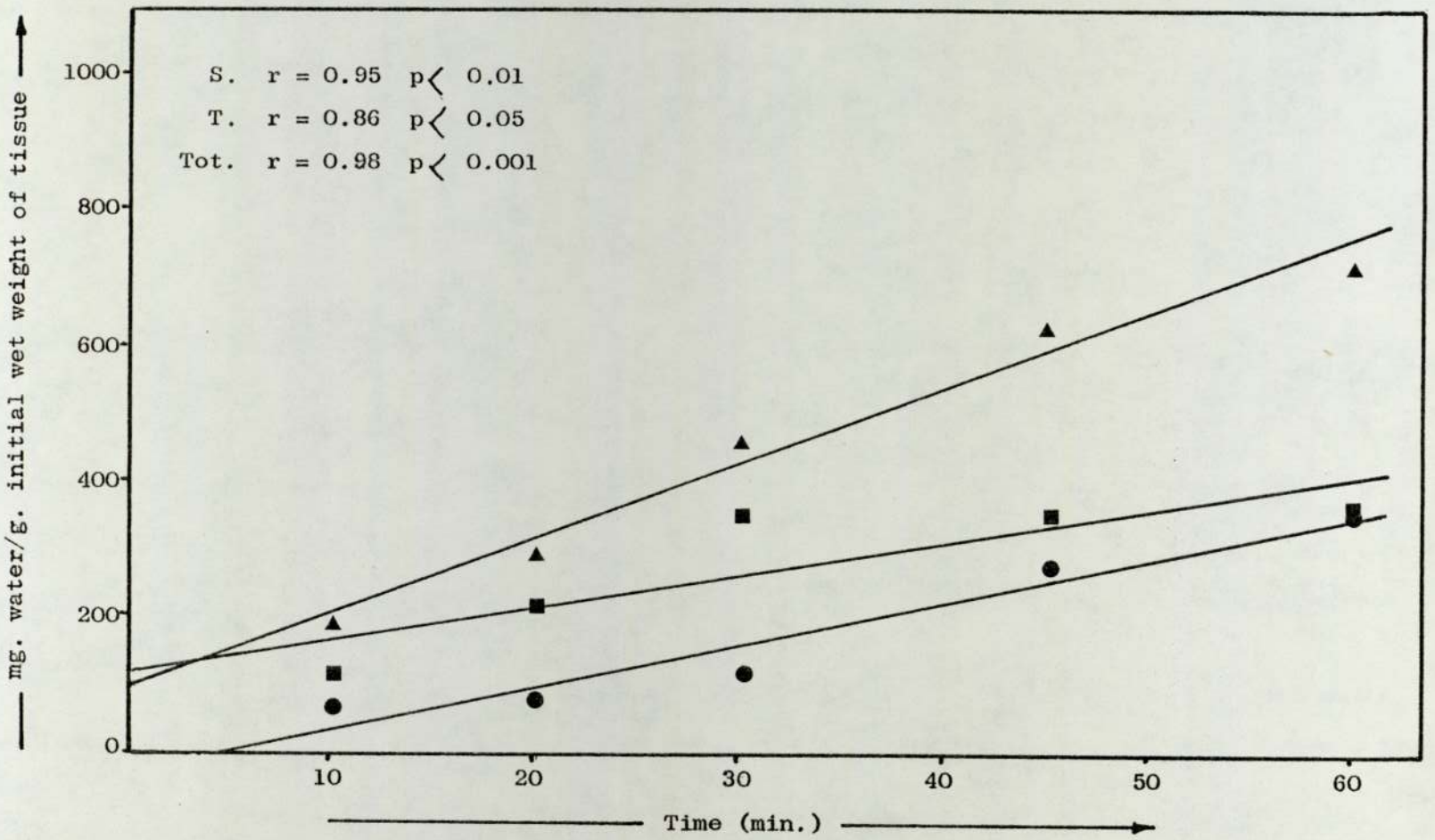


Figure 2.7 Water movement to the serosal compartment and into the intestinal tissue in the ileal region at 37°C
(●) Serosal, (■) Tissue, (▲) Total

The values obtained for tissue uptake and serosal transport of water are comparable with those of other investigators (52), (16), (4). Therefore the tissue appeared to function as a viable preparation. Consequently in all subsequent experiments to monitor viability, water transport was chosen, because it allowed a routine check to be made at the same time as other measurements.

2.7 Methods of Animal Sacrifice and its Effect on Water Transport

According to Levine et al (58), the use of anaesthetics may possibly affect the viability of in vitro tissue preparations and result in reductions in the tissue fluid uptake (tissue uptake). Therefore a comparative study of the water movement across everted sacs prepared from animals sacrificed by three different methods was undertaken. The preparation, incubation and assessment of water movement was carried out as previously described in Sections 2.3 and 2.4.

2.7.a Ether Anaesthetisation

In this method an animal was placed in a glass vacuum desiccator, which contained a quantity of cotton wool soaked in di-ethyl ether (A.R.). After a few minutes the animal became unconscious and was removed from the desiccator. Its abdomen was opened by mid-line incision, and the intestine removed as previously described.

2.7.b Intraperitoneal Injection of Inactin

To anaesthetise, the animals were injected intraperitoneally with Inactin (5 - sec butyl - 5 - ethyl 2 - thiobarbituric acid Promonta). Approximately 100 mg/kg body weight of Inactin was administered in 0.154 M saline. As soon as the animal lost consciousness

the intestine was removed as usual.

2.7.c Stunning and Decapitation

After placing the animal on the surface of a laboratory bench with the minimum possible of disturbance, it was swung suddenly by the tail and stunned with a single blow against the bench. The spinal cord was severed by decapitation and the intestine removed as previously described.

Water movement across the intestine was measured over 30 and 60 minute periods and used to assess the effect of the different methods of sacrifice on sac viability and preparation reproducibility. Although there was not a highly significant difference between the amount of water transported after sacrifice by the three different methods, there was a drop in water movement when the animals were anaesthetised with Ether or Inactin, (Table 2.3). The drop in water movement could be due to both the effects of anaesthetics on the metabolism of the intestine and also, due to the fact that the operation takes several minutes, and therefore the tissue has been exposed to unknown levels of a potentially toxic material. The anaesthesia with Ether has been noted to have a significant reduction effect in glycolysis in everted sac preparations (93). Therefore because water movement is thought to play an important role in the transport of a number of cations, and because of the practical advantage, the stunning method of sacrifice was chosen for use throughout these studies.

2.8 The Use of the Radioisotope ^{51}Cr

The radioisotope ^{51}Cr (as chromic chloride) obtained from

	TIME (Min.)	
	30	60
ETHER ANAESTHETISATION		
DUODENUM	278 (44)	607 (65)
JEJUNUM	478 (62)	709 (61)
ILEUM	388 (65)	514 (33)
INTRAPERITONIAL INJECTION OF INACTIN		
DUODENUM	464 (50)	672 (69)
JEJUNUM	504 (78)	848 (151)
ILEUM	521 (80)	690 (100)
STUNNING		
DUODENUM	702 (191)	849 (68)
JEJUNUM	560 (49)	1022 (162)
ILEUM	464 (76)	723 (154)

Table 2.3 Total water movement for three different methods of sacrifice (mg./g. wet weight of tissue) to the serosal compartment and into the tissue, in the different regions of the rat small intestine after 30 and 60 minutes incubation at 37°C. (S.E. is expressed in parenthesis)

the Radiochemical Centre Amersham, was used as a tracer in all chromium transport studies. ^{51}Cr is a gamma emitter, with a half life of 27.8 days. The amount of radioactivity in both fluid and tissue samples can therefore be directly assessed without the use of scintillants or tissue oxidation techniques.

In a typical experiment ^{51}Cr was added to the mucosal solution containing a known amount of non-labelled chromium chloride ($CrCl_3$, $6H_2O$). After incubation for a fixed time chromium was estimated in 200 µl aliquots of serosal and mucosal solution by counting the 320 Kev gamma emission of ^{51}Cr in a Nuclear Enterprises NE 8312 counter for 10 minutes. Radioactivity in the intestinal tissue was also estimated by placing the entire empty sac in an insert tube and counting as described above.

Total counts were corrected at 24 hour intervals to account for isotope decay from the equation:

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

where A is the counting rate at time t

A_o is the counting rate at time t_o

The theoretical decrease in activity, with time was calculated.

2.9 Calculation of Results

All the results presented are the mean of at least six experimental observations and are expressed in terms of mean ± S.E. of mean. To analyse the results, student's t test was used. The method of least squares was employed to produce regression lines. All the statistical calculations were carried out with an Olivetti Programma 101 Computer. Significancy of the results is denoted by either a single asterisk (*) for $p < 0.05$, or a double asterisk (**) for $p < 0.001$.

CHAPTER 3

TRANSPORT OF CHROMIUM IONS ACROSS THE INTESTINAL TRACT

3.1 Introduction

Although there is a great deal of information published concerning the metabolism of chromium, very little is known about the precise mechanism involved in the transport of chromium across the intestinal epithelium. As a first step to the understanding of the transport mechanism, the rate of chromium transport to the serosal spaces and the uptake of chromium by the intestinal tissue was determined over the concentration range 5×10^{-7} M to 5×10^{-5} M. Three sacs were selected as described in section 2.3 as representative of the distal duodenum (D), mid-jejunum (J), and distal Ileum (I), and used to establish whether there was a preferential site for chromium absorption.

3.2 Rate of Chromium Uptake by the Intestinal Tissue and its Subsequent Passage into the Serosal Compartment

The absorption of trivalent chromium chloride ($CrCl_3$, $6H_2O$) was measured over either 10, 20, 30, 45, or 60 minute periods at 37°C in Krebs-Henseleit bicarbonate buffer containing 20 mM glucose, with an initial mucosal chromium concentration of either 10^{-6} M or 10^{-5} M and ^{51}Cr as a tracer. Chromium transport to the serosal spaces and the uptake by the tissue were assessed as described in section 2.8.

Only a very small percentage of chromium was transported into the serosal compartment after 60 minutes incubation (approximately 0.4%) and the amount of chromium transported into the serosal compartment was not significantly different for

different regions of the small intestine (Table 3.1). The transport of the chromium ion continued in a linear fashion for 60 minutes, across all regions. However the extrapolation of the best-line fit has an intercept on the y-axis (Figure 3.1) and indicates a faster initial rate of absorption to the serosal compartment over approximately the first 10 minutes of incubation.

Over the same time periods, there was a rapid and comparatively much larger uptake of chromium by the intestinal tissue; Approximately 38% of initial mucosal chromium was taken up by the intestinal tissue after 60 minutes incubation in 10^{-6} M Cr^{3+} (Table 3.1, Figure 3.2). At both concentrations investigated the uptake of chromium by the ileal tissue was more than that of either the duodenum or the jejunum. This difference however, was not statistically significant (Table 3.1, Table 3.2).

The rate of chromium uptake by the intestinal tissue was very much greater over the first 10 minutes of incubation than the subsequent 50 minutes incubation period. Approximately 50% of the chromium taken up by the tissue occured during the initial 10 minutes time period. There is some evidence to suggest that the amount of chromium taken up by the intestinal tissue (approximately 38%) comes to equilibrium with the remaining "free" chromium in the mucosal solution (Figure 3.2).

The total amount of water transported across and into the intestinal tissue continued in an approximately linear fashion over 60 minutes incubation (Tables 3.3, 3.4 and Figures 3.3, 3.4). The rate of water movement was independent of chromium concentration and was approximately 500, 1200 and 800 mg/g. initial wet weight of

tissue/hr for the duodenum, jejunum and ileum, respectively. There was a correlation between water transport and chromium transport to the serosal compartment, across the jejunum and ileum (r_J = 0.96, r_I = 0.94) (Figure 3.5). There was however no correlation between water movement into the tissue and chromium uptake of the tissue.

	INCUBATION PERIOD (Min.)				
	10	20	30	45	60
SEROSAL					
DUODENUM	0.8 (0.1)	1.5 (0.1)	2.3 (0.4)	2.8 (0.4)	3.1 (0.5)
JEJUNUM	0.6 (0.2)	1.4 (0.1)	2.2 (0.3)	2.5 (0.3)	3.3 (0.6)
ILEUM	0.5 (0.1)	1.2 (0.2)	1.3 (0.2)	2.2 (0.2)	2.5 (0.3)
TISSUE					
DUODENUM	81 (13)	88 (10)	99 (14)	113 (8)	189 (11)
JEJUNUM	74 (5)	88 (8)	118 (12)	133 (13)	161 (25)
ILEUM	106 (9)	114 (10)	145 (8)	148 (8)	203 (22)

Table 3.1 Transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$), across different regions of the small intestine and the uptake by the tissue after different incubation periods at 37°C expressed as ng. Cr/g. initial wet weight of tissue. (S.E. is expressed in parenthesis)

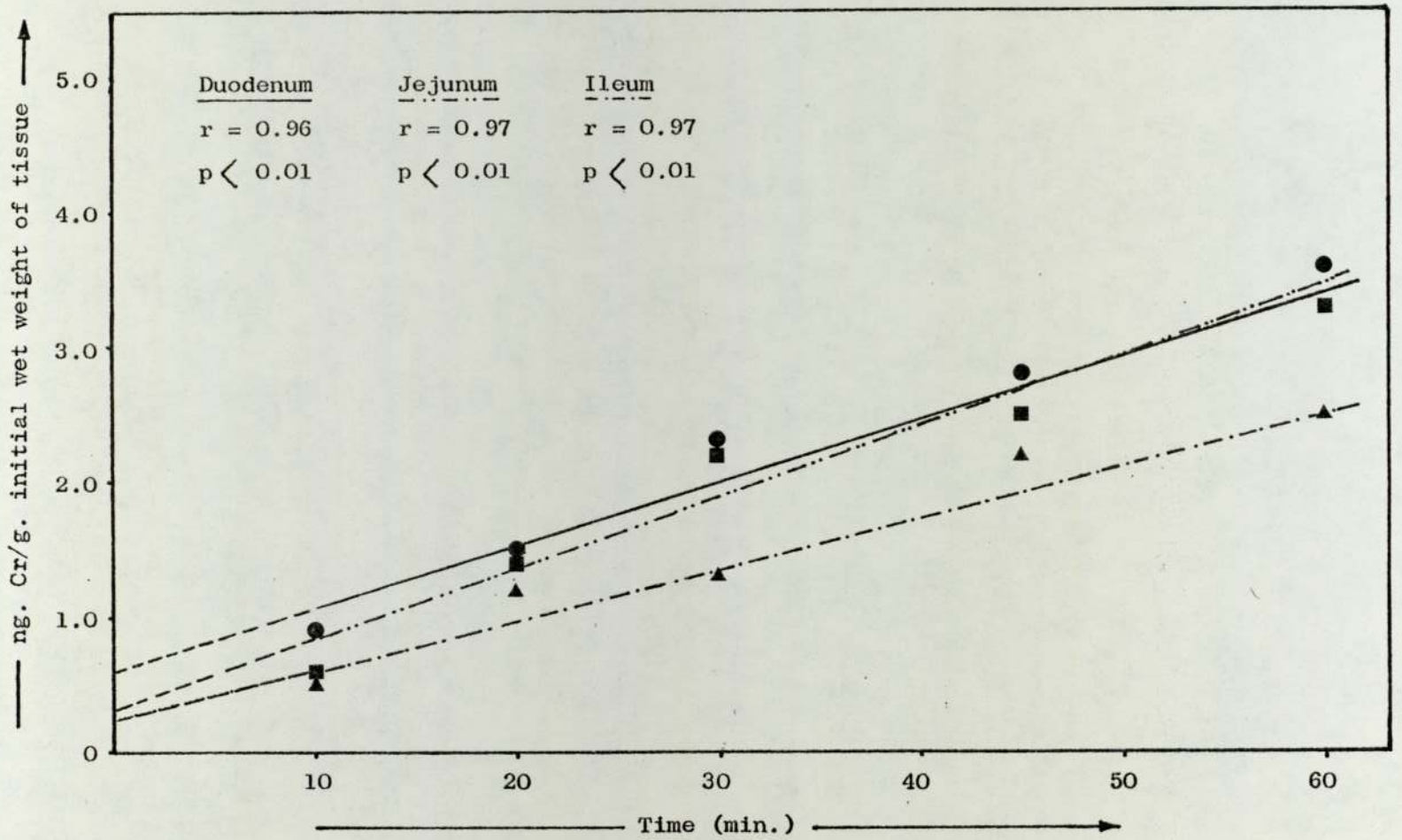


Figure 3.1 Transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) to the serosal compartment across different regions of the small intestine for 10 to 60 minutes at 37°C. (●) Duodenum, (■) Jejunum, (▲) Ileum

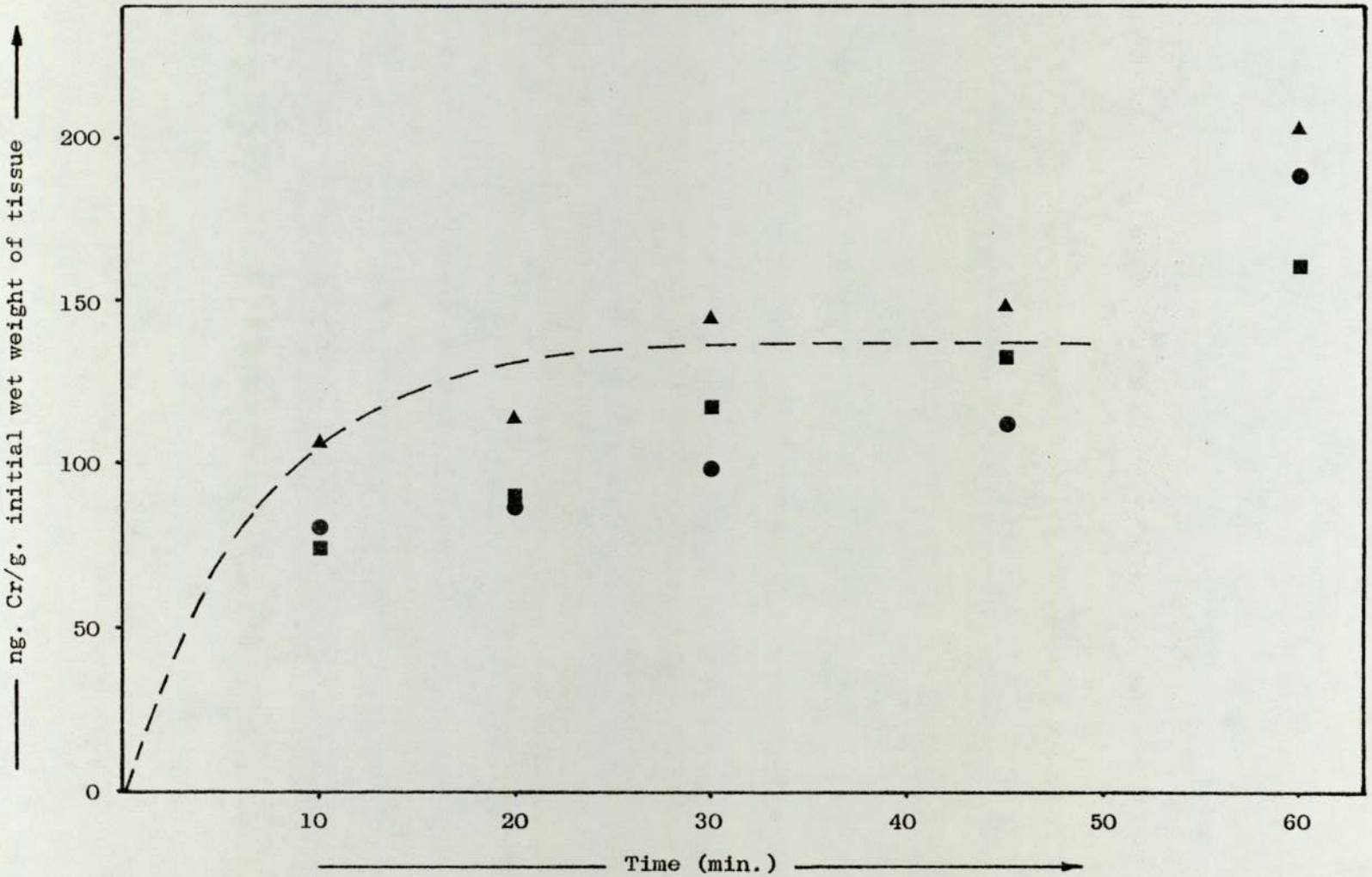


Figure 3.2 Uptake of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) by the intestinal tissue after different incubation periods at 37°C.
(●) Duodenum, (■) Jejunum, (▲) Ileum

	INCUBATION PERIOD (Min.)				
	10	20	30	45	60
SEROSAL					
DUODENUM	6.7 (1.5)	10.4 (2.4)	13.8 (1.9)	17.5 (4.7)	28.4 (4.0)
JEJUNUM	7.1 (1.3)	10.8 (1.6)	20.3 (5.0)	19.8 (6.4)	44.9 (9.5)
ILEUM	6.1 (1.4)	7.0 (1.0)	11.4 (4.0)	7.3 (1.2)	33.5 (9.0)
TISSUE					
DUODENUM	973 (219)	1337 (319)	1239 (182)	1377 (192)	1924 (373)
JEJUNUM	879 (148)	1419 (282)	1715 (243)	1230 (214)	1859 (137)
ILEUM	1080 (173)	1413 (247)	1528 (164)	1494 (267)	2472 (223)

Table 3.2 Transport of 10^{-5} M Cr^{3+} ($CrCl_3$, $6H_2O$) across different regions of the small intestine and the uptake by the tissue after different incubation periods at 37°C expressed as ng. Cr/g. initial wet weight of tissue. (S.E. is expressed in parenthesis)

	INCUBATION PERIOD (Min.)				
	10	20	30	45	60
SEROSAL					
DUODENUM	15 (49)	20 (50)	17 (57)	37 (32)	36 (27)
JEJUNUM	79 (38)	198 (78)	276 (40)	511 (78)	640 (143)
ILEUM	47 (23)	126 (17)	215 (53)	242 (75)	380 (79)
TISSUE					
DUODENUM	277 (51)	512 (52)	434 (16)	490 (42)	532 (55)
JEJUNUM	289 (60)	386 (41)	391 (45)	529 (39)	584 (80)
ILEUM	229 (20)	330 (16)	348 (25)	425 (57)	433 (48)
TOTAL					
DUODENUM	292 (47)	532 (46)	451 (52)	527 (41)	568 (51)
JEJUNUM	368 (51)	584 (73)	667 (50)	1040 (89)	1224 (226)
ILEUM	276 (30)	456 (28)	563 (67)	667 (124)	813 (131)

Table 3.3 Water movement across the different regions of the small intestine and uptake by the tissue, in the presence of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) at 37°C, expressed as mg. water/g. initial wet weight of tissue. (S.E. is expressed in parenthesis)

	INCUBATION PERIOD (Min.)				
	10	20	30	45	60
SEROSAL					
DUODENUM	-42 (47)	86 (59)	29 (26)	38 (27)	93 (54)
JEJUNUM	14 (16)	161 (45)	294 (50)	425 (50)	668 (114)
ILEUM	37 (21)	158 (52)	251 (62)	308 (119)	554 (133)
TISSUE					
DUODENUM	323 (42)	294 (52)	429 (49)	441 (26)	441 (28)
JEJUNUM	278 (42)	341 (50)	359 (32)	460 (13)	529 (34)
ILEUM	285 (77)	368 (62)	389 (74)	392 (76)	521 (74)
TOTAL					
DUODENUM	281 (41)	380 (67)	458 (58)	479 (38)	534 (64)
JEJUNUM	292 (44)	502 (73)	653 (53)	885 (45)	1197 (140)
ILEUM	322 (32)	526 (30)	640 (59)	700 (170)	1075 (166)

Table 3.4 Water movement across the different regions of the small intestine and uptake by the tissue, in the presence of 10^{-5} M Cr^{3+} ($CrCl_3$, $6H_2O$) at 37°C, expressed as mg. water/g. initial wet weight of tissue. (S.E. is expressed in parenthesis)

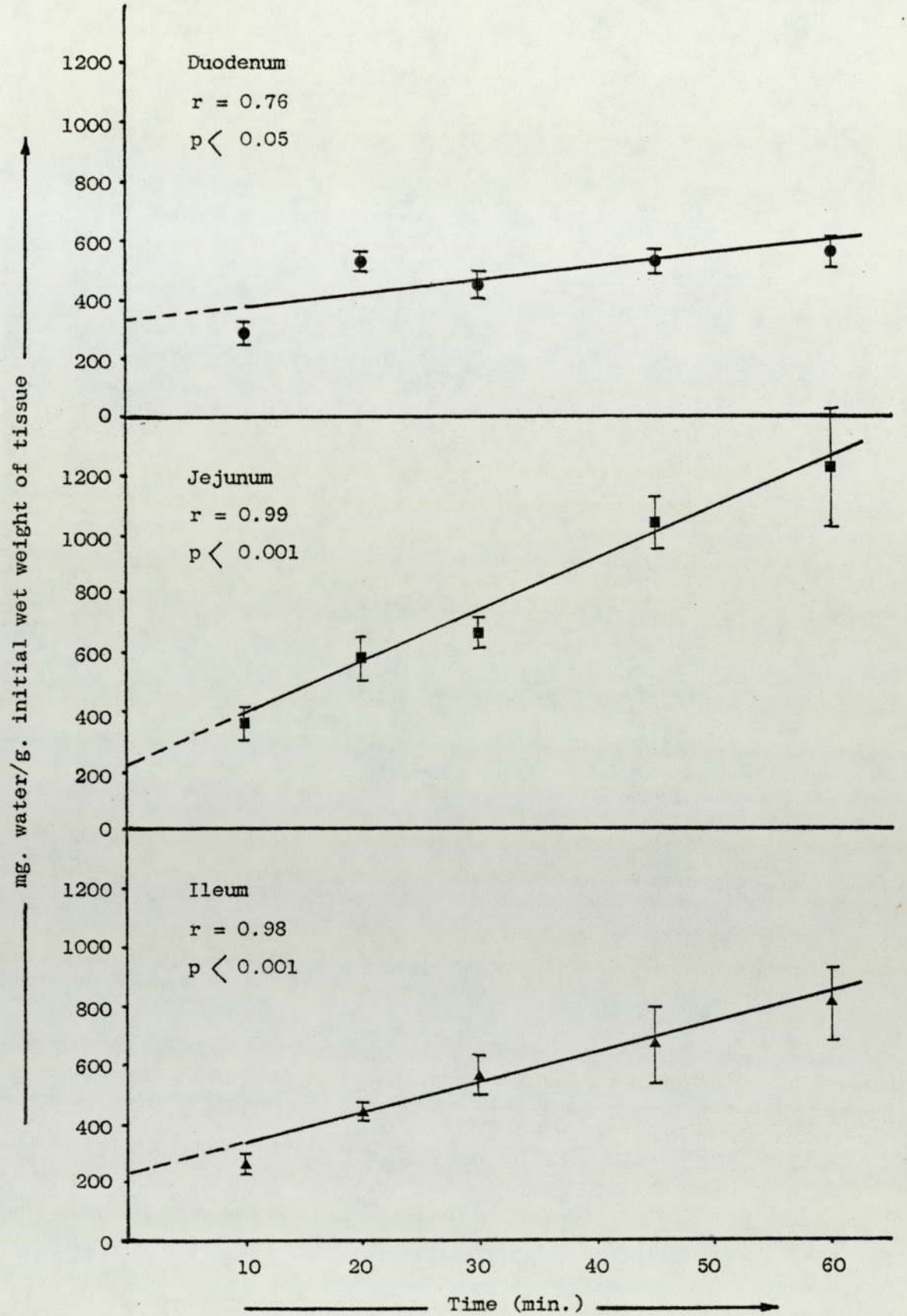


Figure 3.3 Total water movement across the different regions of the small intestine after 30 minutes incubation at 37°C in the presence of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$). Standard error of mean represented by vertical bars.

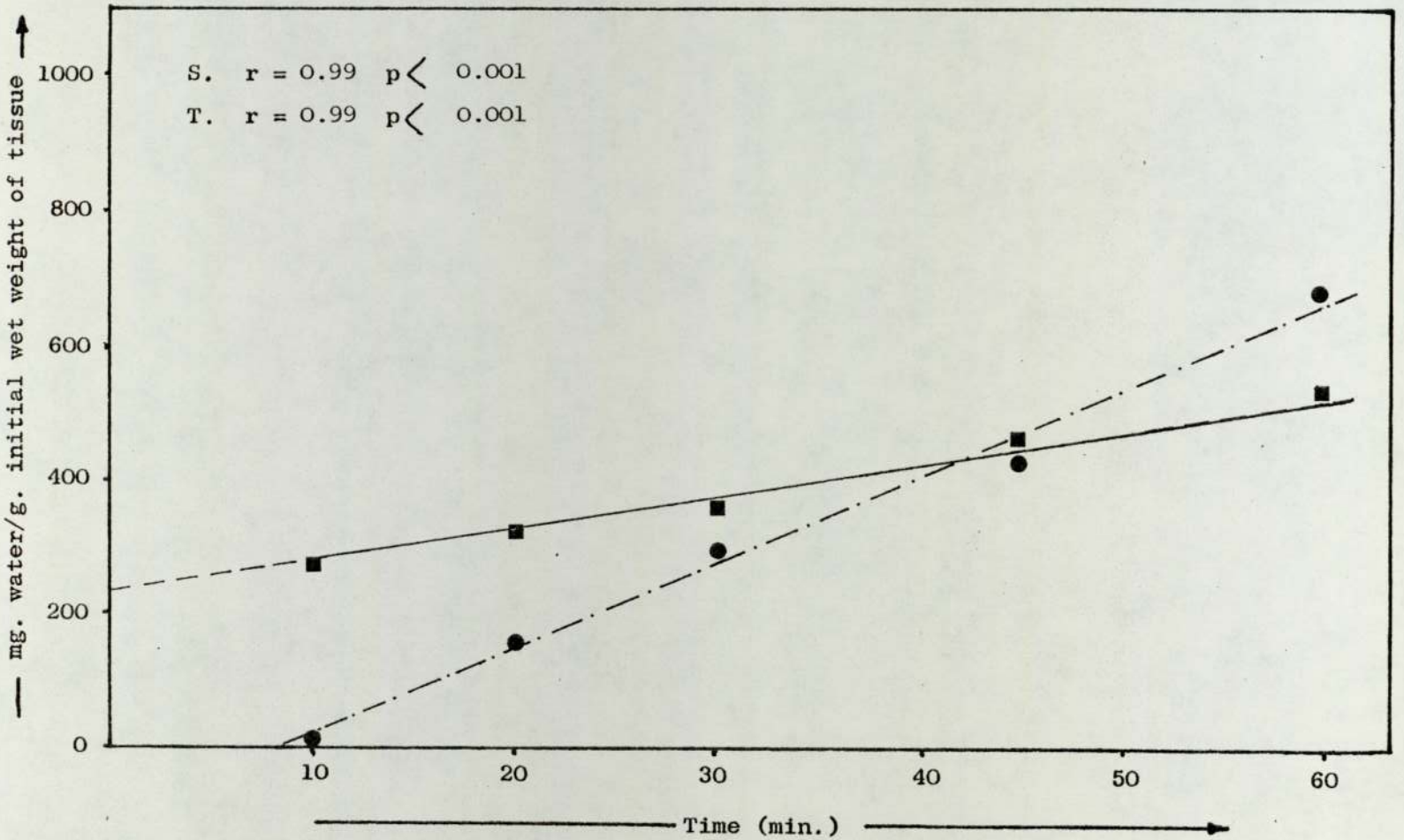


Figure 3.4 Water movement to the serosal compartment (●) and into the intestinal tissue (■) in the jejunal region after different incubation periods at 37°C in the presence of 10^{-5} M Cr^{3+} ($CrCl_3$, $6H_2O$).

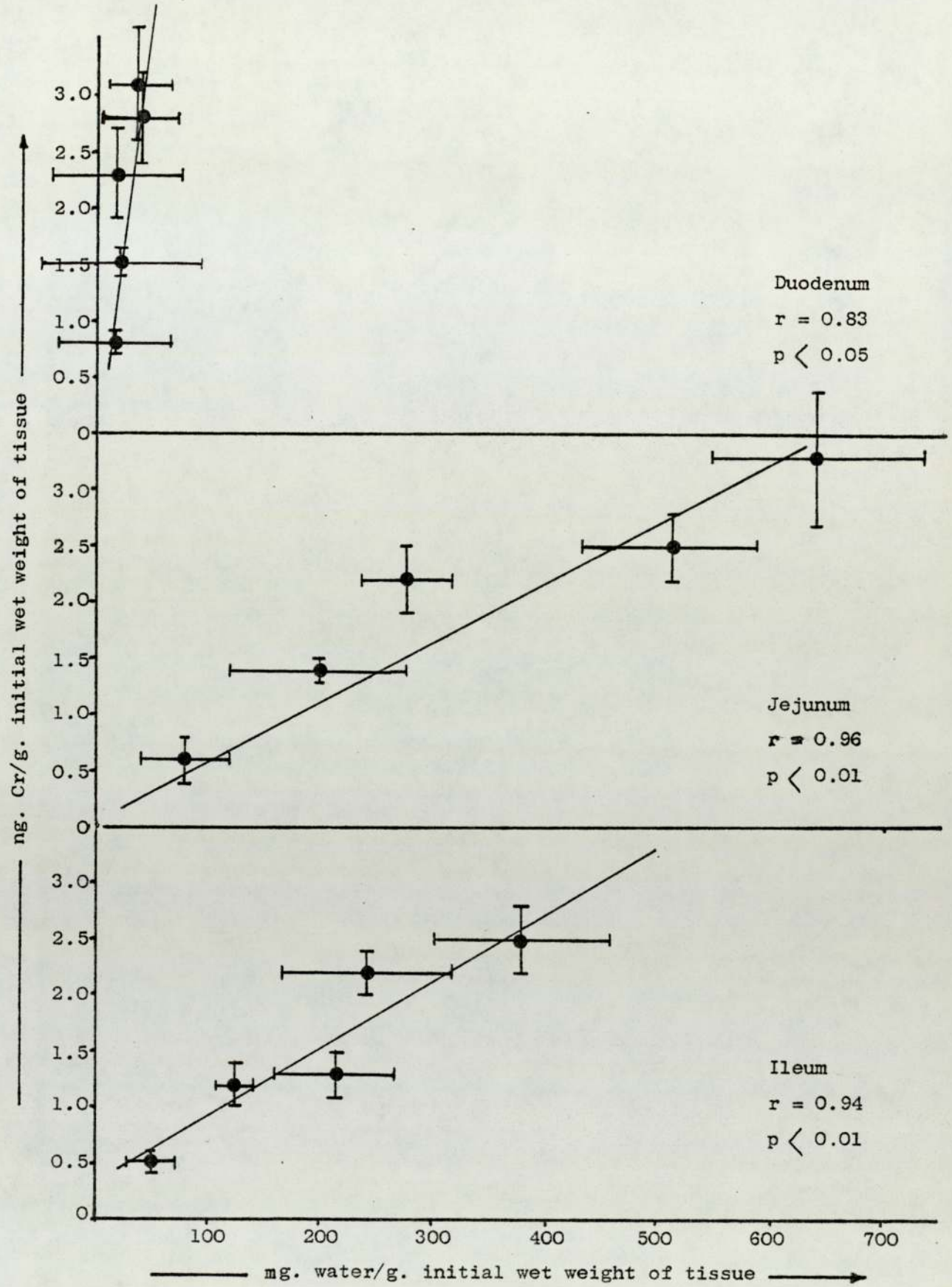


Figure 3.5 Correlation between the transport of water and chromium into the serosal compartment after incubation periods of 10, 20, 30, 45 or 60 minutes at 37°C in 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$).

Standard error of mean appropriately represented by horizontal and vertical bars.

3.3 Effect of Different Concentrations of Chromium in the Mucosal Solution on Tissue Uptake and Movement of Chromium into the Serosal Compartment

The transport of chromium ions to the serosal space and their uptake by the intestinal tissue was measured after either 30 or 60 minutes incubation in Krebs-Henseleit bicarbonate buffer at 37°C over a 100 fold concentration range of mucosal chromium (5×10^{-7} M to 5×10^{-5} M) using ^{51}Cr as tracer. Both water movement and chromium transport to serosal spaces and their uptake by the tissue were assessed as described in Section 2.4 and 2.8.

Transport of chromium to the serosal compartment and chromium uptake by the tissue continued in a linear fashion over a concentration range of 5×10^{-7} M to 10^{-5} M (Tables 3.5, 3.6 and Figures 3.6, 3.7, 3.8, 3.9). The percentage of chromium ions transported to the serosal compartment was independent of mucosal chromium concentration (Table 3.7, 3.8). There was however a greater percentage uptake of chromium by the intestinal tissue at the highest mucosal concentration of chromium investigated (5×10^{-5} M). Transport of water to the serosal compartment was unaffected by the presence of the highest concentration of chromium. Similarly water movement into the intestinal tissue was also largely unaffected (Table 3.9, 3.10).

CONCENTRATION OF CHROMIUM ION

	5×10^{-7} M	10^{-6} M	5×10^{-6} M	10^{-5} M	5×10^{-5} M
SEROSAL					
DUODENUM	0.9 (0.2)	2.3 (0.4)	5.1 (0.4)	13.8 (1.9)	48.1 (9.0)
JEJUNUM	0.9 (0.2)	2.2 (0.3)	5.6 (0.6)	20.3 (5.0)	68.0 (15.0)
ILEUM	0.6 (0.2)	1.3 (0.2)	2.8 (0.4)	11.4 (4.0)	50.2 (10.0)
TISSUE					
DUODENUM	50 (10)	99 (14)	804 (100)	1239 (182)	16239 (1844)
JEJUNUM	54 (7)	118 (12)	953 (154)	1715 (243)	16149 (2939)
ILEUM	76 (8)	145 (8)	1093 (111)	1528 (164)	15091 (1833)

Table 3.5 Transport of different concentrations of Cr^{3+} ($CrCl_3$, $6H_2O$), across different regions of the small intestine after 30 minutes incubation at 37°C, expressed as ng.Cr/g. wet weight of tissue.
(S.E. is expressed in parenthesis)

CONCENTRATION OF CHROMIUM ION

	5×10^{-7} M	10^{-6} M	5×10^{-6} M	10^{-5} M	5×10^{-5} M
SEROSAL					
DUODENUM	1.2 (0.2)	3.1 (0.5)	10.2 (1.3)	28.4 (4.0)	115.4 (20.0)
JEJUNUM	1.6 (0.2)	3.3 (0.6)	10.5 (1.0)	44.9 (9.5)	155.0 (38.0)
ILEUM	1.0 (0.1)	2.5 (0.3)	7.6 (1.0)	33.5 (8.8)	92.0 (17.0)
TISSUE					
DUODENUM	64 (14)	203 (11)	892 (59)	1924 (373)	13169 (2739)
JEJUNUM	62 (8)	161 (25)	1140 (165)	1859 (137)	16481 (3142)
ILEUM	98 (14)	203 (22)	1344 (193)	2472 (223)	16022 (2729)

Table 3.6 Transport of different concentrations of Cr^{3+} ($CrCl_3$, $6H_2O$), across different regions of the small intestine after 60 minutes incubation at 37°C, expressed as ng. Cr/g. initial wet weight of tissue. (S.E. is expressed as parenthesis)

	CONCENTRATION OF CHROMIUM ION				
	5×10^{-7} M	10^{-6} M	5×10^{-6} M	10^{-5} M	5×10^{-5} M
SEROSAL					
DUODENUM	0.34 (0.07)	0.43 (0.07)	0.19 (0.01)	0.25 (0.04)	0.18 (0.03)
JEJUNUM	0.34 (0.07)	0.41 (0.06)	0.21 (0.02)	0.38 (0.09)	0.25 (0.06)
ILEUM	0.22 (0.07)	0.24 (0.04)	0.10 (0.01)	0.21 (0.07)	0.19 (0.04)
TISSUE					
DUODENUM	18.7 (3.7)	18.5 (2.6)	30.1 (3.7)	23.1 (3.4)	60.7 (6.9)
JEJUNUM	20.2 (2.6)	22.0 (2.2)	35.6 (5.8)	32.0 (4.5)	60.3(11.0)
ILEUM	28.4 (3.0)	27.1 (1.5)	40.8 (4.1)	28.5 (3.1)	56.4 (6.9)

Table 3.7 Transport of different concentrations of Cr^{3+} ($CrCl_3$, $6H_2O$), across different regions of the small intestine after 30 minutes incubation at 37°C, expressed as percentage of initial mucosal concentration.

	CONCENTRATION OF CHROMIUM ION				
	5×10^{-7} M	10^{-6} M	5×10^{-6} M	10^{-5} M	5×10^{-5} M
SEROSAL					
DUODENUM	0.45 (0.07)	0.58 (0.09)	0.38 (0.05)	0.53 (0.07)	0.43 (0.07)
JEJUNUM	0.60 (0.07)	0.62 (0.11)	0.39 (0.04)	0.84 (0.18)	0.58 (0.14)
ILEUM	0.37 (0.03)	0.47 (0.06)	0.28 (0.04)	0.62 (0.16)	0.34 (0.06)
TISSUE					
DUODENUM	24.0 (5.2)	37.9 (2.1)	33.3 (2.2)	35.9 (7.0)	49.2(10.2)
JEJUNUM	23.2 (3.0)	30.1 (4.7)	42.6 (6.2)	34.7 (2.6)	61.6(11.7)
ILEUM	31.7 (5.2)	37.9 (4.1)	50.2 (7.2)	46.2 (4.2)	59.9(10.2)

Table 3.8 Transport of different concentrations of Cr^{3+} ($CrCl_3$, $6H_2O$), across different regions of the small intestine after 60 minutes incubation at 37°C, expressed as percentage of initial mucosal concentration.

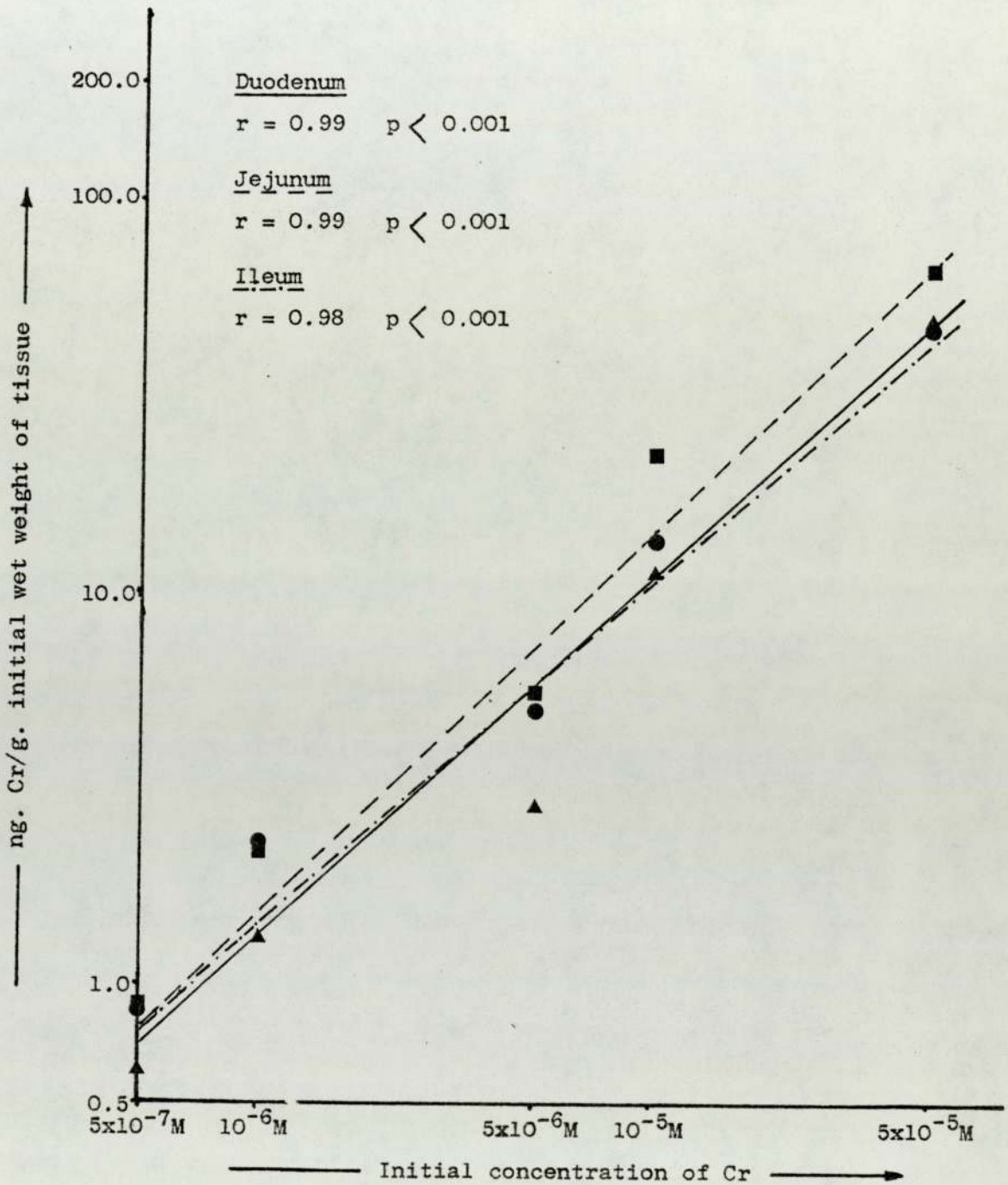


Figure 3.6 Transport of chromium into the serosal compartment after 30 minutes incubation at 37°C with different initial mucosal concentrations of chromium. (●) Duodenum, (■) Jejunum, (▲) Ileum

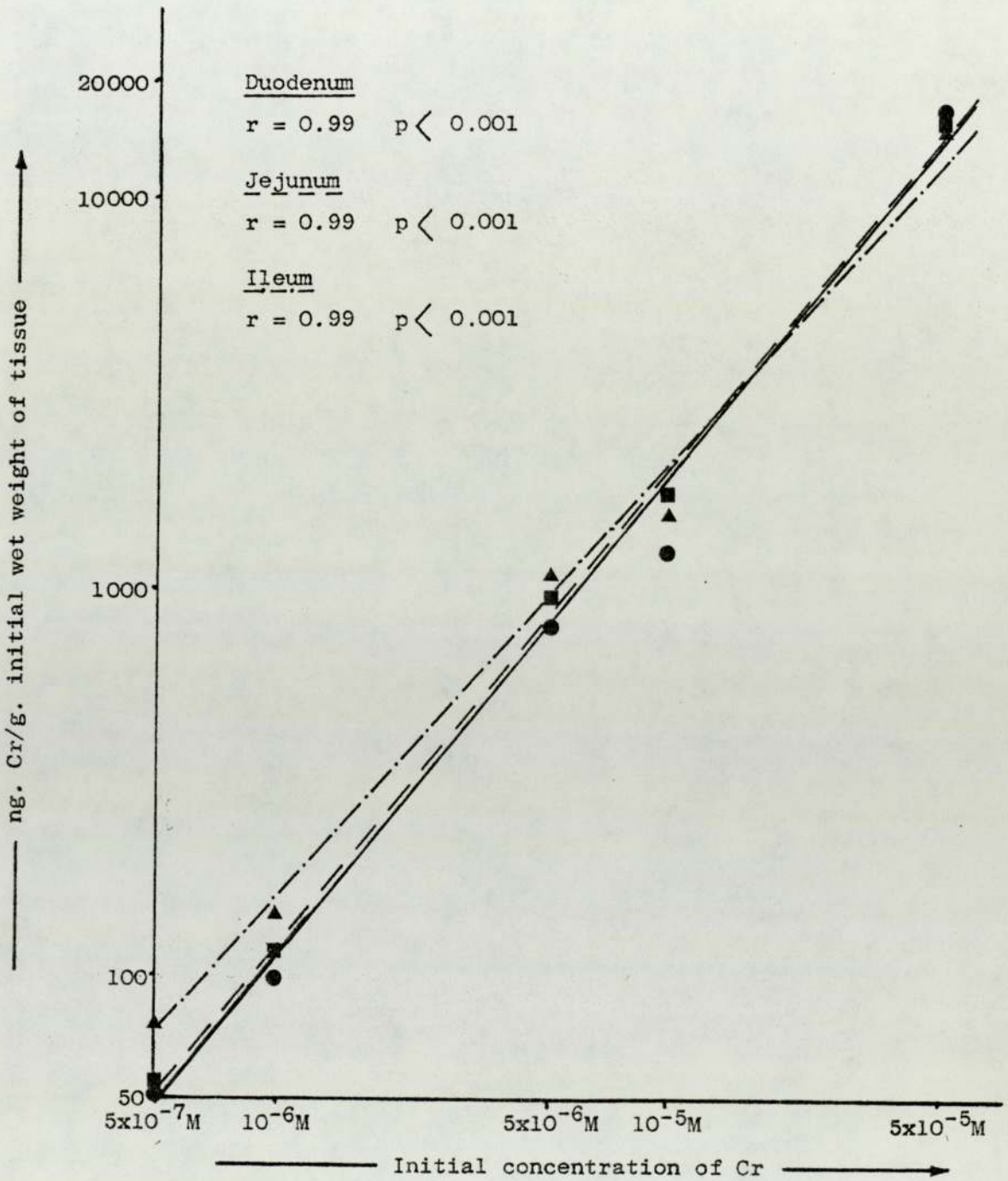


Figure 3.7 Uptake of chromium by the intestinal tissue after 30 minutes incubation at 37°C with different initial mucosal concentrations of chromium. (●) Duodenum, (■) Jejunum, (▲) Ileum

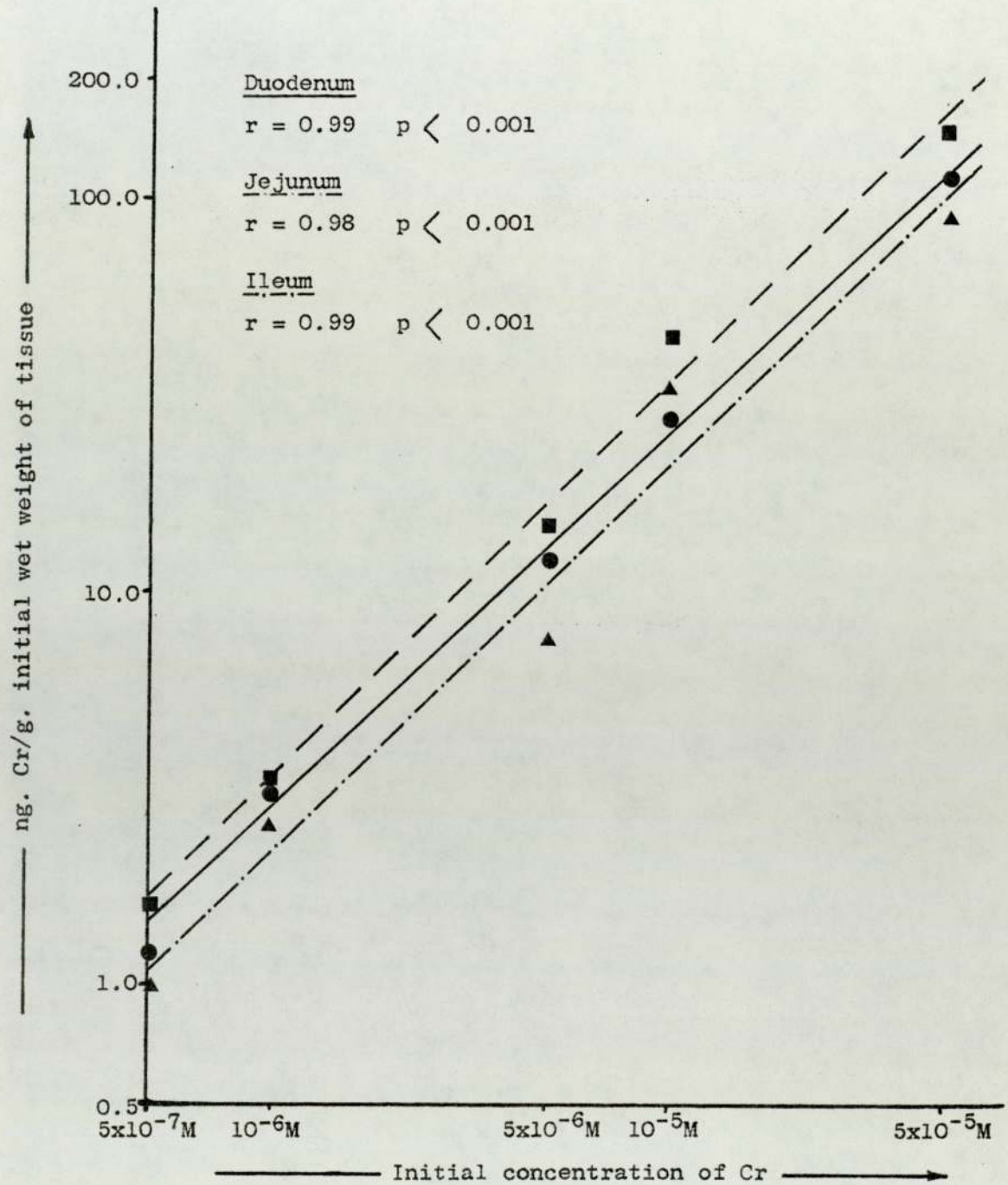


Figure 3.8 Transport of chromium into the serosal compartment after 60 minutes incubation at 37°C with different initial mucosal concentrations of chromium. (●) Duodenum, (■) Jejunum (▲) Ileum

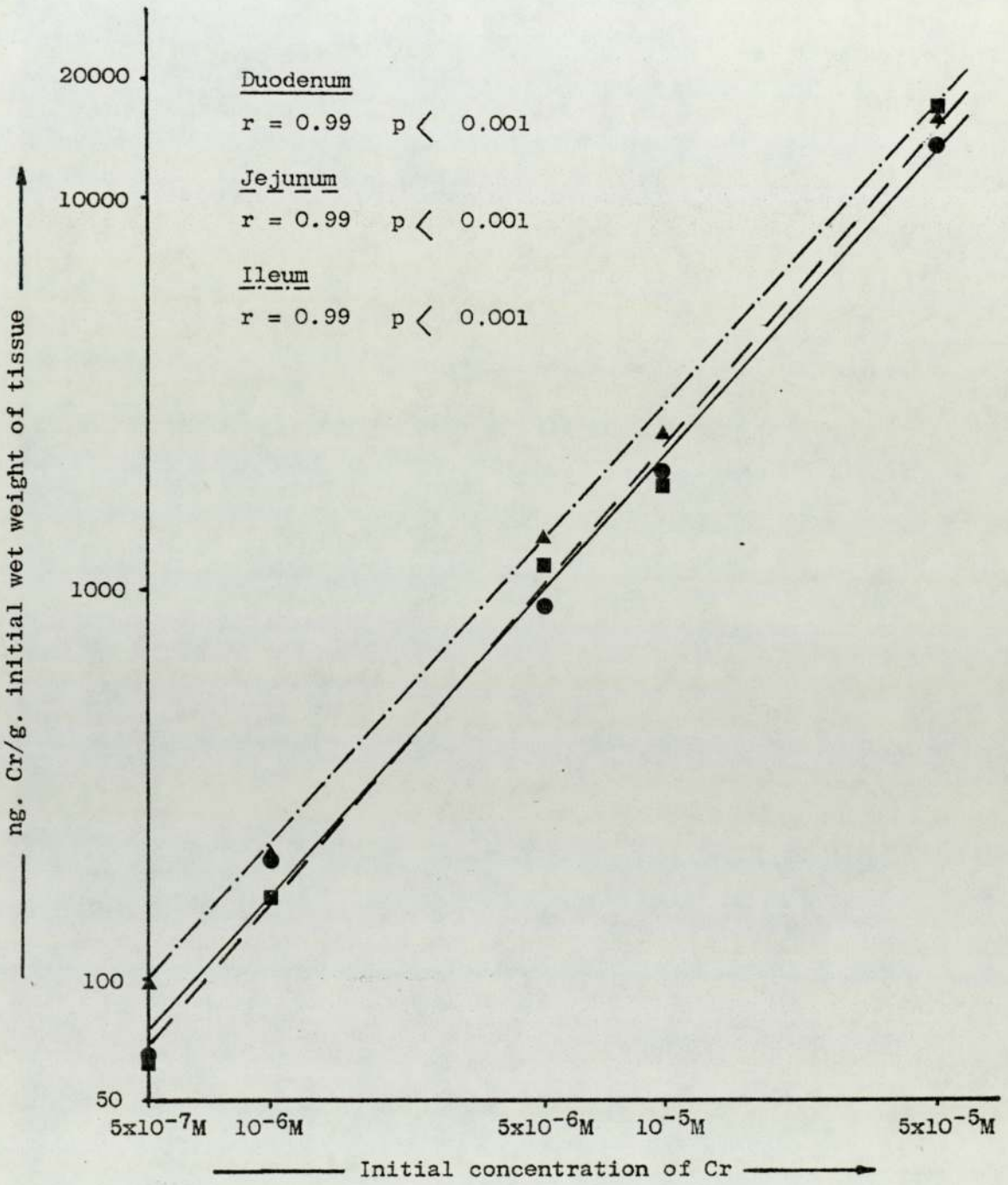


Figure 3.9 Uptake of chromium by the intestinal tissue after 60 minutes incubation at 37°C with different initial mucosal concentrations of chromium. (●) Duodenum, (■) Jejunum, (▲) Ileum

	CONCENTRATION OF CHROMIUM ION				
	5×10^{-7} M	10^{-6} M	5×10^{-6} M	10^{-5} M	5×10^{-5} M
SEROSAL					
DUODENUM	19 (29)	17 (57)	36 (47)	29 (26)	24 (41)
JEJUNUM	177 (59)	276 (40)	362 (46)	294 (50)	395 (149)
ILEUM	153 (12)	215 (53)	189 (81)	251 (62)	295 (50)
TISSUE					
DUODENUM	515 (71)	434 (16)	330 (36)	429 (49)	515 (57)
JEJUNUM	475 (56)	391 (45)	382 (46)	359 (32)	487 (36)
ILEUM	491 (49)	348 (25)	378 (38)	389 (74)	392 (38)

Table 3.9 Transport of water to the serosal compartment and into the intestinal tissue in the presence of different concentrations of Cr^{3+} ($CrCl_3$, $6H_2O$) after 30 minutes incubation at 37°C, expressed as mg. water/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

	CONCENTRATION OF CHROMIUM ION				
	5×10^{-7} M	10^{-6} M	5×10^{-6} M	10^{-5} M	5×10^{-5} M
SEROSAL					
DUODENUM	40 (37)	36 (27)	103 (48)	38 (27)	33 (15)
JEJUNUM	308 (71)	640 (143)	547 (90)	668 (114)	396 (91)
ILEUM	168 (33)	380 (79)	385 (73)	554 (133)	255 (82)
TISSUE					
DUODENUM	613 (63)	532 (42)	485 (60)	441 (26)	583 (33)
JEJUNUM	663 (62)	584 (80)	509 (57)	529 (34)	553 (50)
ILEUM	644 (59)	433 (48)	583 (91)	521 (74)	431 (107)

Table 3.10 Transport of water to the serosal compartment and into the intestinal tissue in the presence of different concentrations of Cr^{3+} ($CrCl_3$, $6H_2O$) after 60 minutes incubation at 37°C, expressed as mg. water/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

3.4 The Nature of the Chromium Interaction with the Intestinal Tissue

As was previously mentioned (Section 3.1, and 3.2) there was a rapid and relatively large uptake of chromium by the intestinal tissue. Since this uptake may be one of the factors that influences the transport of chromium into the serosal compartment it is important to establish whether the interaction of chromium with the intestinal tissue is permanent or exchangeable with luminal chromium ions.

A series of experiments was carried out to determine whether the bound chromium can be removed easily by various washing procedures and also to investigate whether the interaction between chromium and the intestinal tissue is ionic or covalent in nature. Everted sacs prepared from the duodenum, jejunum and ileum, were incubated as described previously (Section 2.3) at 37°C for 30 minutes in 10 ml of Krebs-Henseleit bicarbonate buffer containing 10^{-6} M chromium chloride and the ^{51}Cr as tracer. After incubation the sacs were transferred to 10 ml. of either Krebs-Henseleit bicarbonate buffer or Krebs-Henseleit bicarbonate buffer containing 10^{-6} M chromium chloride or Krebs-Henseleit bicarbonate buffer and 10^{-6} M Ethylene Diamine Tetra Acetic acid (E.D.T.A), for a further 30 minutes incubation period. None of the washing solutions contained the radioactive tracer ^{51}Cr.

200 μl samples were taken from the mucosal media after either 5, 10, 20, or 30 minutes washing in the non-radioactive buffer. The loss of ^{51}Cr from the tissue to the washing media after each time interval, as well as the remaining chromium

associated with tissue after 30 minutes washing was assessed by counting the γ radiation of ^{51}Cr as described previously (Section 2.8).

When the tissue was washed with Krebs-Henseleit bicarbonate buffer alone, there was a gradual increase in the amount of chromium removed from the tissue with time, but the total amount removed was relatively small. Approximately 1 - 3% chromium was removed after 5 minutes. There appeared to be a greater percentage of chromium removed after 30 minutes from the duodenum (17%) than either the jejunum or ileum (5%) (Table 3.11, Figure 3.10).

In the presence of 10^{-6} M Ethylene Diamine Tetra Acetic acid (E.D.T.A.) there was a rapid removal of chromium from the tissue over the first 5 minutes of incubation (Figure 3.10) (4.5 - 13.5%, compared with approximately 1 - 3% in the presence of Krebs-Henseleit bicarbonate buffer alone). Little more chromium was removed after this period.

When 10^{-6} M chromium replaced E.D.T.A. in the buffer incubation media, significantly more chromium ($p < 0.02$) was removed from the tissue (Figure 3.10). 11.5 - 26.5% of chromium was removed over the first 5 minutes and from thereafter the percentage removed stayed approximately the same. During the initial 5 minutes of washing chromium was preferentially removed from the duodenum and jejunum rather than the ileum. However the difference was reduced after longer washing periods.

	WASHING PERIOD (Min.)			
	5	10	20	30
K.H.B.				
DUODENUM	1.9 (0.9)	1.8 (1.4)	8.2 (3.6)	16.9 (4.8)*
JEJUNUM	2.2 (1.0)	3.1 (1.4)	1.5 (1.5)	10.0 (5.5)*
ILEUM	1.1 (1.2)	0.6 (0.6)	3.7 (3.8)	4.8 (2.5)*
K.H.B. + E.D.T.A. (10^{-6} M)				
DUODENUM	10.8 (2.2)	12.8 (2.9)	12.0 (2.8)	14.4 (3.0)
JEJUNUM	9.8 (1.7)	9.1 (2.5)	11.5 (2.6)	9.3 (1.3)
ILEUM	5.9 (2.5)	7.0 (2.0)	10.0 (1.7)	7.8 (2.1)
K.H.B. + Cr^{3+} (10^{-6} M)				
DUODENUM	23.5 (3.3)	20.9 (3.2)	21.7 (4.3)	20.9 (2.5)
JEJUNUM	17.6 (1.6)	18.1 (1.8)	18.8 (2.8)	21.5 (2.4)
ILEUM	13.1 (1.1)	15.5 (1.7)	18.5 (1.4)	14.7 (1.5)

Table 3.11 Percentage of Cr^{3+} ($CrCl_3$, $6H_2O$) that was removed from the total intestinal tissue uptake, after different periods of washing the tissue in different washing mediums.
(S.E. is expressed in parenthesis)
(K.H.B. = Krebs-Henseleit bicarbonate buffer)

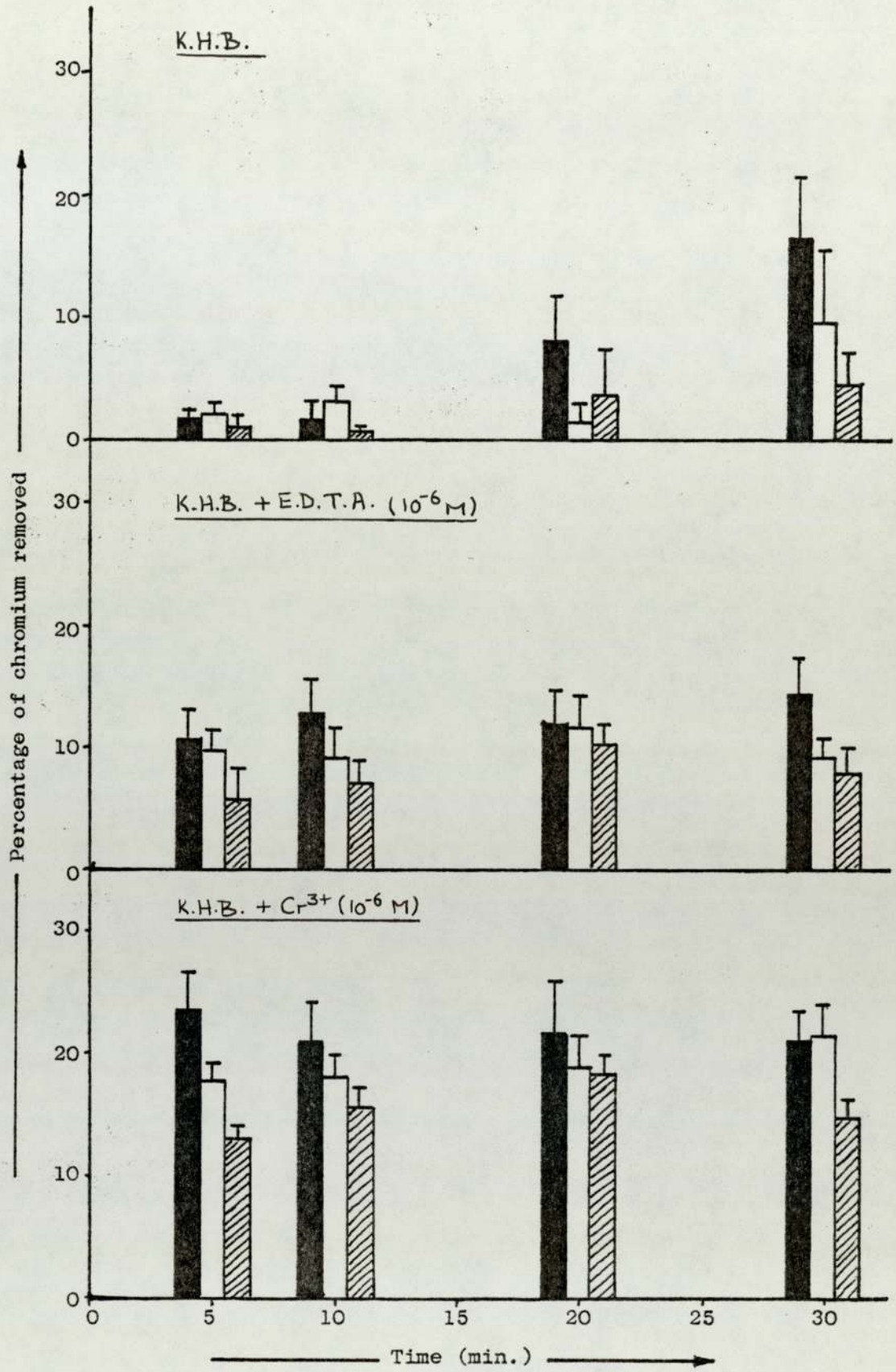


Figure 3.10 Percentage of chromium removed from the intestinal tissue after different periods of washing, using various washing media. (■) Duodenum, (□) Jejunum, (▨) Ileum Standard error of mean represented by vertical bars.

3.5 Effect of Mucosal Volume on the Transport of Cr^{3+} ions

Time based studies suggested that the tissue uptake of Cr^{3+} came to an equilibrium with the mucosal solution after approximately 30 minutes. In the previously reported studies 10 ml of Krebs-Henseleit bicarbonate buffer was used to incubate each section of the small intestine (approximately 500 mg in weight). Under normal physiological conditions the ratio of fluid inside the small intestine to tissue volume is much smaller. Therefore a series of experiments were carried out to investigate the effect of reduced mucosal volumes on tissue uptake of chromium as well as the transport of chromium across the intestinal barrier.

Mucosal volumes of 10, 5, 4, 3, 2, and 1 mls were used to incubate everted sacs, and assessments of chromium transport to the serosal compartment, tissue uptake of chromium, and water transport measurements were made as described earlier.

There was no significant difference in the amount of chromium transported to the serosal compartment across different regions of the intestine as mucosal volume decreased when expressed as ng.Cr/g. initial wet weight of tissue (Table 3.12). However if the amount of chromium is expressed in terms of the percentage of the available chromium in the initial mucosal volume, it can be seen that there was an increase in the amount of chromium transported to the serosal compartment as well as the amount taken up by the intestinal tissue, as the mucosal volume decreased. The same trend was observed for all three regions of the small intestine (Table 3.13).

It was not possible to measure the transport of chromium using smaller volumes than 1 ml. The ratio of mucosal fluid to

tissue, using the smallest experimental volume (1 ml) was still larger than the normal physiological condition. Therefore the increase in the percentage of chromium ions transported across the epithelium or taken up by tissue will be even more under normal physiological conditions. By extrapolation, the data suggests that under normal physiological conditions more than 95% of chromium could be bound to the intestinal tissue. The data also suggests that the amount of chromium appearing in the serosal compartment will be larger under normal physiological conditions.

MUCOSAL VOLUME (ml.)

	1	2	3	4	5	10
SEROSAL						
DUODENUM	0.9 (0.2)	0.9 (0.2)	1.2 (0.2)	1.1 (0.3)	0.9 (0.2)	1.3 (0.2)
JEJUNUM	1.0 (0.2)	1.0 (0.2)	1.3 (0.4)	1.5 (0.3)	0.8 (0.2)	1.4 (0.1)
ILEUM	0.6 (0.2)	0.7 (0.2)	1.3 (0.3)	0.9 (0.2)	0.5 (0.1)	0.7 (0.2)
TISSUE						
DUODENUM	26 (3)**	33 (4)**	36 (5)**	58 (10)*	60 (11)*	97 (8)
JEJUNUM	35 (4)**	46 (8)**	46 (6)**	60 (7)**	67 (13)**	114 (6)
ILEUM	34 (4)**	48 (8)**	65 (7)**	87 (7)**	73 (7)**	136 (23)

Table 3.12 Effect of the initial mucosal volume on transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) into the serosal compartment and the amount of chromium taken up by the intestinal tissue across different regions of the small intestine, expressed as ng. Cr/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

MUCOSAL VOLUME (ml.)

	1	2	3	4	5	10
SEROSAL						
DUODENUM	1.7 (0.4)	0.8 (0.2)	0.7 (0.4)*	0.5 (0.1)**	0.3 (0.1)**	0.2 (0.0)**
JEJUNUM	1.9 (0.4)	0.9 (0.2)	0.8 (0.5)*	0.7 (0.1)**	0.3 (0.1)**	0.2 (0.0)**
ILEUM	1.1 (0.4)	0.6 (0.2)	0.8 (0.5)*	0.4 (0.1)*	0.2 (0.0)**	0.1 (0.0)
TISSUE						
DUODENUM	48 (5.6)**	31 (3.7)**	22 (3.1)*	27 (4.7)	22 (4.1)	18 (1.5)
JEJUNUM	65 (7.5)**	43 (7.5)**	28 (3.7)*	28 (3.3)*	25 (4.9)	21 (1.1)
ILEUM	63 (7.5)**	45 (7.5)**	40 (4.4)**	40 (3.3)*	27 (2.6)	25 (4.3)

Table 3.13 Effect of the initial mucosal volume on transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) into the serosal compartment and the amount of chromium taken up by the intestinal tissue, across different regions of the small intestine, expressed as percentage of initial mucosal chromium.

3.6 The Effect of Altering the Energy Supply on the Transport of Chromium

If the transport mechanism for chromium across the small intestine is energy dependent, transport will be inhibited in the absence or reduction of energy supply. In a series of experiments the energy supply was altered by incubating everted sacs in either the absence of oxygen or in a reduced glucose media or at sub-optimum temperature, and the effect on chromium transport examined.

3.6.a The Effect of Different Concentrations of Glucose in the Bathing Media on the Transport of Cr^{3+}

The transport of 10^{-6} M chromium across the small intestinal wall and the uptake of it by the intestinal tissue was measured after either 30 or 60 minutes incubation in Krebs-Henseleit bicarbonate buffer containing a range of glucose concentration 0 - 20 mM (the usual amount of glucose in the buffer in previous control experiments was 20 mM).

When the preparations were incubated for up to 60 minutes with different glucose concentrations there was no effect on chromium transport to the serosal compartment for all regions of the intestine investigated. There was however a significant decrease ($p < 0.001$) in the amount of chromium taken up by the tissue after 60 minutes in the absence of glucose concentration by all three regions (Table 3.15). The total water transported to the serosal compartment and that taken up by the tissue was unaffected by the concentration changes of glucose in the bathing media (Table 3.16). However there was generally less movement of water in the absence of glucose.

	GLUCOSE CONCENTRATION (mM.)			
	0.0	5.0	10.0	20.0
SEROSAL				
DUODENUM	1.7 (0.2)	3.1 (0.8)	2.0 (0.5)	2.3 (0.4)
JEJUNUM	1.6 (0.3)	3.2 (0.4)	L.5 (0.4)	2.2 (0.3)
ILEUM	1.2 (0.2)	1.3 (0.1)	1.5 (0.5)	1.3 (0.2)
TISSUE				
DUODENUM	92 (12)	88 (7)	73 (5)*	99 (14)
JEJUNUM	107 (12)	108 (18)	77 (5)	118 (12)
ILEUM	116 (10)*	168 (18)	123 (12)	145 (8)

Table 3.14 Effect of different concentrations of glucose in bathing medium on the transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$), across the intestinal wall and uptake of it by the intestinal tissue in different regions of the small intestine after 30 minutes incubation at 37°C, expressed as ng. Cr/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

	GLUCOSE CONCENTRATION (mM.)			
	0.0	5.0	10.0	20.0
SEROSAL				
DUODENUM	3.4 (0.3)	1.9 (0.2)	2.2 (0.1)	2.4 (0.5)
JEJUNUM	3.5 (0.1)	3.0 (0.6)	3.1 (0.7)	2.9 (0.4)
ILEUM	2.2 (0.3)	2.5 (0.4)	2.4 (0.3)	3.3 (0.6)
TISSUE				
DUODENUM	107 (9)**	147 (17)*	98 (10)**	225 (27)
JEJUNUM	111 (7)**	146 (18)	111 (6)**	189 (17)
ILEUM	131 (10)	168 (27)	176 (19)	161 (25)

Table 3.15 Effect of different concentrations of glucose in bathing medium on the transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$), across the intestinal wall and uptake of it by the intestinal tissue in different regions of the small intestine after 60 minutes incubation at 37°C, expressed as ng. Cr/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

	GLUCOSE CONCENTRATION (mM.)			
	0.0	5.0	10.0	20.0
SEROSAL				
DUODENUM	9 (24)	99 (121)	-15 (17)	17 (57)
JEJUNUM	114 (61)	486 (72)	121 (31)	276 (40)
ILEUM	59 (20)	281 (35)	114 (34)	215 (53)
TISSUE				
DUODENUM	385 (71)	350 (42)	448 (17)	434 (16)
JEJUNUM	448 (27)	433 (51)	438 (34)	391 (45)
ILEUM	371 (32)	449 (77)	353 (44)	348 (25)
TOTAL				
DUODENUM	394 (51)	449 (96)	433 (26)	451 (52)
JEJUNUM	562 (62)	919 (83)	559 (40)	667 (50)
ILEUM	430 (38)	730 (38)	467 (29)	563 (67)

Table 3.16 Transport of water in the presence of different concentrations of glucose after 30 minutes incubation at 37°C, expressed as mg. water/g. initial wet weight of tissue.
(S.E. is expressed in parentheses)

3.6.b The Effect of Sub-optimum Temperature on the Transport of 10^{-6} M Cr^{3+}

The transport of 10^{-6} M chromium chloride across the small intestinal wall and also the uptake of it by the intestinal tissue was measured over a 30 minute incubation period in Krebs-Henseleit bicarbonate buffer at either 27°C or 37°C (as described in Section 2).

As illustrated in Table 3.17, there was no significant effect of temperature on the amount of chromium either transported to the serosal compartment or taken up by the tissue by all three regions of the small intestine. However there was a significant ($p < 0.001$) reduction of water movement when a sub-optimum temperature of 27°C was utilized (Table 3.18).

3.6.c The Effect of Anoxia on the Transport of Cr^{3+}

The effect of anoxia on the transport of the chromium ion into the serosal compartment and that taken up by the intestinal tissue was measured. For the control experiments the bathing medium was continuously gassed by a mixture of 95% O_2 and 5% CO_2 (as described in Section 2.4). For the anoxic experiments a mixture of 95% N_2 and 5% CO_2 was used. All other conditions were the same as for the control experiments.

There was no difference in the amount of chromium transported to the serosal compartment after 30 minutes incubation using either nitrogen or oxygen gassing (Table 3.19). On the other hand the amount of chromium taken up by the intestinal tissue was significantly reduced ($p < 0.01$) in the absence of oxygen by all

three different regions of the rat small intestine (Table 3.19). Total water movement was also reduced significantly in the absence of oxygen in all regions of the small intestine ($p < 0.05$), (Table 3.20).

	TEMPERATURE OF INCUBATION		Q_{10} VALUE
	37°C	27°C	
SEROSAL			
DUODENUM	1.6 (0.4)	1.4 (0.1)	1.14
JEJUNUM	1.5 (0.4)	1.4 (0.2)	1.07
ILEUM	0.9 (0.1)	0.8 (0.1)	1.12
TISSUE			
DUODENUM	62 (5)	78 (12)	0.79
JEJUNUM	82 (7)	73 (8)	1.11
ILEUM	100 (7)	78 (5)	1.28

Table 3.17 Effect of sub-optimum temperature on transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) to the serosal compartment and uptake of it by intestinal tissue, across different regions of the small intestine after 30 minutes incubation, expressed as ng. Cr/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

	TEMPERATURE OF INCUBATION	
	37°C	27°C
SEROSAL		
DUODENUM	-98 (40)	-35 (21)
JEJUNUM	446 (53)	191 (38)**
ILEUM	246 (29)	39 (20)**
TISSUE		
DUODENUM	472 (59)	267 (12)*
JEJUNUM	494 (49)	257 (42)**
ILEUM	361 (25)	168 (25)**
TOTAL		
DUODENUM	374 (52)	232 (20)*
JEJUNUM	940 (86)	448 (30)**
ILEUM	607 (37)	238 (28)**

Table 3.18 Effect of sub-optimum temperature on movement of water to the serosal compartment and into the tissue after 30 minutes incubation expressed as mg. water/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

	GASSING MIXTURE	
	CO_2/O_2	CO_2/N_2
SEROSAL		
DUODENUM	1.7 (0.4)	1.9 (0.4)
JEJUNUM	1.8 (0.2)	1.6 (0.3)
ILEUM	1.7 (0.2)	1.5 (0.4)
TISSUE		
DUODENUM	123 (8)	73 (13)**
JEJUNUM	135 (14)	62 (10)**
ILEUM	180 (26)	78 (18)**

Table 3.19 Effect of anoxia on the serosal transport and the tissue uptake of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) after 30 minutes incubation at 37°C.
(S.E. is expressed in parenthesis)

	GASSING MIXTURE	
	CO_2/O_2	CO_2/N_2
SEROSAL		
DUODENUM	63 (40)	-52 (56)
JEJUNUM	452 (86)	99 (25)**
ILEUM	266 (65)	22 (15)**
TISSUE		
DUODENUM	322 (34)	322 (105)
JEJUNUM	386 (54)	490 (36)
ILEUM	295 (26)	246 (26)
TOTAL		
DUODENUM	385 (44)	270 (74)
JEJUNUM	838 (127)	589 (42)
ILEUM	561 (57)	268 (17)**

Table 3.20 Effect of anoxia on water movement to serosal compartment and into the intestinal tissue after 30 minutes incubation at 37°C, expressed as mg. water/g. initial wet weight of tissue. (S.E. is expressed in parenthesis)

3.7 Discussion

The *in vitro* everted sac preparation has been used to obtain the kinetic data reported in this chapter. The kinetic data can be utilized to develop a model which describes the transport of chromium across the small intestine. However when interpreting the kinetic data, it is necessary to recognise the difference between *in vitro* experimental conditions and the normal physiological conditions.

Under normal physiological conditions a substance has only the epithelial barrier to negotiate before passing into the blood or lymphatic system. On the other hand in the *in vitro* everted sac preparation substances absorbed at the mucosal surface have to pass through submucosal and smooth muscle layers before reaching the serosal fluid. Another important difference between physiological conditions and those that exist during *in vitro* techniques is that under normal conditions, when substances pass the epithelia they enter the capillaries and are rapidly carried out so that no concentration gradient builds up. In *in vitro* everted sac preparations relatively large concentrations of absorbed substances may build up in the small volume of serosal fluid; consequently the transport process probably proceeds under less favourable conditions.

The transport of chromium ions into the serosal compartment was very slow, across all regions of the small intestine investigated. There was no significant difference in the amount transported across different regions which suggests there is no

specific site for chromium absorption in the rat intestinal tract.

There was a linear transport of chromium ions to the serosal compartment with time within the concentration range 5×10^{-7} M to 5×10^{-5} M. This observation together with the fact that the amount transported to the serosal compartment is dependent upon the initial concentration of chromium in the bathing medium, and that the alteration of energy supply had no affect on the transport of chromium to the serosal compartment, suggests that the transport proceeds by passive diffusion.

There was a reasonable correlation between water transport and chromium transport to the serosal compartment. The correlation was best across the jejunal-ileal region ($r = 0.94$).

In contrast to the serosal appearance of chromium, the uptake of chromium by the intestinal tissue was comparatively large. The rate of uptake by the tissue was extremely rapid during the first 10 minutes of incubation. Indeed there appears to be good evidence for a "plateau" in the tissue uptake of chromium between 10 and 50 minutes. The 60 minute observation (Figure 3.2) does not appear to fit the plateau, however inspection of the results obtained at other mucosal concentrations (Table 3.7 to 3.8) indicates that the percentage of chromium taken up by the intestinal tissue after 60 minutes incubation is approximately the same as that after 30 minutes incubation. Irregularity in the plateau effect may be due to the uptake of water by the intestinal tissue (see Table 3.3 and 3.4). It would appear that when the tissue takes up a greater amount of water than normal, it also takes up more chromium. Whether chromium uptake is directly related to the extra water movement, or to a subsequent increase in the number of available surface reaction

sites is not clear. However this large and rapid uptake of chromium together with the fact that there is a very poor correlation between water movement into the tissue and chromium uptake by the tissue, indicates that the interaction of the chromium ion with the intestinal tissue is probably a surface adsorption phenomenum.

Reduction of the mucosal volume increased the amount of chromium taken up by the intestinal tissue. Under normal physiological conditions the uptake of chromium is probably far greater than has been reported in these <u>in vitro</u> studies. In the experimental system 10 ml of fluid was used to incubate approximately 0.5 g. of tissue, (a volume to weight ratio of 20). However under normal physiological conditions the ratio is much smaller as the luminal volume is approximately 500 ml and intestinal tissue weight approximately 1650 g. (a ratio of 0.3). An equilibration may occur between the amount of chromium taken up by the tissue and the amount of chromium in the mucosal fluid. One may describe an equilibration constant for the system as

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

Where K = equilibration constant

X = percentage of chromium taken up by the tissue

M = mass of tissue

$100 - X$ = percentage of chromium remaining in the mucosal fluid

V = volume of mucosal fluid

If $\bar{V}$ represents the ratio of V/M (V in cm^3 and M in g.) then

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

The equilibration relationship therefore suggests that as the volume is reduced and consequently $\bar{V}$ is reduced, the percentage of chromium bound to the tissue increases. This in fact is what was observed experimentally. The following value for K can be derived by substituting experimental observation values into the equilibration equation.

$$K_{Duodenum} = 4.39 \pm 0.30$$

$$K_{Jejunum} = 5.32 \pm 0.22$$

$$K_{Ileum} = 6.67 \pm 0.90$$

Using the derived value for K, it was found that the amount of chromium bound to the tissue under normal physiological conditions is probably greater than 95%.

The fact that more than 95% of chromium is taken up by the intestinal tissue is probably the main reason why so very little chromium is absorbed into the body cavity. Indeed the chromium-tissue interaction possibly is the most important factor in controlling the amount of chromium absorbed.

The washing experiments revealed that the exchange of chromium with all three different washing procedures was slow and incomplete, which suggests that chromium is tenaciously bound to the intestinal tissue. The fact that washing the tissue with a solution containing 10^{-6} M Cr^{3+} only removed 27% of the interacted

chromium, indicates that the interaction is mainly in the form of covalent bonding.

The reduced incubation temperature did not affect the amount of chromium taken up by the tissue. On the other hand there was a significant reduction of intestinal tissue uptake of chromium in the absence of oxygen or when glucose concentrations were reduced. As the supply of both glucose and oxygen affect the production of A.T.P., these observations indicate that the chromium ions may be bound to phosphate groups on the intestinal tissue. Therefore any factors which interfere with the production of A.T.P. or inhibit A.T.P.ases will subsequently decrease the amount of chromium bound to the intestinal tissue.

CHAPTER 4

THE ROUTE OF CHROMIUM TRANSPORT ACROSS THE INTESTINAL MEMBRANE

4.1 Introduction

Experiments reported in Chapter 3 suggest that chromium ions are transported across the gastrointestinal tract by passive diffusion. There are two ways that a cation may cross the intestinal epithelium;

1) An intracellular or transcellular route whereby the ion has to cross two cell membranes.
2) An extracellular pathway situated at the Zonula Occludens, (19), (61).

Barry, Diamond and Wright (5) have demonstrated that changes in hydrogen and calcium ion concentration are two of the parameters that will alter the integrity of the extracellular route. Therefore experiments were undertaken to find out the possible route chromium cation utilises to cross the intestinal barrier, and understand the influence of hydrogen and calcium ion concentration on chromium transport.

4.2 The Effect of Different Concentrations of Calcium Ions on the Transport of Chromium across the Small Intestine

Experiments were carried out using everted sacs prepared from different regions of the small intestine as described in Section 2.3. The prepared sacs were incubated at 37°C for 30 minutes in 10 ml of Krebs-Henseleit bicarbonate buffer, containing different concentrations of Ca^{2+} (0.0 mM, 0.6 mM, 1.2 mM, 2.4 mM and 4.8 mM) and 10^{-6} M Cr^{3+} and ^{51}Cr as tracer. After incubation the amount of chromium transported to the serosal compartment and taken up by the

intestinal tissue was assessed as described in Section 2.8.

In the presence of reduced calcium concentrations the transport of the chromium into the serosal compartment was increased for all regions of the small intestine. The increase became statistically significant ($p<0.05$) compared to the control value ($[Ca^{2+}]$ = 2.4 mM), in the total absence of calcium ions (Table 4.1, Figure 4.1). Water transport was also increased with reduced calcium ion concentration, although the increase was not significant (Table 4.2). The amount of chromium taken up by the intestinal tissue decreased as the calcium ion concentration decreased (Table 4.1, Figure 4.2). Uptake of water by the intestinal tissue was not affected by changes in the calcium ion concentration (Table 4.2).

4.3 The Effect of Different Concentrations of Hydrogen Ions on the Transport of Chromium across the Small Intestine

Everted sacs from the different regions of the small intestine were prepared as described previously. The sacs were incubated for 30 minutes at 37°C in Krebs-Henseleit bicarbonate buffer, containing 10^{-6} M chromium ions and ^{51}Cr as tracer. The pH of the buffer was adjusted to either 2.4, 3.4, 4.4, 5.4, 6.4, 7.4, 8.4, 9.4, or 10.4, by the addition of either hydrochloric acid or sodium hydroxide. The transport of chromium ions to the serosal compartment and the amount taken up by the intestinal tissue was assessed as described in Chapter 2.

There was a gradual decrease in the amount of chromium

transported to the serosal compartment as the pH of the mucosal bathing medium increased from 2.4 to 10.4 (Table 4.3, Figures (4.3), (4.4) and (4.5). The difference was significant for all regions of the intestine ($p < 0.05$) compared to the control value of pH = 7.4, at extreme acid pH. The amount of chromium taken up by the intestinal tissue increased as the pH increased from 2.4 to 10.4, and also became statistically significant compared with control value of pH = 7.4 at pH of 2.4 ($p < 0.02$) (Table 4.4, Figures (4.6), (4.7) and (4.8). Water transport to the serosal spaces was minimal at extreme pH values (Table 4.5). The uptake of water by the tissue was unaffected by changes in pH (Table 4.6).

CALCIUM CONCENTRATION

	0.0 mM	0.6 mM	1.2 mM	2.4 mM	4.8 mM
SEROSAL					
DUODENUM	4.0 (0.6)*	3.2 (0.4)	2.8 (0.6)	2.7 (0.3)	1.4 (0.1)**
JEJUNUM	5.2 (0.7)*	4.0 (0.6)	3.7 (0.8)	2.9 (0.2)	1.6 (0.4)*
ILEUM	3.0 (0.8)	2.5 (0.8)	2.3 (0.9)	1.5 (0.2)	1.4 (0.4)
TISSUE					
DUODENUM	73 (18)	104 (22)	111 (19)	78 (14)	147 (33)
JEJUNUM	66 (8)	107 (15)	164 (29)	103 (22)	176 (23)*
ILEUM	99 (26)	96 (12)	180 (25)	102 (19)	137 (24)*

Table 4.1 Transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) to the serosal compartment and uptake of it by the intestinal tissue after 30 minutes incubation in the presence of different calcium concentrations at 37°C. The amount of chromium is expressed as ng. Cr/g. wet weight of tissue. (S.E. is expressed in parenthesis)

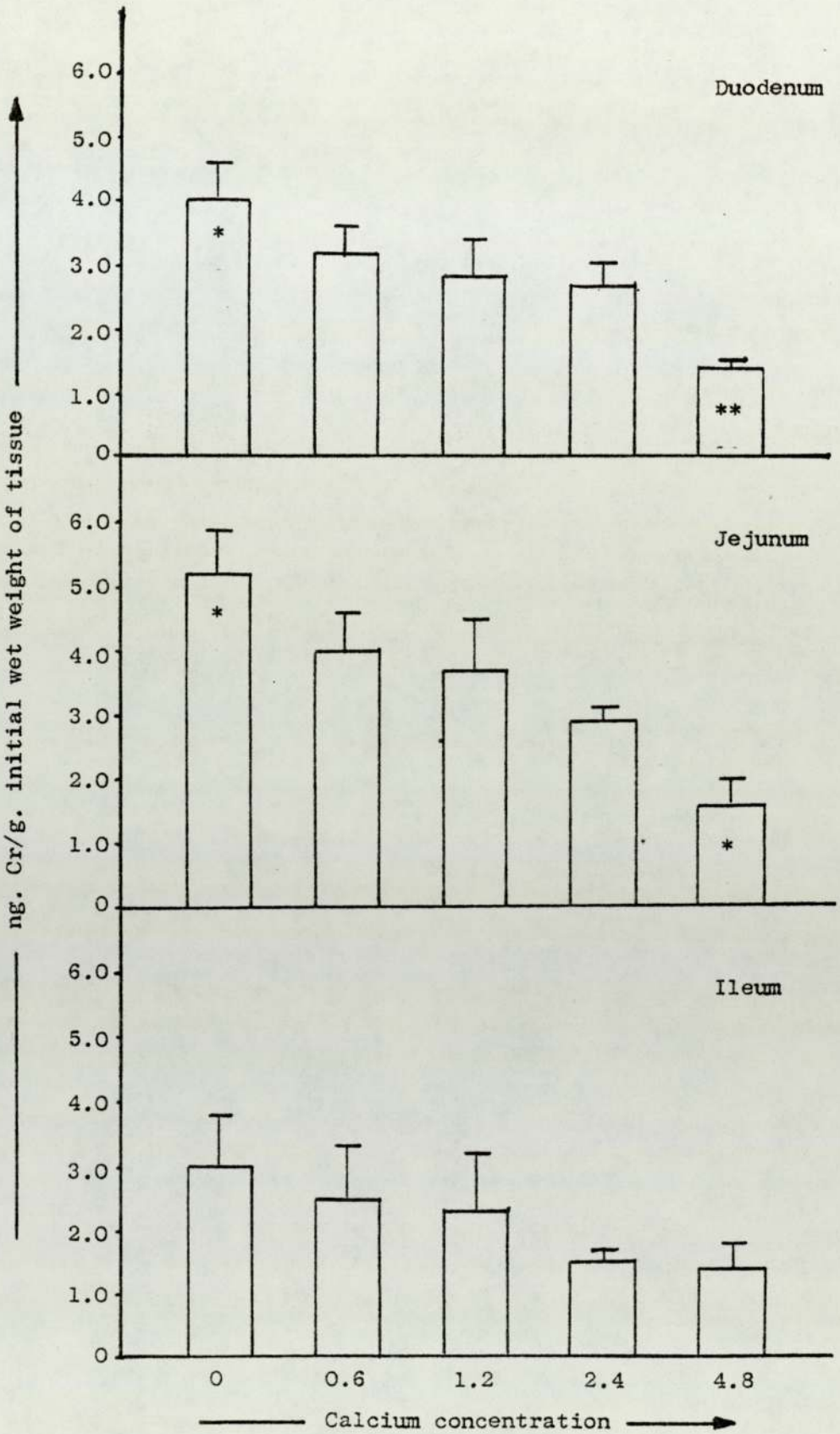


Figure 4.1 The effect of different concentrations of calcium ions on the transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) to the serosal compartment after 30 minutes incubation at 37°C. Standard error of mean represented by vertical bars.

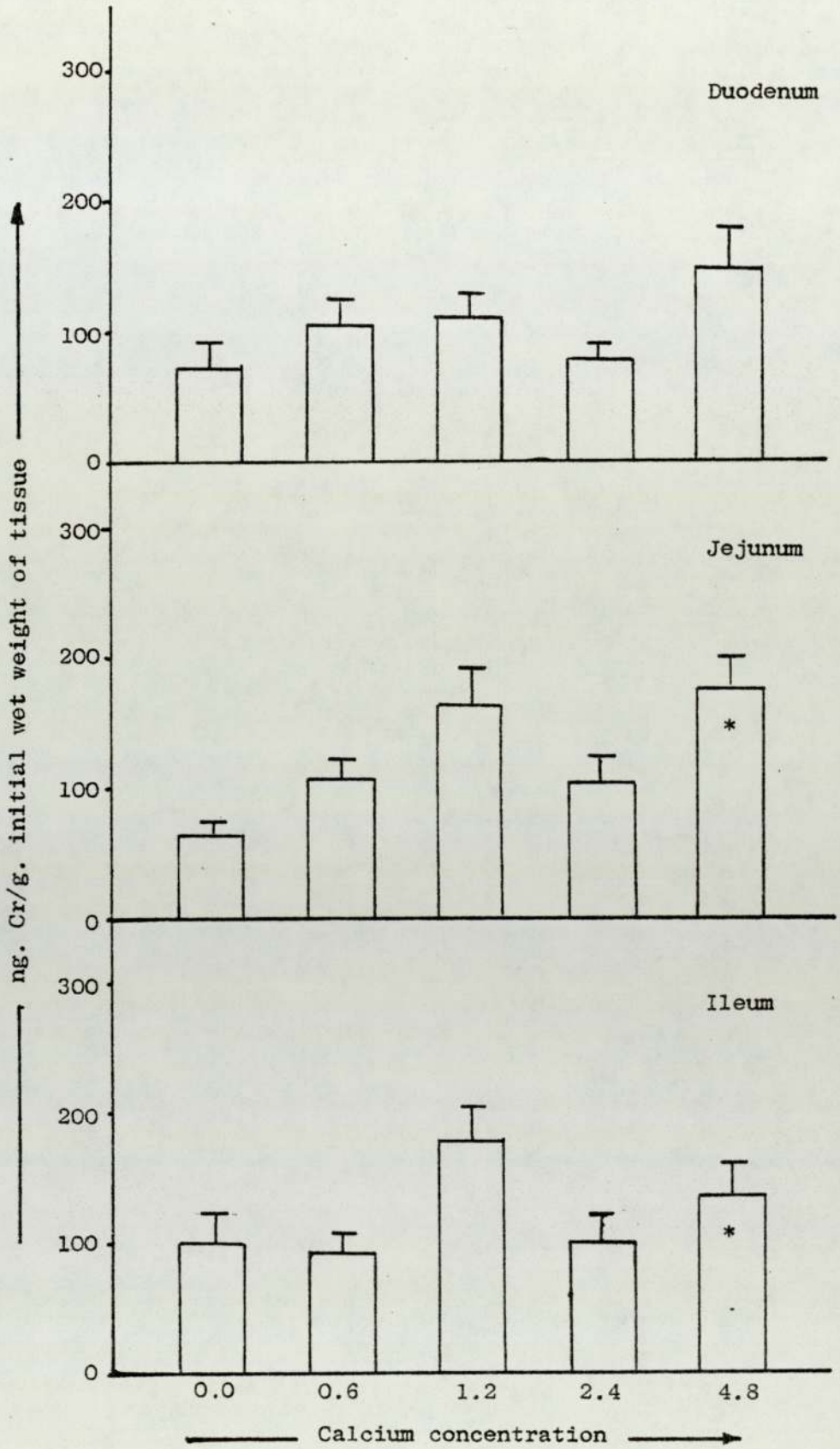


Figure 4.2 The effect of different concentrations of calcium ions on the amount of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) taken up by the intestinal tissue after 30 minutes incubation at 37°C.
Standard error of mean represented by vertical bars.

CALCIUM CONCENTRATION

	0.0 mM	0.6 mM	1.2 mM	2.4 mM	4.8 mM
SEROSAL					
DUODENUM	9 (26)	15 (23)	9 (27)	-43 (35)	16 (13)
JEJUNUM	439 (80)	459 (112)	533 (74)	328 (98)	212 (106)
ILEUM	254 (51)	189 (48)	287 (56)	170 (71)	115 (63)
TISSUE					
DUODENUM	429 (72)	525 (49)	551 (57)	630 (37)	487 (46)
JEJUNUM	415 (39)	603 (56)	468 (54)	647 (58)	601 (72)
ILEUM	428 (47)	444 (56)	432 (52)	477 (39)	526 (87)
TOTAL					
DUODENUM	438 (66)	540 (51)	560 (57)	587 (39)	503 (33)
JEJUNUM	854 (81)	1062 (146)	1001 (111)	975 (146)	813 (83)
ILEUM	682 (65)	633 (83)	719 (98)	647 (102)	641 (118)

Table 4.2 Water movement across the different regions of the small intestine and uptake by the tissue, in the presence of different calcium concentrations after 30 minutes incubation at 37°C, expressed as mg. water/g. initial wet weight of tissue.
(S.E. is expressed as parenthesis)

pH VALUE	DUODENUM	JEJUNUM	ILEUM
2.4	5.6 (0.6)*	5.4 (0.8)**	6.7 (0.4)**
3.4	3.1 (0.4)	2.5 (0.2)*	1.8 (0.2)*
4.4	3.6 (0.7)	4.5 (0.6)**	1.8 (0.2)*
5.4	2.1 (0.3)	3.2 (0.5)*	1.8 (0.3)*
6.4	2.2 (0.3)	3.5 (0.5)*	2.2 (0.4)*
7.4	2.4 (0.8)	1.5 (0.3)	1.0 (0.1)
8.4	1.1 (0.2)	1.0 (0.2)	0.9 (0.3)
9.4	1.9 (0.5)	1.1 (0.3)	1.4 (0.5)
10.4	2.0 (0.6)	0.9 (0.0)	0.9 (0.1)

Table 4.3 The effect of pH on transport of Cr^{3+} ($CrCl_3$, $6H_2O$) to the serosal compartment, after 30 minutes incubation at 37°C. The amount of chromium is expressed as ng. Cr/ g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

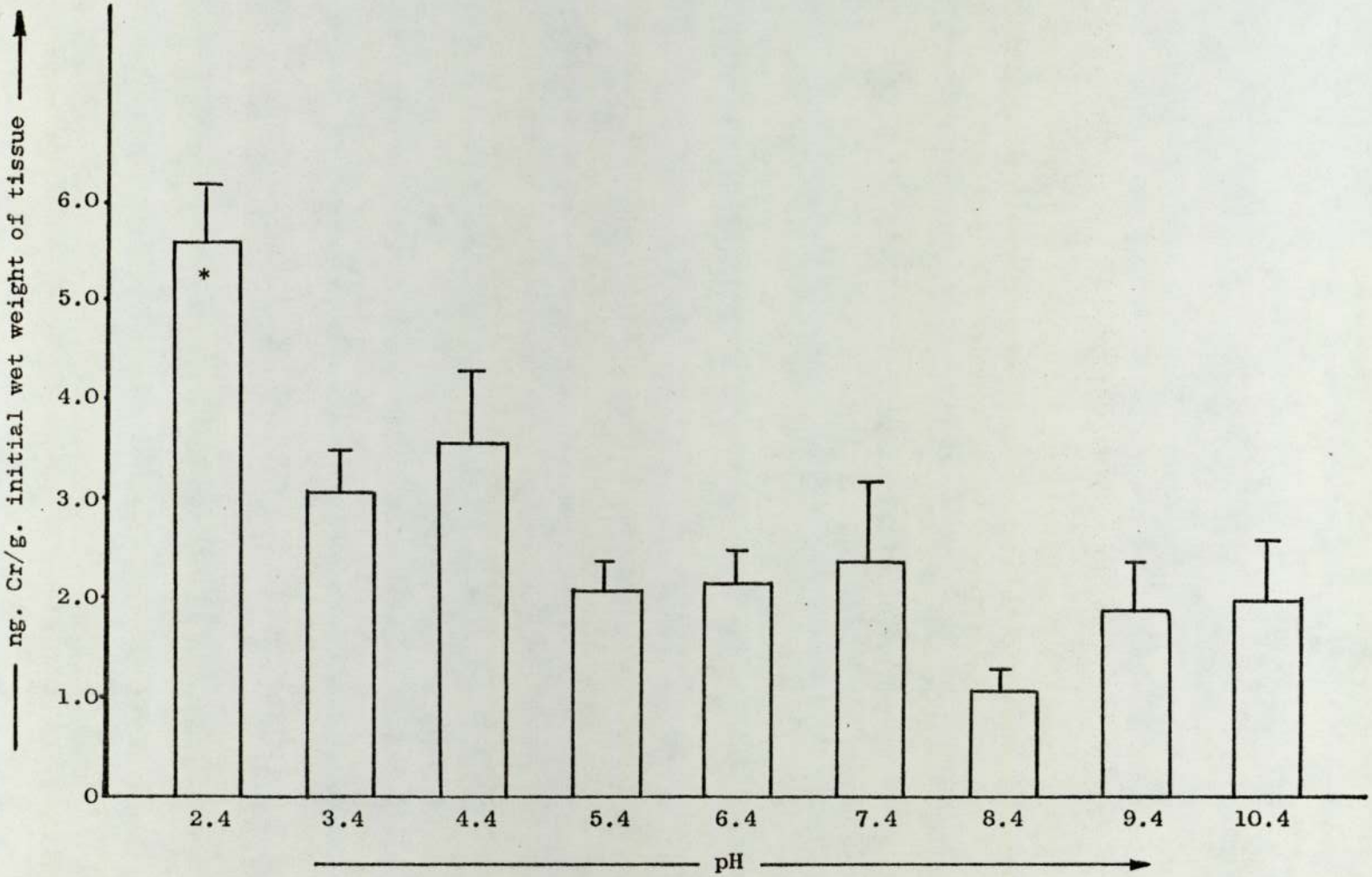


Figure 4.3 The effect of different concentrations of hydrogen ions on transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) to the duodenal serosal compartment after 30 minutes incubation at 37°C.
Standard error of mean represented by vertical bars.

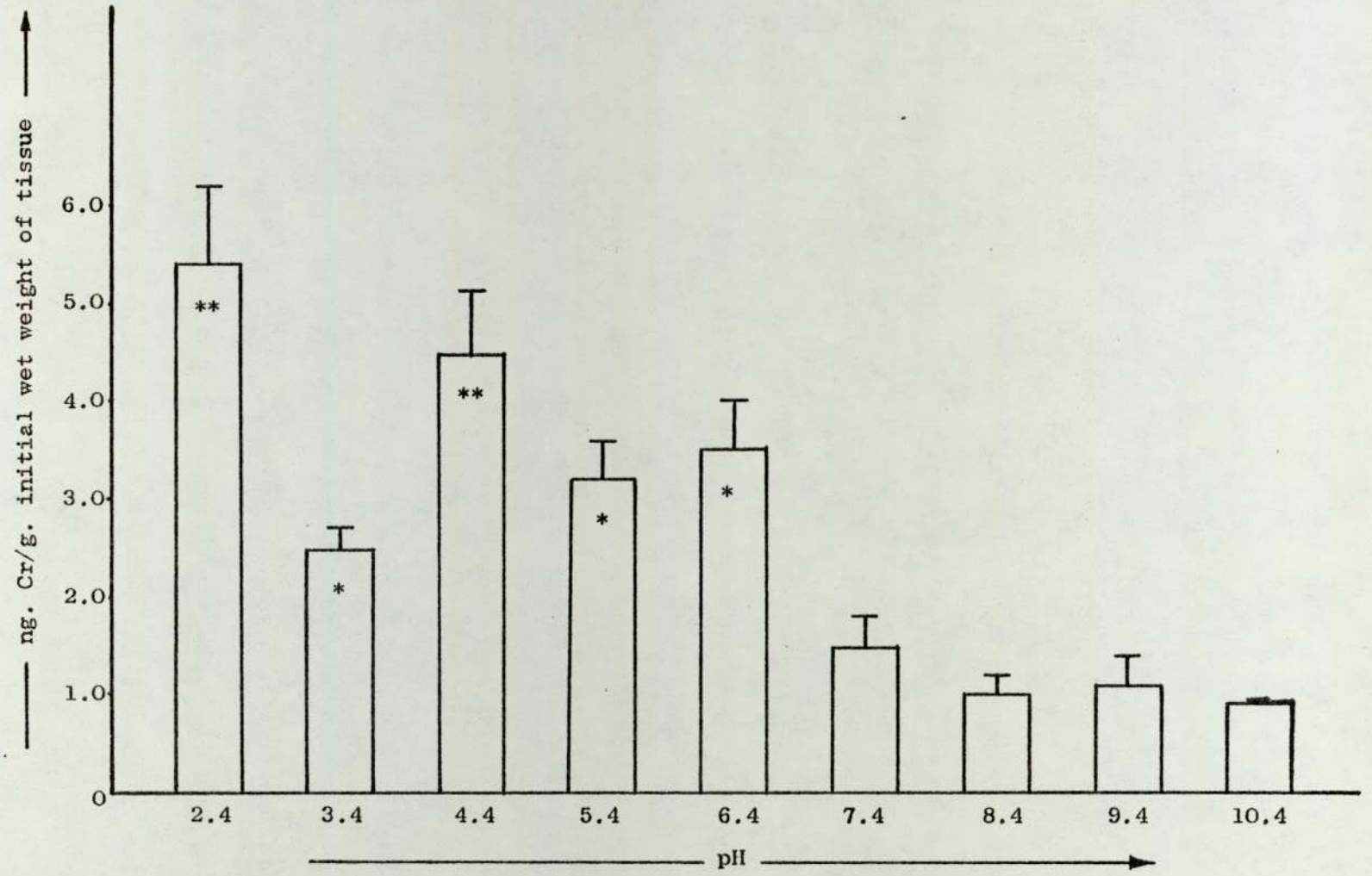


Figure 4.4 The effect of different concentrations of hydrogen ions on transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) to the jejunal serosal compartment after 30 minutes incubation at 37°C.
Standard error of mean represented by vertical bars.

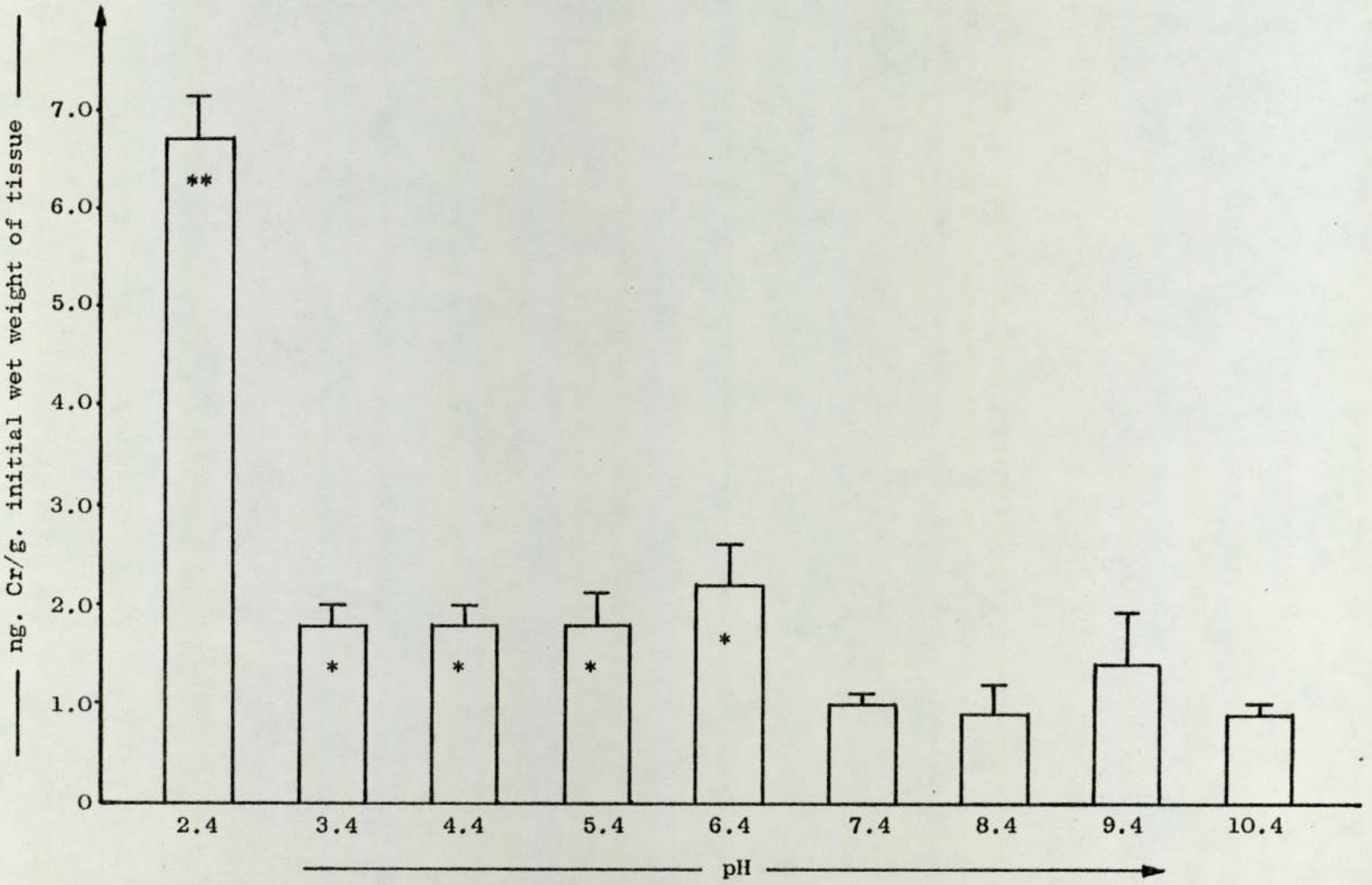


Figure 4.5 The effect of different concentrations of hydrogen ions on transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) to the ileal serosal compartment after 30 minutes incubation at 37°C.
Standard error of mean represented by vertical bars.

pH VALUE	DUODENUM	JEJUNUM	ILEUM
2.4	65 (5)*	61 (5)*	59 (3)*
3.4	179 (15)	135 (12)	181 (17)
4.4	100 (4)	127 (6)	136 (24)
5.4	96 (11)	114 (8)	157 (35)
6.4	111 (8)	150 (12)	212 (17)
7.4	124 (23)	136 (20)	144 (23)
8.4	354 (50)**	383 (76)*	344 (24)**
9.4	442 (39)**	561 (86)**	613 (51)**
10.4	343 (58)**	532 (67)**	633 (83)**

Table 4.4 The effect of pH on the amount of Cr^{3+} ($CrCl_3,6H_2O$) taken up by the intestinal tissue, after 30 minutes incubation at 37°C. The amount of chromium is expressed as ng. Cr/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

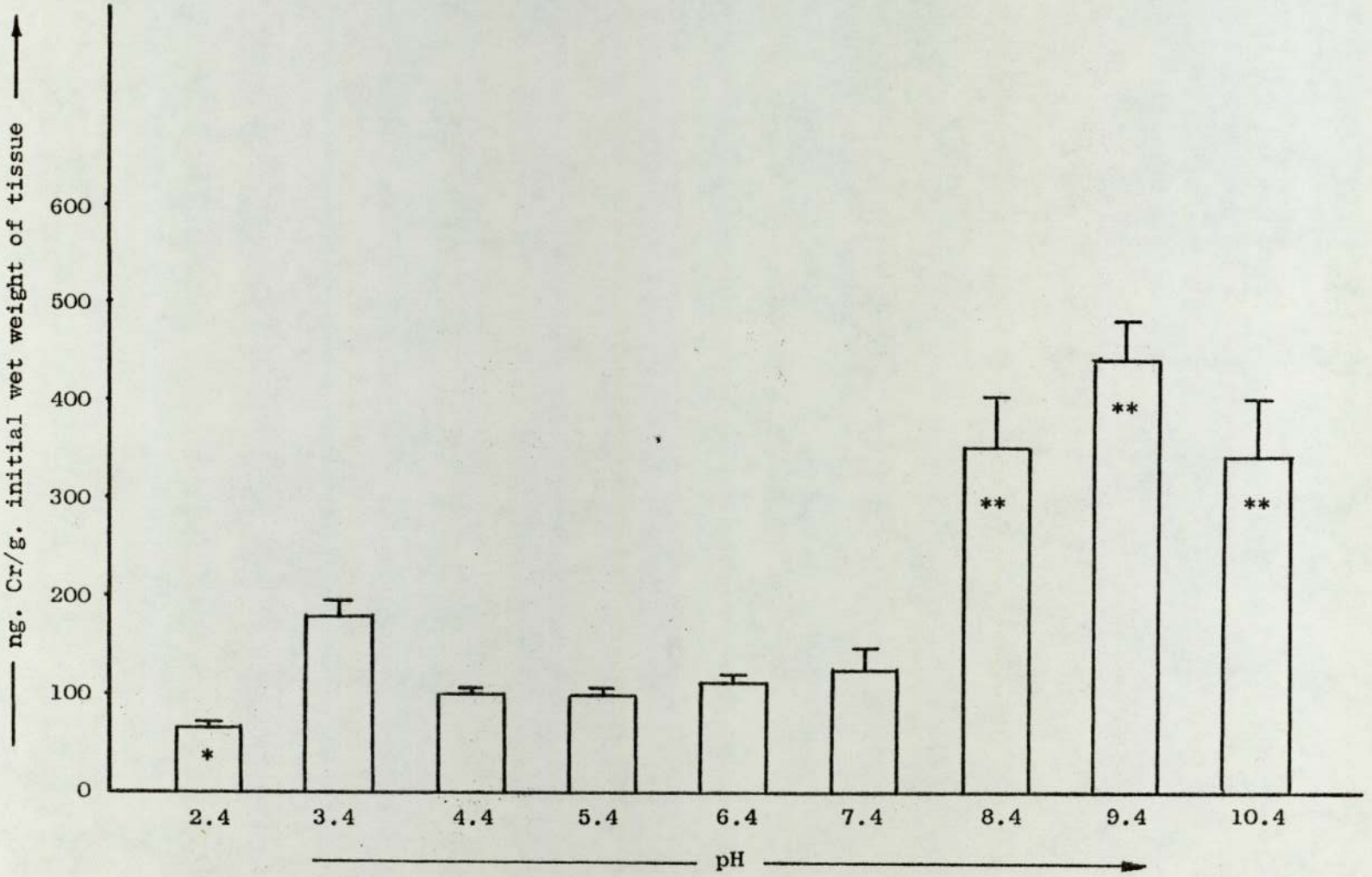


Figure 4.6 The effect of different concentrations of hydrogen ions on the amount of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) taken up by the duodenal tissue after 30 minutes incubation at 37°C.
Standard error of mean represented by vertical bars.

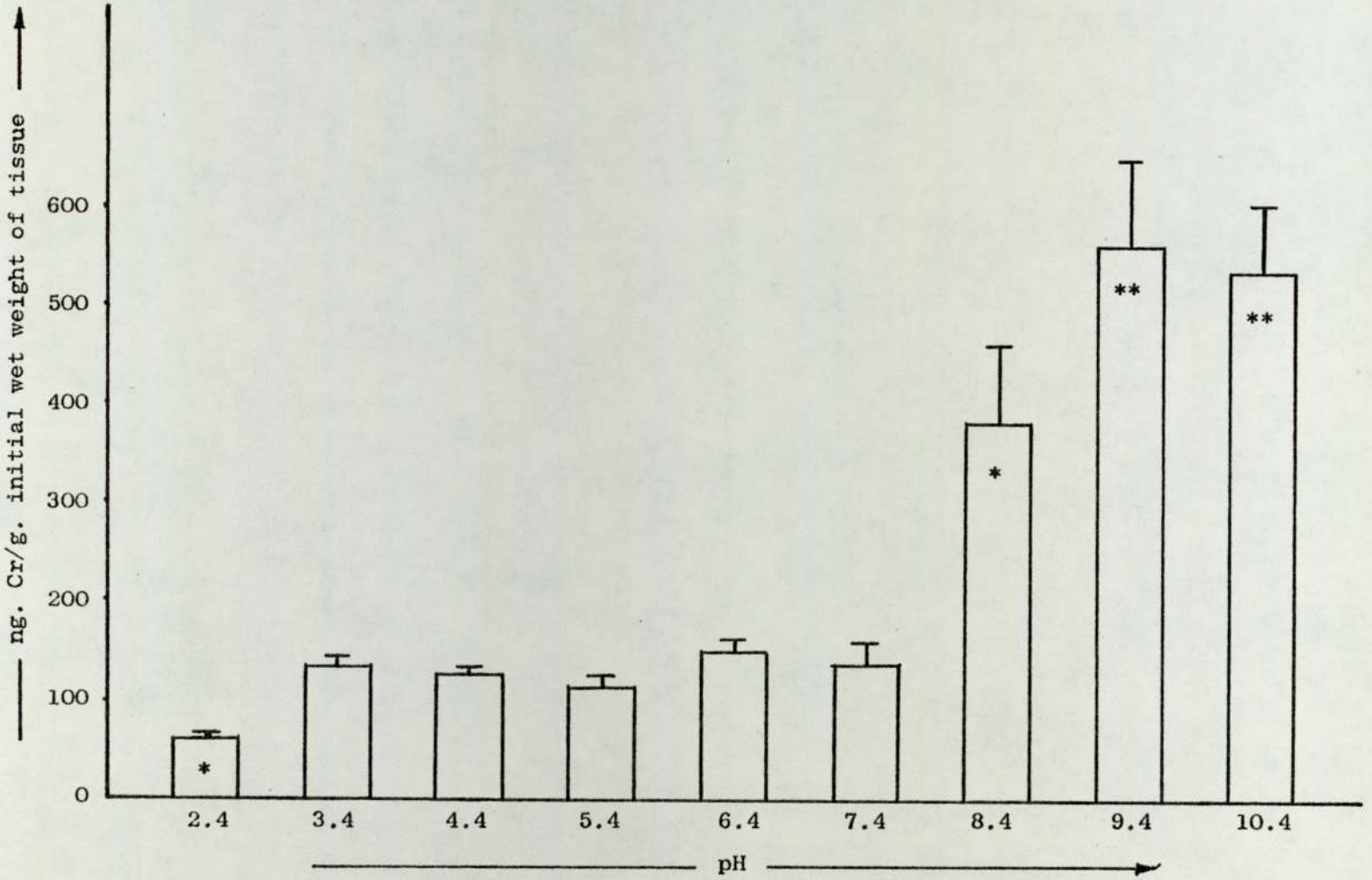


Figure 4.7 The effect of different concentrations of hydrogen ions on the amount of 10^{-6} M Cr^{3+} ($CrCl_3, 6H_2O$) taken up by the jejunal tissue after 30 minutes incubation at 37°C.
Standard error of mean represented by vertical bars.

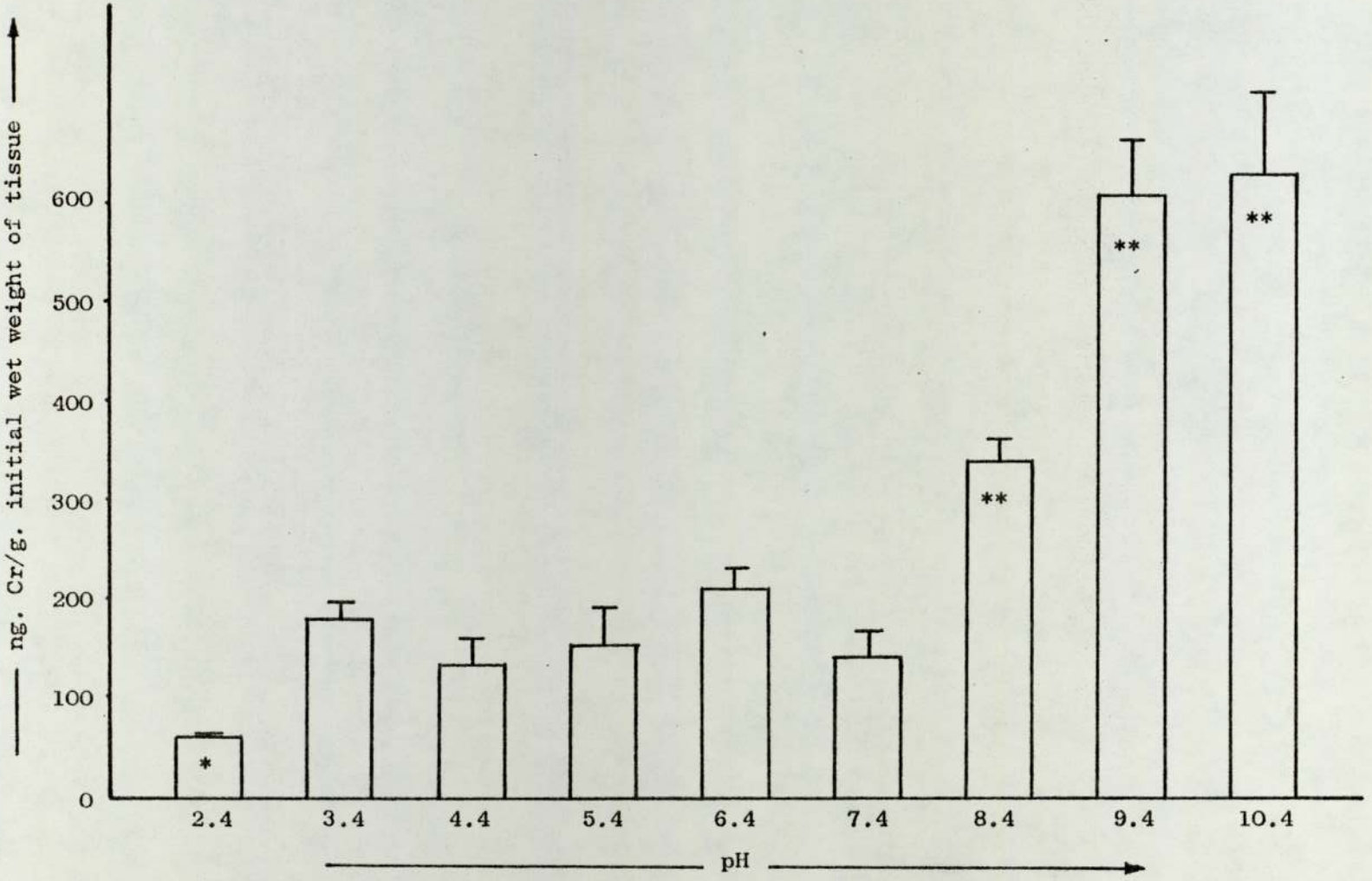


Figure 4.8 The effect of different concentrations of hydrogen ions on the amount of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) taken up by the ileal tissue after 30 minutes incubation at 37°C.
Standard error of mean represented by vertical bars.

pH VALUE	DUODENUM	JEJUNUM	ILEUM
2.4	-178 (63)*	-112 (24)**	-11 (42)**
3.4	-44 (34)	132 (23)*	84 (11)**
4.4	0 (26)	256 (38)	160 (29)*
5.4	-47 (33)	291 (59)	216 (21)
6.4	-39 (23)	453 (20)	448 (28)*
7.4	42 (35)	438 (96)	284 (50)
8.4	58 (44)	456 (66)	165 (44)
9.4	-22 (30)	389 (75)	132 (43)*
10.4	-87 (34)*	327 (94)	87 (23)

Table 4.5 The effect of pH on transport of water to the serosal compartment, after 30 minutes incubation at 37°C. The amount of water is expressed as mg. water/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

pH VALUE	DUODENUM	JEJUNUM	ILEUM
2.4	478 (24)	506 (31)	360 (46)
3.4	441 (33)	359 (29)**	285 (31)
4.4	417 (26)	371 (69)*	326 (40)
5.4	458 (35)	452 (42)	295 (15)
6.4	453 (20)	527 (38)	404 (31)
7.4	445 (69)	534 (37)	361 (45)
8.4	361 (38)	455 (45)	369 (60)
9.4	383 (41)	403 (53)*	369 (66)
10.4	302 (41)	340 (34)**	277 (12)

Table 4.6 The effect of pH on the movement of water to the intestinal tissue after 30 minutes incubation at 37°C. The amount of water is expressed as mg. water/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

4.4 Discussion

Some epithelia, such as the urinary bladder display a high transmural resistance, and their extracellular junctions are designated as "Tight". There are other epithelia such as gall bladder and intestinal epithelia which are relatively "Leaky epithelia" because they possess low resistance transepithelial extracellular pathways (84), (25), (71). These pathways (Zonulae Occludens) are formed between adjacent cells and are 10 - 16 Å in diameter. They maintain electroneutrality and are cation selective (84), (71). In freeze etching studies of some epithelia, Claude and Goodenough (41) and Staehelin (89) found globular subunits associated with the Zonulae Occludens, and it was observed that the leakiness of an epithelia is dependent upon the numbers of globular subunits. "Leaky epithelia have a less complex network of globular subunits.

The rate that a solute crosses the intestinal epithelium via an extracellular route is dependent upon the nature of the solute and the arrangement of Zonulae Occludens and underlying lateral spaces between cells (36). The pathways or tight junctions are like channels lined with dipolar groups orientated so that anionic groups are directed into the channel (5). The minimum concentration of calcium required to maintain attachment between cells is approximately 0.25 mM (63). Therefore calcium probably plays an essential role in maintaining the integrity of the Zonulae Occludens by interaction with anionic groups on adjacent cellular membranes.

The transport of chromium ions across epithelia of the small intestine was markedly increased in the absence of calcium (Table 4.1).

It appears that the gradual removal of calcium ions has an increasing effect on the permeability of the intestinal membrane to chromium, probably at the site of the Zonulae Occludens. The data therefore suggests that chromium may be transported across the epithelial membrane via an extracellular pathway.

Increasing hydrogen ion concentration in the bathing medium resulted in an increase in the amount of chromium transported to the serosal compartment (Figure 4.3). Several authors (102), (71) have predicted that the anionic groups responsible for the cation selectivity of the extracellular channel have a pK_a of approximately 3 - 4. The reduction of negative charges inside the channels at extreme acid pH therefore permits relatively more cationic movement. The observation again suggests that chromium may be transported via an extracellular route.

There was a decrease in the amount of chromium taken up by the tissue in all regions of the small intestine at extremely high hydrogen ion concentrations. If chromium is mainly bound to phosphate groups on the surface of the intestine (see Page 79) any factors affecting the nature of the phosphate groups will affect the uptake of chromium by the intestinal tissue. At a pH of 2.4 dihydrogen phosphate groups $\left[H_2PO_4^-\right]$ are predominantly present and chromium uptake by the tissue is minimal. As the pH value approaches neutrality gradually the proportion of hydrogen phosphate ions $\left[HPO_4^{2-}\right]$ increases and more chromium is taken up. Under alkali conditions, $\left[PO_4^{3-}\right]$ groups predominate and the uptake of chromium ions by the tissue reaches a maximal value (Figures 4.4, 4.6, 4.8).

These data offer good support for the hypothesis that chromium is mainly bound to the surface of the intestinal tissue as the insoluble phosphate species.

CHAPTER 5

THE EFFECT OF SYNTHETIC AND NATURAL CHELATING AGENT ON THE ABSORPTION OF CHROMIUM IONS IN THE INTESTINAL TRACT

5.1 Introduction

As described in Chapter 4 the most likely way that chromium ions can pass across the intestinal barrier is via an extracellular route, through the spaces between epithelial cells. Absorption of ionised chromium via an intracellular route, directly through the lipoidal layers of the intestinal barrier, seems unlikely. However there could be a possibility of chromium passing directly through the intestinal barrier if it could form a lipid soluble complex with a dietary compound or intestinal secreted products. In this study the effect of a) a synthetic chelating agent, b) a common dietary constituent, and c) a mixture of two bile salts, on the transport of chromium across the intestinal tract and its uptake by the intestinal tissue, was investigated.

5.2 The Effect of a Synthetic Chelating Agent (E.D.T.A.) on the Absorption of Chromium across the Intestinal Tract

To investigate the effect of a synthetic chelating agent on the absorption of chromium, Ethylene Diamine Tetra Acetic Acid (E.D.T.A.) was used. E.D.T.A. is a well known and effective chromium chelating agent; it has six atoms (four oxygen and two nitrogen) through which it can bond to a metal ion (Figure 5.1). E.D.T.A. forms very stable metal chelates with practically every metal in the periodic table; the ratio of metal ions chelated/ chelating molecule is almost invariably 1. The investigation was carried out using everted sacs prepared as described in Chapter 2. The sacs were incubated at 37°C for 30 minutes in

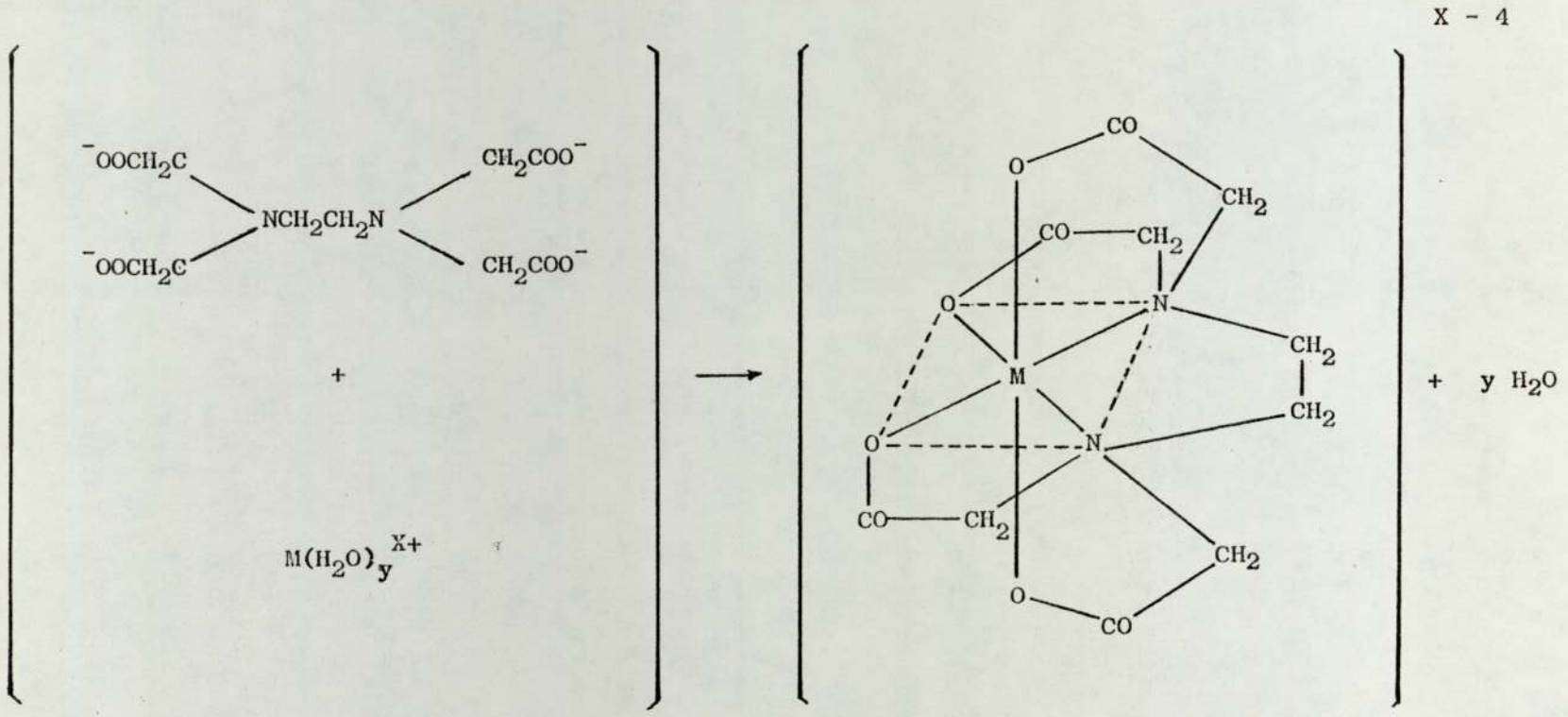


Figure 5.1 Interaction of Ethylene Diamine Tetra Acetic Acid with a metal ion.

the 10 ml of Krebs-Henseleit bicarbonate buffer containing 10^{-6} M Cr^{3+}, 1 μCi of ^{51}Cr and different concentrations of E.D.T.A. (10^{-7} M to 10^{-4} M). The transport of chromium by the intestinal tissue was measured as described in Section 2.8.

Over the concentration range of E.D.T.A. investigated there was no significant difference in the amount of chromium ions absorbed across the intestinal barrier (Table 5.1 and Figure 5.2). The uptake of chromium ions by the intestinal tissue was less at higher concentrations of E.D.T.A. This decrease on uptake was observed in all three different regions of the small intestine (Table 5.1 and Figure 5.3), but the difference was not highly significant. No effect of E.D.T.A. on water movement was observed.

5.3 The Effect of Citric Acid on the Absorption of Chromium across the Intestinal Tract

Everted sacs from different regions of the small intestine were prepared as described in Chapter 2, and incubated for 30 minutes at 37°C in Krebs-Henseleit bicarbonate buffer containing either 0.0 M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, or 10^{-3} M of citric acid, 10^{-6} M of Cr^{3+} and 1 μCi of ^{51}Cr. The uptake of chromium by the intestinal tissue and its transport to the serosal compartment was measured as described in Section 2.8.

The amount of chromium passing to the serosal compartment increased as the concentration of citric acid increased (Table 5.2, Figure 5.4). The difference became statistically significant ($p < 0.01$) at 10^{-4} M citric acid concentration. Citric acid had a greater effect across the ileum and jejunum than the duodenum.

	E.D.T.A. CONCENTRATION				
	0.0 M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
SEROSAL					
DUODENUM	1.8 (0.3)	2.0 (0.3)	1.6 (0.3)	1.3 (0.3)	1.6 (0.2)
JEJUNUM	1.8 (0.3)	2.0 (0.3)	1.8 (0.2)	1.7 (0.4)	2.0 (0.3)
ILEUM	1.0 (0.2)	1.0 (0.1)	0.8 (0.2)	0.9 (0.1)	0.9 (0.2)
TISSUE					
DUODENUM	115 (10)	94 (8)	107 (14)	103 (7)	74 (13) *
JEJUNUM	143 (13)	125 (10)	115 (11)	122 (8)	99 (12) *
ILEUM	159 (20)	140 (5)	170 (19)	196 (26)	117 (21)

Table 5.1 The effect of E.D.T.A. on transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$), to the serosal compartment and its uptake by the intestinal tissue, after 30 minutes incubation at 37°C. The amount of chromium is expressed as ng. Cr/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

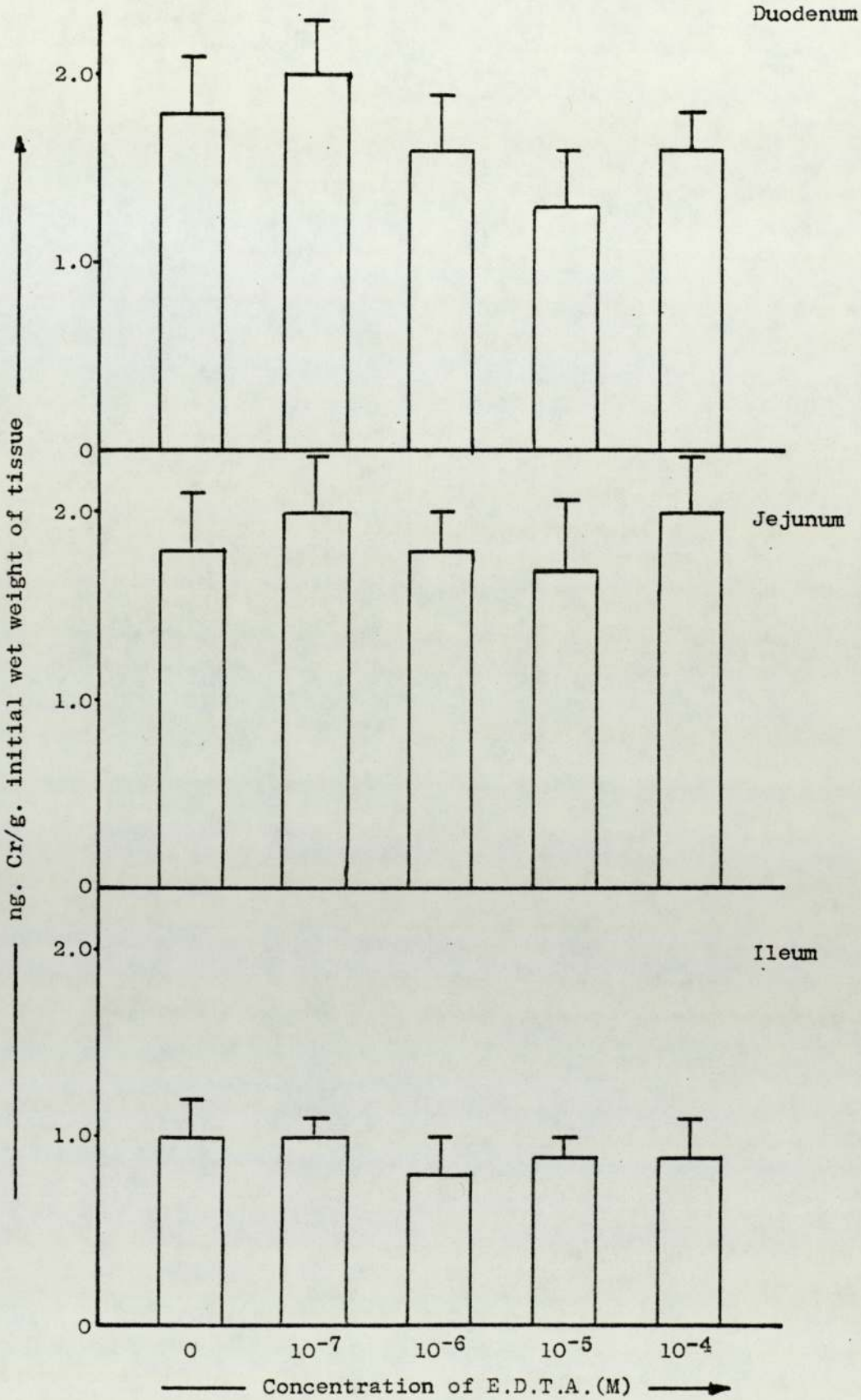


Figure 5.2 The effect of different concentrations of E.D.T.A. on transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) to the serosal compartment after 30 minutes incubation at 37°C.
Standard error of mean represented by vertical bars.

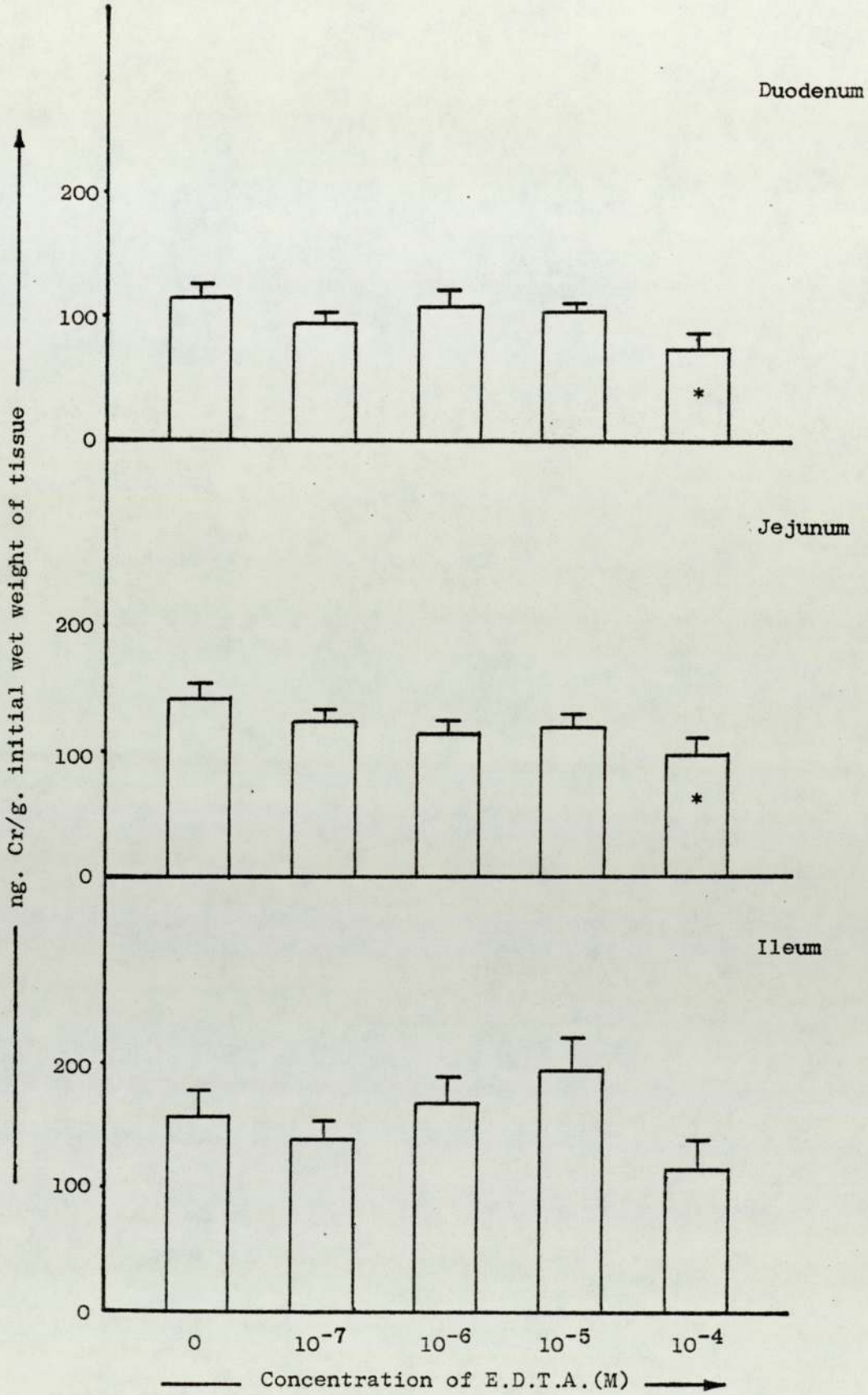


Figure 5.3 The effect of different concentrations of E.D.T.A. on the amount of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) taken up by the intestinal tissue after 30 minutes incubation at 37°C.
Standard error of mean represented by vertical bars.

CITRIC ACID CONCENTRATION

	0.0 M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
SEROSAL						
DUODENUM	1.7 (0.3)	1.7 (0.2)	2.0 (0.2)	2.6 (0.3)*	3.3 (0.4)*	3.9 (0.9)*
JEJUNUM	2.0 (0.5)	2.2 (0.4)	1.5 (0.3)	3.2 (0.5)	6.1 (0.3)**	5.9 (1.0)*
ILEUM	1.2 (0.1)	1.0 (0.1)	1.4 (0.3)	2.5 (0.5)*	3.0 (0.3)**	3.0 (0.3)**
TISSUE						
DUODENUM	101 (23)	113 (22)	88 (3)	64 (13)	27 (1)*	18 (2)*
JEJUNUM	124 (23)	120 (17)	110 (16)	82 (13)	33 (2)*	25 (1)**
ILEUM	139 (17)	146 (24)	96 (12)*	81 (16)*	34 (4)**	20 (1)**

Table 5.2 The effect of citric acid on the transport of 10^{-6} M Cr^{3+} ($CrCl_3,6H_2O$), to the serosal compartment and its uptake by the intestinal tissue, after 30 minutes incubation at 37°C. The amount of chromium is expressed as ng. Cr/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

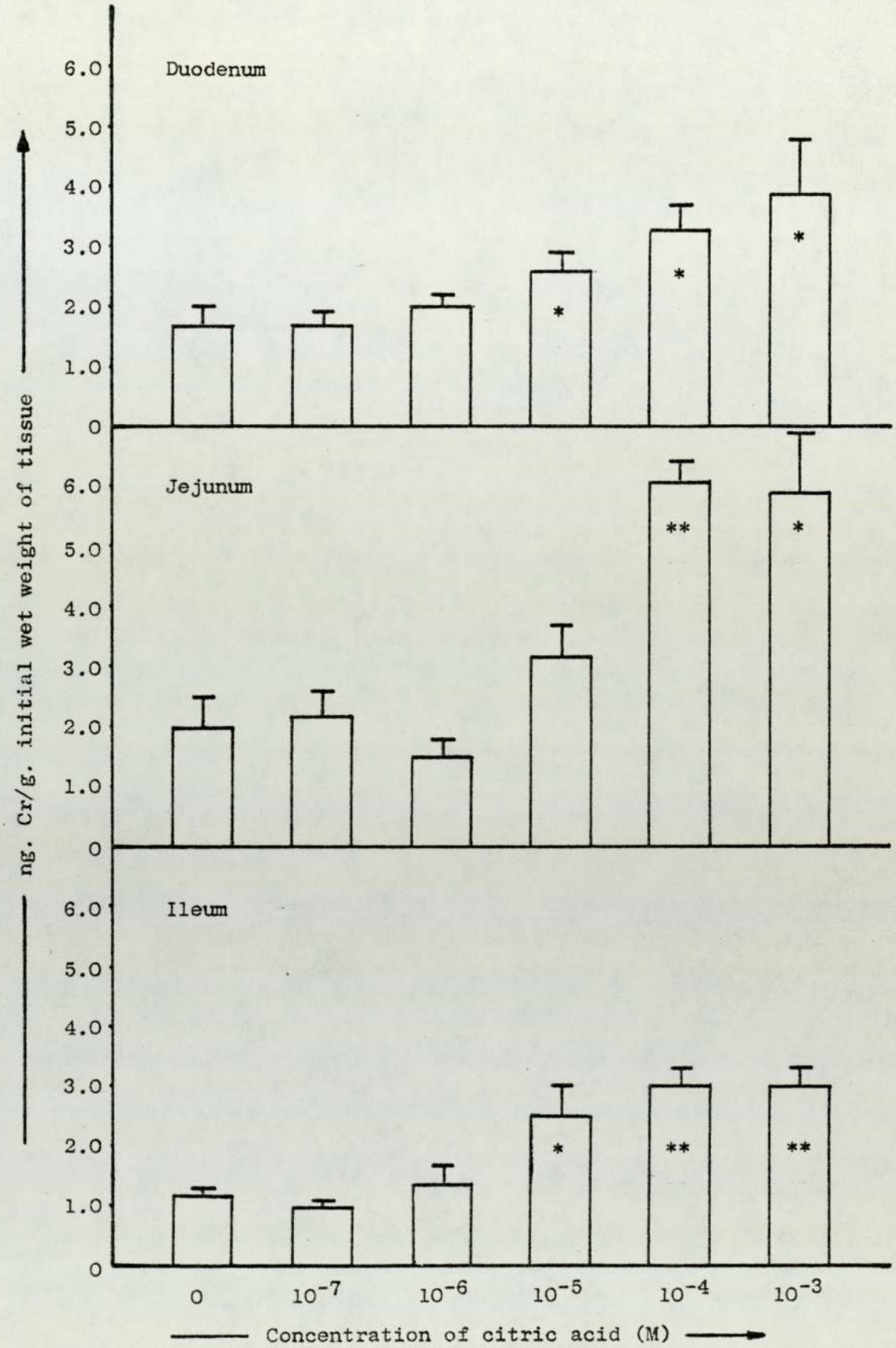


Figure 5.4 The effect of different concentrations of citric acid on the transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) to the serosal compartment after 30 minutes incubation at 37°C.
Standard error of mean represented by vertical bars.

Citric acid also produced a dramatic decrease in the amount of chromium taken up by the tissue at all three different regions of the small intestine (Table 5.2 and Figure 5.5). The effect became statistically significant at a citric acid concentration of 10^{-4} M ($p < 0.01$ for duodenum, $p < 0.01$ for jejunum, and $p < 0.001$ for ileum). Total water transport was unaffected by increases in the concentration of citric acid (Table 5.3).

5.4 The Effect of Bile Salts on the Absorption of Chromium across the Intestinal Tract

Bile is both an excretory medium and a digestive juice (78). Bile salts facilitate the digestion and the absorption of fats, and are the principal end product and excretory form of cholesterol. There are several groups of bile salts, the proportion of which varies from species to species. In man, cholic, chenodeoxycholic and decoxycholic acids are the most important bile acids. Cholic and chendeoxycholic acid are both primary bile acids, synthesized de novo by the liver; deoxycholic acid is secondary acid which is altered by bacteria in the intestine (Figure 5.6). Bile as it leaves the liver contains 2.3 percent solid material, half of which is bile salts. Blaxter and Cowie (13), Klaassen and Shoeman (54) indicated that bile is an important excretory pathway for a cationic metal (lead). These investigations suggest that bile, or some of its components may form metal complexes.

In this study a mixture of sodium cholate and sodium deoxycholate was used to investigate the effect of bile salts on chromium absorption. Everted sacs prepared from three different

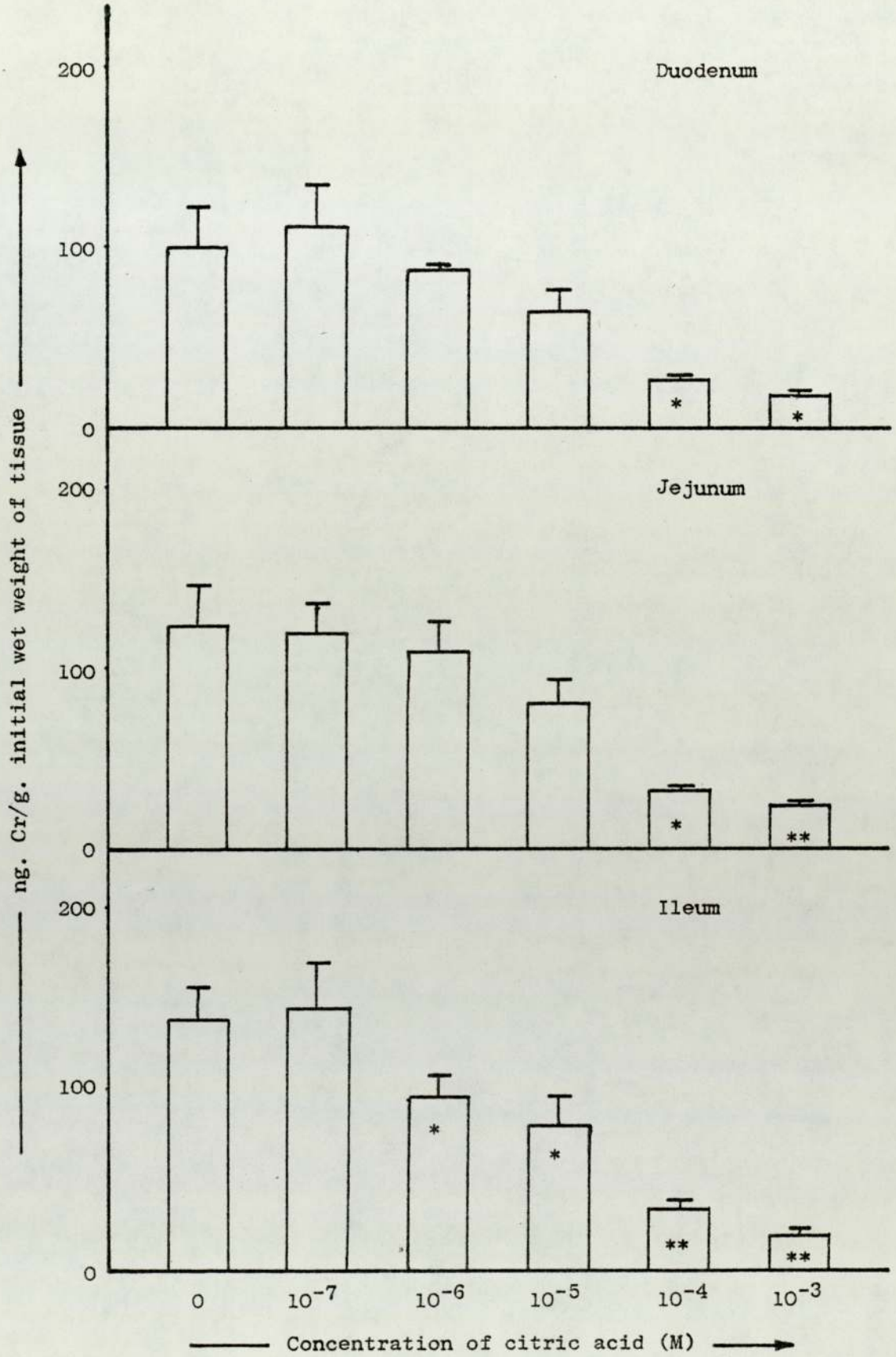
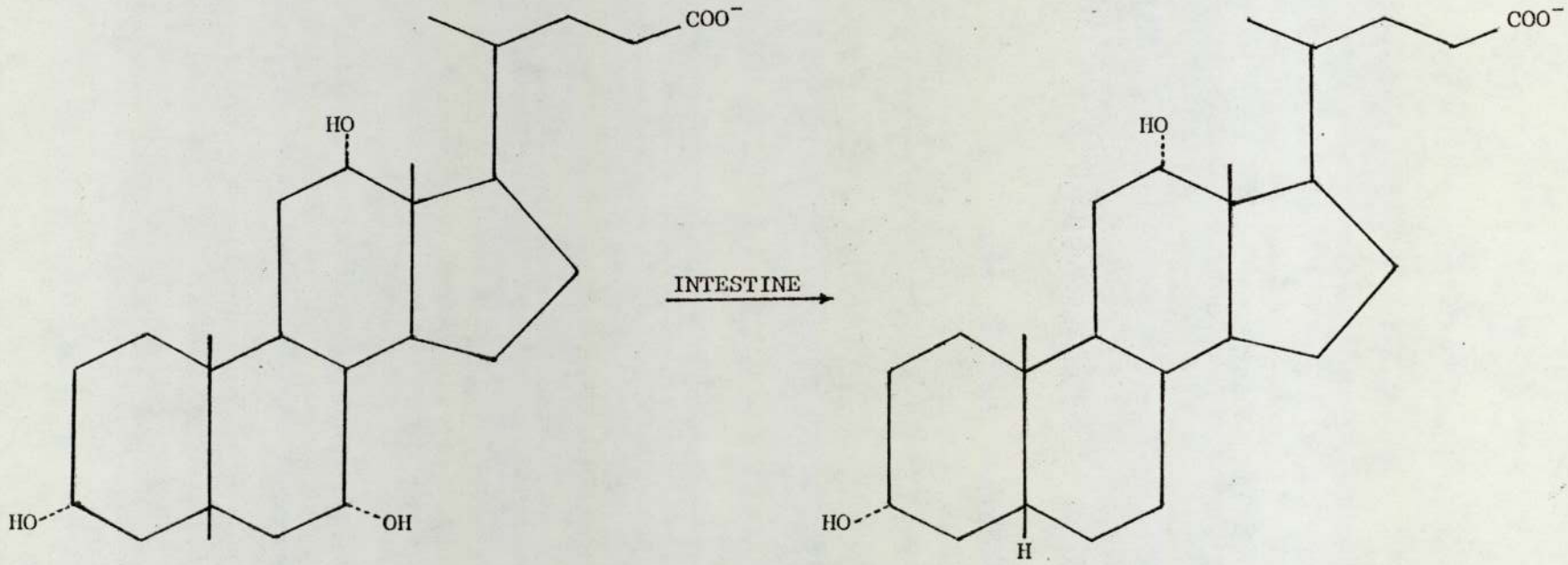


Figure 5.5 The effect of different concentrations of citric acid on the amount of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) taken up by the intestinal tissue after 30 minutes incubation at 37°C.

CITRIC ACID CONCENTRATION

	0.0 M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
SEROSAL						
DUODENUM	85 (37)	18 (42)	15 (22)	111 (50)	81 (19)	109 (64)
JEJUNUM	558 (92)	522 (79)	474 (82)	476 (70)	529 (36)	520 (109)
ILEUM	265 (48)	189 (52)	287 (36)	264 (52)	399 (40)	309 (62)
TISSUE						
DUODENUM	585 (42)	527 (42)	602 (21)	573 (33)	600 (59)	556 (60)
JEJUNUM	628 (44)	709 (51)	710 (13)	747 (42)	685 (52)	628 (129)
ILEUM	543 (43)	524 (71)	497 (43)	524 (48)	538 (26)	474 (56)
TOTAL						
DUODENUM	670 (52)	545 (34)	617 (25)	684 (92)	681 (63)	665 (66)
JEJUNUM	1186 (115)	1231 (119)	1184 (82)	1223 (77)	1214 (74)	1148 (93)
ILEUM	808 (82)	713 (54)	784 (70)	788 (79)	937 (64)	783 (104)

Table 5.3 The effect of different concentrations of citric acid on the movement of water to the serosal compartment and to the intestinal tissue, after 30 minutes incubation at 37°C. The amount of water expressed as mg. water/g. initial wet weight of tissue. (S.E. is expressed in parenthesis)



Cholate

Deoxycholate

Figure 5.6 Alteration of cholate to deoxycholate by the bacteria in the intestine.

regions of the rat small intestine were incubated for 30 minutes at 37°C in Krebs-Henseleit bicarbonate buffer, containing different concentrations of bile salts from 0.0 M to 10^{-3}M. The sacs were removed after incubation and the amount of chromium transported to the serosal compartment and also the intestinal tissue uptake of chromium was measured as described in Section 2.8.

The amount of chromium transported to the serosal compartment was approximately the same over the bile salt concentration range (10^{-7} M to 10^{-3} M) (Table 5.4 and Figure 5.7). There was also no significant difference in the tissue uptake of chromium when the concentration of bile salts was less than 10^{-3} M (Table 5.4 and Figure 5.8). However when the everted sac preparation was incubated in the presence of 10^{-3} M bile salts there was a significant decrease ($p < 0.05$) in the amount of chromium taken up by the intestinal tissue.

The water transport to the serosal compartment as well as the movement of water to the intestinal tissue was unaffected by the change in bile salt concentration from 10^{-7}M to 10^{-4} M, but further increases in the concentration of bile salt produced a dramatic decrease in the water movement both to the serosal compartment and into the tissue (Table 5.5).

BILE SALTS CONCENTRATION

	0.0 M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
SEROSAL						
DUODENUM	1.6 (0.5)	1.7 (0.2)	1.6 (0.4)	1.5 (0.2)	1.3 (0.2)	1.6 (0.4)
JEJUNUM	1.5 (0.1)	1.4 (0.3)	1.5 (0.3)	2.1 (0.3)	1.3 (0.1)	1.2 (0.2)
ILEUM	0.7 (0.1)	1.1 (0.2)	0.8 (0.2)	1.0 (0.2)	1.0 (0.1)*	0.8 (0.1)
TISSUE						
DUODENUM	82 (12)	73 (9)	86 (5)	74 (7)	97 (12)	60 (6)
JEJUNUM	103 (14)	106 (10)	115 (11)	119 (10)	111 (10)	71 (6)*
ILEUM	97 (7)	124 (9)*	145 (7)*	129 (10)*	109 (12)	77 (5)*

Table 5.4 The effect of bile salts on the transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) to the serosal compartment and its uptake by the intestinal tissue, after 30 minutes incubation at 37°C. The amount of chromium is expressed as ng. Cr/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

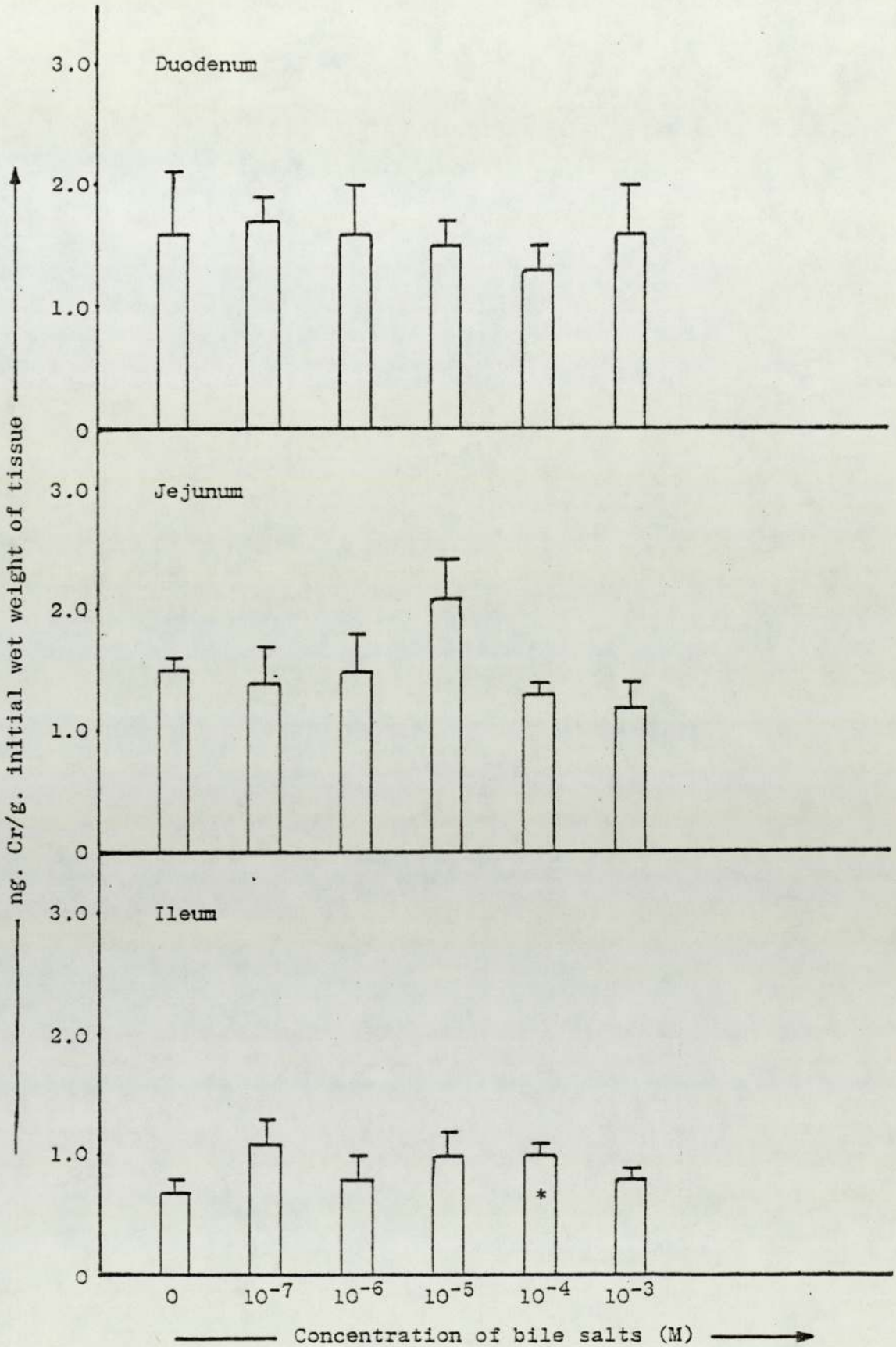


Figure 5.7 The effect of bile salts (sodium deoxycholate) on the transport of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) into the serosal compartment after 30 minutes incubation at 37°C.
Standard error of mean represented by vertical bars.

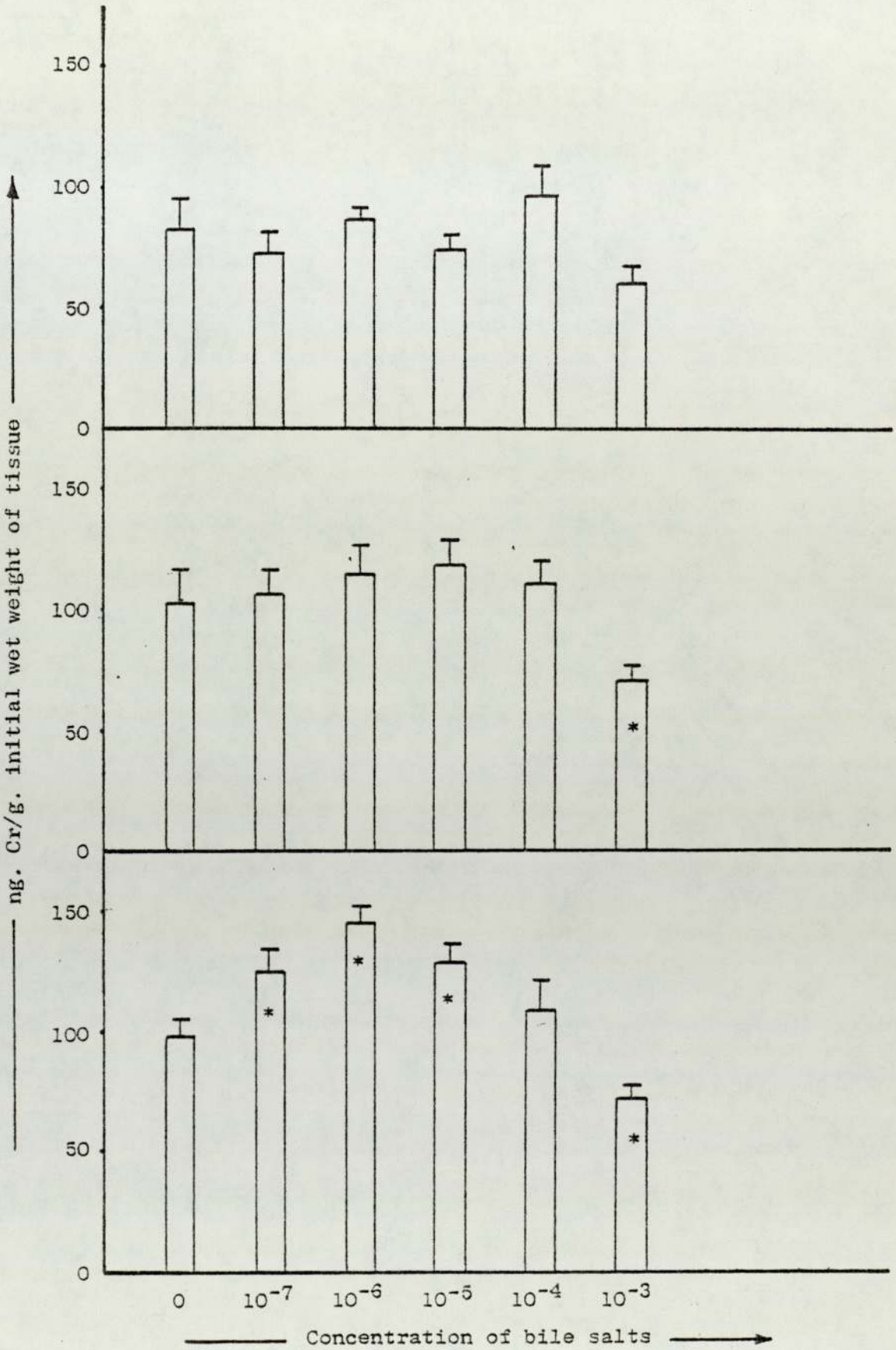


Figure 5.8 The effect of bile salts (sodium deoxycholate) on the tissue uptake of 10^{-6} M Cr^{3+} ($CrCl_3$, $6H_2O$) after 30 minutes incubation at 37°C.
Standard error of mean represented by vertical bars.

BILE SALTS CONCENTRATION

	0.0 M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
SEROSAL						
DUODENUM	41 (31)	-20 (34)	60 (96)	-37 (14)	-13 (35)	6 (42)
JEJUNUM	486 (80)	504 (96)	421 (56)	416 (113)	461 (104)	90 (48)**
ILEUM	194 (33)	299 (47)	220 (49)	238 (33)	326 (49)	42 (43)*
TISSUE						
DUODENUM	502 (25)	423 (27)	511 (46)	410 (51)	526 (64)	399 (32)*
JEJUNUM	638 (56)	497 (84)	567 (39)	504 (102)	562 (64)	374 (41)*
ILEUM	501 (42)	418 (51)	365 (34)	372 (65)	454 (46)	253 (24)**
TOTAL						
DUODENUM	543 (36)	403 (54)	571 (62)	373 (47)	513 (43)	405 (79)
JEJUNUM	1124 (123)	1001 (143)	988 (85)	920 (124)	1023 (103)	464 (47)
ILEUM	695 (75)	717 (70)	585 (71)	610 (87)	780 (69)	295 (39)

Table 5.5 The effect of bile salts on the transport of water to the serosal compartment and into the tissue of the small intestine, after 30 minutes incubation at 37°C. The amount of water is expressed as mg. water/g. initial wet weight of tissue.
(S.E. is expressed in parenthesis)

5.5 Discussion

Three different chemicals known to associate with chromium ions have been utilized to investigate their effect on the transport of chromium across the rat small intestine. The extent to which the presence of E.D.T.A. in the mucosal solution influences chromium transport across the intestinal barrier depends upon at least two factors, one is the number of chromium-E.D.T.A. complexes formed, and the other is the ability of the complex itself to pass across the epithelia. Furthermore E.D.T.A. chromium complexes do not form neutral species, and as a result although there is a general reduction in the charge associated with chromium, the overall charge of the complex (-1) may still inhibit lipid solubility and therefore transport via an inter cellular route.

As the concentration of E.D.T.A. was increased in the mucosal solution, the uptake of chromium by the intestinal tissue decreased, however although consequently the amount of chromium available for transport increases there was not a significant difference in the amount of chromium transported to the serosal compartment.

The uptake of chromium by the intestinal tissue was unaffected in the presence of low concentration of bile salts, (10^{-7} M to 10^{-4} M). At the critical micellar concentration of bile salts (10^{-3} M) however there was a sudden and relatively large decrease in the amount of chromium taken up by the intestinal tissue. High concentrations of deoxycholate are often employed in

the dissolution of membranes and in the isolation of membrane components (17). Coleman et al (18) suggests a model for the removal of lipid and protein from the surface of the membrane. They reported that the treatment of plasma membrane preparations, in isotonic solution, with high concentration of deoxycholate, or cholate, brought about almost total removal of phospholipids. The fact that the water movement both to the serosal compartment and into the intestinal tissue decreased (Table 5.5) in the presence of 10^{-3} M and higher concentrations of deoxycholate suggests that the tissue surface has been stripped off. As the tissue uptake of chromium was severely reduced, this observation gives good indirect support for the hypothesis of surface adsorption of chromium to phosphate-rich lipid components. However despite the fact that a large decrease in tissue uptake of chromium results in more chromium being available for transport, there was no increase in the amount of serosal chromium. Coleman et al (18) reported that after deoxycholate treatment plasma membrane had lost its membrane-like appearance and had become granular and disorganised with only occasional tight junctions remaining. This evidence may explain the absence of an increase in the serosal transport of chromium. Further, because of the relatively large mucosal volume used throughout these studies, any decrease in the tissue binding of chromium will result in only a very small increase in the mucosal concentration of chromium. Therefore although chromium transport to the serosal compartment is dependent upon the mucosal concentration of chromium, it is unlikely with this in vitro system that there will be a large increase in the amount of serosal

chromium.

The presence of citric acid in the mucosal bathing medium decreased the amount of chromium taken up by the intestinal tissue. The effect became significant ($p < 0.01$) at a citric acid concentration of 10^{-4} M. The amount of chromium transported to the serosal compartment was also significantly ($p < 0.01$) increased at citric acid concentration of 10^{-4} M. This may be the result of transport of the extra chromium available, but this seems unlikely for the reason stated earlier. It is more likely that the complex formed is more lipid soluble, and that the general transport properties of citric acid enhance absorption, possibly via an intracellular route. However further experimentation with sub-membrane components is needed in order to clarify the precise route of transport.

Although it is difficult to extrapolate from the in vitro experimentation to normal physiological conditions, it is probable that dietary chelating agents in the intestinal lumen do not affect the transport of chromium unless they are able to neutralize the overall charges associated with chromium ion.

CHAPTER 6

DISCUSSION

A Model to describe the Transport of Chromium across the Small Intestine

Chromium has been recognised as an essential element both for animals and man (81), (8), (67). In 1959 it was found to be necessary for the maintenance of normal glucose tolerance in rats (86). A severe degree of chromium deficiency in rats leads to a syndrome indistinguishable from diabetes mellitus (96). Chromium has been found in most tissues and appears to be required for the action of insulin in controlling glucose metabolism. It also occurs in microsomes and nucleus. It functions in the form of Cr^{3+} in these organelles to stabilise the D.N.A. double helix (59). Hambidge (46) suggests the possibility that at least some forms of diabetes, particularly adult-onset, may be associated with chromium deficiency or some aberration of chromium metabolism. The input of chromium by the respiratory system in normal environmental conditions but not industrial, is negligible, because of the very low concentration of chromium in the air and the insolubility of airborne chromium in the lungs. The interaction of chromium with the skin has been reported (79), but there has been no reliable report of chromium absorption through the skin (26).

Consequently a knowledge of the precise mechanism and route by which the chromium cation cross the epithelial membrane is essential, if one is to attempt to understand the factors which influence chromium absorption. The object of this study was therefore to determine in some detail the mechanism whereby inorganic chromium is transported from the mammalian gut lumen to the body interior.

Secondly it was hoped to define the conditions such as dietary states, and metabolic interactions as well as any disease states in which the entry of the chromium cation could be decreased or increased.

The fact that data reported in this study and also the reports of other investigators (30), (29) suggests very low amounts of chromium are transported from the gastro-intestinal tract into the body, must be taken into account to develop a model for chromium transport. The *in vitro* everted sac preparation has been used throughout this study. Therefore the differences between *in vitro* preparations and the normal physiological conditions have also to be considered, in attempting to provide information about the movement of chromium cations across the small intestinal barrier.

It is clear that the *in vitro* everted sac preparation does not precisely duplicate the events that take place *in vivo*. Under normal physiological conditions any substance that has passed the epithelial barrier would then enter the blood vascular system or lymph system and subsequently be distributed throughout the body. However in an *in vitro* everted sac preparation, substances absorbed at the mucosal surface have to pass through submucosal and smooth muscle layers before entering the serosal compartment. One of the other major differences that exist between *in vitro* preparations and normal physiological conditions is the effect of concentration gradients for solute movement, that occur especially with the everted sac preparation because of the small serosal volume (see Page 75).

Kinetic studies demonstrated that unchelated chromium is transported from the gut lumen to the serosal compartment at a slow

but similar rate at all sites in the rat small intestine. The rate of transport varied linearly with the mucosal chromium concentration across the jejunum and ileum and was related to water movement. The rate of transport was also independent of energy supply. Therefore the data suggests that the transport of chromium across the intestinal barrier is not an active energy dependent form of transport, but is more likely to be a simple passive diffusion process.

In the absence of calcium or under very acidic conditions, the rate of chromium transport to the serosal compartment was increased. According to Schultz (84), the characteristic properties of 'leaky epithelia' are due to low resistance transepithelial extracellular pathways. The pathways which maintain electroneutrality are cation selective channels of 10-16Å (84) (71). Therefore the permeability of the intestinal membrane is dependent upon the arrangement of the zonulae occludentes and underlying lateral spaces between cells (36), and also the nature of the solute. Zonulae occludentes may be thought of as channels lined with dipolar groups orientated so that their anionic groups are directed into the channel (5). Wright and Diamond (102) and Malenkov and Melikyants (63), reported the requirement of calcium for the maintenance of adhesion between cells. Calcium may therefore play an essential role in the maintenance of the integrity of the zonulae occludentes by interaction with anionic groups on adjacent cellular membranes. The fact that the absence of calcium increased chromium transport may therefore suggest an extra cellular transport of chromium through the epithelial barrier, possibly at the site of

the zonulae occludentes. Wright and Diamond (102) and Moreno and Diamond (71), suggested that the anionic groups that are responsible for the cation selectivity of the extracellular channels have a pK_a of 3-4. Relatively more cationic movement would be permitted with a reduction in anionic charge within the channels. Therefore the observation of increased chromium transport, with increased hydrogen ion concentration may again support the idea of extracellular transport of chromium across the epithelial sheet.

When chromium was complexed with citric acid, the rate of transport was increased, probably due to the increase in lipid solubility of the complex. This different potential transport route means that chelated chromium compounds may be more toxic to epithelial cells than free chromium cations. This observation is however more important in the industrial situation, where the level of exposure to chromium is higher.

In contrast to the slow transepithelial movement of chromium, there was a rapid and large amount of chromium taken up by the intestinal tissue. The amount of chromium taken up by the tissue is proportional to the concentration of chromium in the gut, and is not affected by temperature. The uptake is decreased in the absence of glucose and under anoxic conditions, and with high concentrations of bile salts. The absence of glucose, and anoxic conditions affects the production of A.T.P. and indirectly affects the production of phosphate groups. It is therefore possible to suggest that chromium interacts with tissue phosphate groups present in the glycocalyx and lipid membrane. Bile salts

at a concentration above their critical micellar concentration, are known to solubilize phospholipids and phosphoproteins (18). Therefore the effect of high concentrations of bile salts on chromium transport is probably due to the fact that the glycocalyx and perhaps the lipoprotein components of the cell membrane have been lost, and therefore less chromium is taken up by the tissue (see Section 5.5).

All the data taken together suggests that chromium is absorbed onto the surface of intestinal tissue probably in the form of chromium phosphate. The proportion of chromium in the system, bound to the tissue, increases as the fluid-tissue ratio decreases. Therefore under normal physiological conditions where the ratio of fluid to tissue is about 0.3 (11), the amount of chromium bound to the tissue is approximately 95% (see Page 78). Consequently the bound chromium is not available for transport into the body and is probably lost in the faeces, and as a result a large proportion of the intestinal chromium is not normally absorbed. This again emphasises the specialised nature of the surface of the gut mucosa, as it potentially is able to exclude both non-essential and indeed essential ions. It does not mean however that chromium cannot be absorbed across other lipid membranes within the body, as complexation of chromium will promote transport into cells (e.g. Red Blood Cells), (42).

The binding of chromium to the intestinal tissue appears to play an important role in the mechanism of absorption. It is clear that under conditions in which the binding of chromium to the tissue is reduced, there will be a subsequent increase in the amount of

chromium available for transport. For example, the natural process of bile excretion, may in fact help to strip chromium from the surface of the intestine and subsequently aid absorption. Therefore one can suggest that certain groups in the population who have a discontinuous excretion of bile, (due to an interrupted intake of fat, or for surgical reasons, e.g. cannulation of the bile duct), may absorb less chromium and may consequently suffer from a relative body deficiency of chromium. Similarly, people who have a high carbohydrate intake, would be expected to have a subsequent increase in the amount of A.T.P. and phosphate groups in the surface of the mucosa. They would therefore have less intake of chromium into the body as a result of the higher uptake of chromium by the intestinal tissue. These are in fact exactly the groups of people who need chromium to counteract any possible glucose intolerance.

In general one can summarise the factors that affect the tissue uptake of chromium and its subsequent absorption as:-

a) When the glycocalyx is diminished,

b) When enzyme activity of A.T.P.ase is reduced,

and c) When the supply of A.T.P. is reduced.

Further clarification and refinement of the model proposed in this study can be obtained with the following experimentation:-

1. In order to confirm that a) the major route of transport for the chromium cation is via the extracellular spaces, and b) that the major portion of chromium is absorbed onto the surface of the epithelial membrane, cell fractionation procedures could be carried out. Everted sacs could be incubated in chromium solutions and a

mucosal scrape carried out, in order to confirm that the majority of the chromium was associated with the brush border region of the intestinal preparation rather than the muscle layers. Subsequent separation of the epithelial cells will give an approximate figure for the amount of chromium that is associated with the extracellular spaces. Further sub-fractionation of the cellular component into the cytoplasmic fractions, the organelles and the debris which will contain the cell membrane including the glycocalyx will give approximate distribution of the chromium.

2. A better method to determine the overall distribution of chromium across the epithelial sheet would be to use an autoradiographic technique. This however would not quantify the amount of chromium in each fraction. The everted sac preparation would be again incubated for various periods of time with ^{51}Cr for subsequent fixation.

3. The effect of dietary factors on the intestinal absorption of chromium could also be investigated, for example, the effect of Vitamin D on the absorption of chromium would add some relevant information concerning the proposed model. Vitamin D is known to be involved in the transport of calcium ions (100), as it affects the binding of calcium to calcium binding proteins in the epithelial cell membrane. As the calcium binding proteins form a group of compounds which are an alternative site for the interaction of chromium with the intestinal tissue, one can in fact investigate the potential interaction of chromium with calcium binding proteins, or the replacement of calcium by chromium in calcium binding proteins, by varying the Vitamin D intake in the

experimental animals. Vitamin D is also thought to affect the acidity of the surface microclimate (Lucas, M.. Private Communication). Therefore by varying the Vitamin D status of the animal one could also investigate whether or not the presence or absence of acid microclimate affects chromium transport.

4. Interaction of chromium with the phosphate groups can further be investigated by examining the affect of inhibition of A.T.P.ase or alkaline phosphatase on the uptake of chromium by intestinal tissue. This can be achieved by reducing the concentration of Mg^{2+}, Na^{+}, K^{+}, or Zn^{2+}.

However the model presented in this study suggests a physiological mechanism for chromium transport, and indicates the importance of chromium-tissue interaction in the movement of chromium across the intestinal barrier. The model also predicts the conditions which will affect chromium absorption.

BIBLIOGRAPHY

1. Aisen, P.; Aasa, R. and Redfield, A.C.
The chromium, manganese, and cobalt complexes of transferrin
J. Biol. Chem. 244 4628-4633 (1969)

2. Baetjer, A.M.; Damron, C. and Budacz, V.
The distribution and retention of chromium in men and animals
A.M.A. Arch. of Ind. Health 20 136-150 (1959)

3. Baker, R.D.; Searle, G.W. and Nunn, A.S.
Glucose and sorbose absorption at various levels of rat small intestine
Am. J. Physiol. 200 301-304 (1961)

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

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

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

7. Barry, R.J.C. and Eggenton, J.
In: Intestinal absorption. Biomembranes 4B
Plenum press Chap. 18
Ed: Smyth, D.H. (1974)

8. Beyerman, K.
The analytical behaviour of very small amounts of chromium
Part I Z. Anal. Chem. 196 4- 33 (1962)
Part II Z. Anal. Chem. 191 345-369 (1962)

9. Bidstrup, D.L. and Case, R.A.M.
Carcinoma of the lung in workmen in the bichromates producing industry in Great Britain
Brit. J. Ind. Med. 13 260-264 (1956)

10. Bihler, I.; Hawkins, K.A. and Crane, R.K.
Studies on the mechanism of intestinal absorption.VI.The specificity and other properties of Na^+-dependent entrance of sugars into intestinal tissue under anaerobic conditions, in vitro
Biochim. Biophys. Acta 59 94-102 (1962)

11. Biochemical handbook
Ed: C. Long Section IV p. 675 E. & F.M. Spon Ltd., London (1971)

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

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

14. Bowen, H.J.M.
Trace elements in biochemistry
New York: Academic p.163 (1966)

15. Bresler, S.E. and Rozentsveig, N.A.
Proteolytic enzymes metalloproteins
Biokhimiya 16 84-94 (1951)

16. Coleman, I.P.L.
The gastro intestinal absorption of lead
PhD. thesis, Department of Chemistry
The University of Aston in Birmingham (1979)

17. Coleman, R.
Solubilization of membrane components
Biochem. Soc. Trans. 2 813 (1974)

18. Coleman, R.; Holdsworth, G. and Vyvoda, O.S.
Membrane alterations as basis of liver injury
Eds: H. Popper; L. Bianchi, and W. Reutter Chapter 9 143-156 (1977)

19. Creamer, B.
In: Intestinal absorption. Biomembranes 4A
Plenum press Chap. 1 (1974)

20. Curran, G.L.

Effect of certain transition group elements on hepatic synthesis of cholesterol in the rat

J. Biol. Chem. 210 765-770 (1954)

21. Curran, P.F.

Ion transport in intestine and its coupling to other transport processes

Fed. Proc. 24 993-999 (1965)

22. Davidson, I.W.F. and Blackwell, W.L.

Changes in carbohydrate metabolism of squirrel monkeys with chromium dietary supplementation

Proc. Soc. Exp. Biol. Med. 127 66-71 (1968)

23. Davies, I.J.T.

The clinical significance of the essential biological metals

Chap. VI (1972)

24. Diamond, J.M.

Standing-gradient model for fluid transport in epithelia

Fed. Proc. 30 6-13 (1971)

25. Diamond, J.M.

Tight and leaky junctions of epithelia

Fed. Proc. 33 2220-2224 (1974)

26. Di Ferrante, E. (Ed.)
Trace metals, exposure and health effects
Proceeding of Research Seminar, University of Surrey, Guildford
p. 139 (1978)

27. Doisy, R.J.; Jastremski, M.S. and Greenstein, F.L.
Metabolic effects of glucose tolerance factor and trivalent chromium in normal and genetically diabetic mice
Excerpta Med. Found. Int. Cong. Ser. 280 155 (Abstr.) (1973)

28. Doisy, R.J.; Streeten, D.H.P.; Levine, R.A. and Chodos, R.B.
Effect of metabolism of chromium in normals, elderly subjects and diabetics. In Proc. 2nd Annual Conf. "Trace substances in environmental health"
Ed: D.D. Hemphill Vol.II, p 75 University of Missouri Columbia (1968)

29. Doisy, R.J.; Streeten, D.H.P.; Souma, M.L.; Kalafer, M.E.; Rekant, S.L. and Dalakas, T.G.
Metabolism of 51-chromium in human subjects
In: "Newer trace elements in nutrition"
Ed: W. Mertz and W.E. Cornatzer Chap. 8, Dekker, New York (1971)

30. Donaldson, R.M. and Barreras, F.
Intestinal absorption of trace quantities of chromium
J. Lab. Clin. Med. 68 484-493 (1966)

31. Faust, R.G.
The effects of anoxia and lithium ions on the absorption of D-glucose by rat jejunum in vitro
Biochim. Biophys. Acta. 60 604-614 (1962)

32. Feldman, F.J.; Knoblock, E.C. and Purdy, W.C.
The determination of chromium in biological materials by atomic absorption spectroscopy
Anal. Chim. Acta. 38 489-497 (1967)

33. Fishbein, L.
Environmental metallic carcinogens: An overview of exposure levels
J. Toxicology. Enviro. Health 2 77-109 (1976)

34. Foster, R.F.
Environmental behaviour of Cr and Np
In: Proc. Nat. Symp. Radioecology, Ed: Frot Collins 569-576 (1961)

35. Freund, H.; Atamian, S. and Fischer, J.E.
Chromium deficiency during total parenteral nutrition
J. Am. Med. Assoc. (JAMA) 241 496-498 (1979)

36. Fromter, E. and Diamond, J.M.
Route of passive ion permeation in epithelia
Nature, New Biol. 235 9-13 (1972)

37. Fukai, R.
Valence state of chromium in sea water
Nature 213 901 (1967)

38. Fuwa, K.; Wacker, W.E.C.; Druyan, R.; Bartholomay, A.T. and Vallee, B.L.
Nucleic acid and metals. II. Transition metals as determinats of the conformation of ribonucleic acids
Proc. Natl. Acad. Sci. U.S. 46 1298-1307 (1960)

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

40. Glinsmann, W.H. and Mertz, W.
Effect of trivalent chromium on glucose tolerance
Metabolism 15 510-520 (1966)

41. Goodenough, D.A. and Revel, J.P.
A line structual analysis of intercellular junctions in the mouse liver
J. Cell. Biol. 45 272-279 (1970)

42. Gray, S.J. and Sterling, K.
The tagging of red cells and plasma proteins with radioactive chromium
J. Clin. Invest. 29 1604-1619 (1950)

43. Gromican, A.
Inorganic elements in food used in hospital menus
J. Amer. Diet. Ass. 56 397-403 (1970)

44. Hafty, J.

Residue methods for common minor elements

Geological Survey Water-Supply Paper 1540-A

45. Hambidge, K.M.; Rodgerson, D.O. and O'Brien, D.

Concentration of chromium in the hair of normal children and children with juvenile diabetes mellitus

Diabetes 17 517-519 (1968)

46. Hambidge, K.M.

Chromium nutrition in man

Am. J. Clin. Nutr. 27 505-514 (1974)

47. Harris, D.C.

Different metal-binding properties of the two sites of human transferrin

Biochem. 16 560-564 (1977)

48. Wilson, T.H.

Intestinal absorption

Saunders, W.B. and Co. p.2 (1962)

49. Hopkins, L.L. and Schwarz, K.

Chromium (III) binding to serum proteins specifically siderophilin

Biochem. Biophys. Acta. 90 484-491 (1964)

50. Hopkins, L.L.

Distribution in the rat of physiological amounts of injected Cr^{51}(III) with time

Am. J. Physiol. 209 731-735 (1965)

51. House, C.R.

Water transport in cells and tissues

Monographs of the physiological society

Ed: Davson, H.; Greenfield, A.D.M.; Whittam, R. and Brindley, G.S. (1974)

EDWARD ARNOLD (Publishers) Ltd. London

52. Jackson, M.J. and Cassidy, M.M.

Studies on intestinal fluid transport. II. The location of fluid accumulated in the wall of rat jejunum during incubation in vitro

Biochem. Biophys. Acta. 211 436-447 (1970)

53. Kimiaki Sumino; Kiyoko Hayakawa; Takako Shibata; Shoji Kitamura

Heavy metal in normal Japanese tissues, amounts of 15 heavy metals in 30 subjects

Arch. Environ. Health 30 487-494 (1975)

54. Klaassen, C.D. and Shoeman, D.W.

Biliary excretion of lead in rats, rabbits and dogs

Toxicol. App. Pharmac. 29 434-446 (1974)

55. Krebs, H.A. and Henseleit, K.
Untersuchungen über die harnestoffbildung in tierköper
Zeitschrilt fur Physiolog Chemie 210 33-36 (1932)

56. Langard, S. and Norseth, T.
A cohort study of bronchial carcinomas in workers producing chromate pigments
Brit. J. Ind. Med. 32 62-65 (1975)

57. Levine, R.A.; Streeten, D.H.P. and Doisy, R.J.
Effect of oral chromium supplementation on the glucose tolerance of elderly human subjects
Metabolism 17 114-128 (1968)

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

59. Levis, A.G.; Blanchi, V.; Tamino, G. and Pegoraro, B.
Cytotoxic effects of hexavalent and trivalent chromium on mammalian cells in vitro
Brit. J. Cancer 37 386-392 (1978)

60. Lucas, M.L.
Acidification in the rat proximal jejunum
PhD.thesis, Department of Chemistry
The University of Aston in Birmingham (1974)

61. Machen, T.E.; Erlig, D. and Wooding, F.B.P.
Permeable junctional complexes, The movement of lanthanum across rabbit gall bladder and intestine
J. Cell Biol. 54 302-312 (1972)

62. Mackenzie, R.D.; Anwar, R.A.; Byerrum, R.U. and Hoppert, C.A.
Absorption and distribution of Cr^{51} in the albino rat
Arch. Biochem. Biophy. 79 200-205 (1959)

63. Malenkov, A.G. and Melikyants, A.G.
Ion permeabiltiy and strength of cell contacts
J. Memb. Biol. 36 97-113 (1977)

64. Mertz, W. and Schwarz, K.
Relation of glucose tolerance factor to impaired intravenous glucose tolerance of rats on stock diets
Am. J. Physiol. 196 614-618 (1959)

65. Mertz, W.; Roginski, E.E. and Schwarz, K.
Effect of trivalent chromium complexes on glucose uptake by epididymal fat tissue of rats
J. Biol. Chem. 236 318-322 (1961)

66. Mertz. W.
Biological role of chromium
Fed. Proc. 26 186-193 (1967)

67. Mertz, W.

Chromium occurrence and function in biological systems

Am. Physiol. Soc. 49 163-239 (1969)

68. Mertz, W.

Human requirements: Basic and optimal

Ann. N.Y. Acad. Sci. 199 191-199 (1971)

69. Mertz, W.

Biological function of chromium nicotinic acid-complexes

Fed. Proc. Fed. Amer. Soc. Exp. Biol. 33 659 (Abstr.) (1974)

70. Metal Statistics

A publication of American metal market

Fairchild Publication, INC. p.55 Chromium Profile

6th Annual Edition (1976)

71. Moreno, J.H. and Diamond, J.M.

Nitrogenous cations as probes of permeation channels

J. Memb. Biol. 21 197-259 (1975)

72. Newman, H.A.I.; Leighton, R.I.; Lanese, R.R. and Freeland, N.A.

Serum chromium and angiographically determined coronary artery disease

Clin. Chem. 24/4 541-544 (1978)

73. Onkelinx, G.
Compartment analysis of metabolism of chromium (III) in rats of various ages
Am. J. Physiol. 232 E478-E484 (1977)

74. Public Health Service Drinking Water Standards
Public Health Rept. 7 370-372 (1962)

75. Razzaque, A.
Transport of 5-methyltetrahydrofolic acid across the rat jejunum
PhD. thesis, Department of Chemistry
The University of Aston in Birmingham (1975)

76. Robertson, D.E.
Role of contamination in trace element analysis of sea water
Anal. Chem. 40 84A-86A (1968)

77. Rollinson, C.L.; Rosenbloom, E. and Lindsay, L.
Reactions of chromium III with biological substances
In: Proc. 7th Intern. Congr. Nutrition. N.Y. Pergamon
5 692-698 (1967)

78. Ruch, T.C. and Patton, H.D. (Eds.)
Physiology and Biophysics 19th Edition
W.B. Saunders Company, Philadelphia and London
Chap. 49 p 992 (1960)

79. Samitz, M.H. and Katz, S.
A study of the chemical reaction between chromium and skin
J. Invest. Dermatol. 43 35-43 (1964)

80. Sargent III, T.; Lim, T.K. and Jenson, R.L.
Reduced chromium retention in patients with hemochromatosis, a possible basis of hemochromatic diabetes
Metabolism 28 70-79 (1979)

81. Schroeder, H.A.; Balassa, J.J. and Tipton, I.H.
Abnormal trace metal in man: chromium
J. Chronic Diseases 15 941-964 (1962)

82. Schroeder, H.A.
The role of chromium in mammalian nutrition
Am. J. Clin. Nutr. 21 230-244 (1968)

83. Schroeder, H.A.; Balassa, J.J. and Vinton, W.H.
Chromium, cadmium and lead in rats: effects of life span, tumors, and tissue levels
J. Nutr. 86 51-56 (1965 A)

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

85. Shwarz, K. and Mertz, W.
A glucose tolerance factor and its differentiation from factor 3
Arch. Biochem. Biophys. 72 515 (1957)

86. Shwarz, K. and Mertz, W.
Chromium III and the glucose tolerance factor
Arch. Biochem. Biophys. 85 292-295 (1959)

87. Smyth, D.H.
In: Recent advances in physiology
Churchill Ltd. (1963)

88. Smyth, D.H.
Electric potential and fluid transfer in the rat intestine
Ecerpta Medica Monography on Nuclear Medicine and Biology
1 195-205 (1966)

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

90. Stickland, L.H.
Activation of phosphoglucomutase by metal ions
Biochem. J. 44 190-197 (1949)

91. Sunderman, F.W.
A review of the carcinogenicities of nickel, chromium and arsenic compounds in man and animals
Prev. Med. 5 279-284 (1970)

92. Tabor, E.C. and Warren, W.V.
Distribution of certain metals in the atmosphere of some American cities
A.M.A. Arch. Ind. Health 17 145-151 (1958)

93. Taylor, C.B.
The effect of acetyltrimethyl ammonium bromide and some related compounds on transport and metabolism in intestine of the rat in vitro
J. Physiol. 165 199-218 (1963)

94. Tipton, I.H.
Distribution of trace metals in the human body
In: Metal Binding in Medicine
Ed: M.J. Seven Philadelphia: Lippincott 27-42 (1960)

95. Toepfer, E.W.; Mertz, W.; Roginski, E.E. and Polansky, M.M.
Chromium in foods in relation to biological activity
J. Agr. Food Chem. 21 69-73 (1973)

96. Toepfer, E.W.
Separation from yeast of chromium containing material possessing glucose tolerance factor (G.T.F) activity
Fed. Proc. 33 659 (1974)

97. Visek, W.J.; Whitney, I.B.; Kuhn III, U.S.G. and Comar, C.L.
Metabolism of Cr^{51} by animal as influenced by chemical state
Proc. Soc. Exp. Biol. Med. 84 610-615 (1953)

98. Wacker, W.E.C. and Vallee, B.L.
Chromium, manganese, nickel and other metals in RNA
Fed. Proc. 18 345 (1959)

99. Wacker, W.E.C. and Vallee, B.L.
Nucleic acid and metals. I. chromium, manganese, nickel, iron and other metals in ribonucleic acid from divers biological sources
J. Biol. Chem. 234 3257-3262 (1959)

100. Wasserman, R.H. (Ed.)
The transfer of calcium and strontium across biological membranes
Acad. press N.Y. p. 211 (1963)

101. Wilson, T.H. and Wiseman, G.
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 (1954)

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