STUDIES ON THE INTESTINAL ABSORPTION OF RADIOACTIVE STRONTIUM

by

Paul M. Thyvalikakath, M.B.B.S., M.Sc.

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Department of Experimental Surgery
McGill University
Montreal, Canada

April, 1965

PREFACE

The present investigation is the continuation of the studies done as M.Sc. student in this department. I had the good fortune to benefit from the data and experience that had accumu - lated in this department from the studies by my predecessors on the carcinogenic effects and metabolism of radiostrontium.

I am grateful to Dr. D.R. Webster, Professor of Experimental Surgery, for providing me with facilities to carry out this work in his department at McGill University and his willingness to buy new equipment that has helped a great deal in this study. My sincere thanks go to Dr. S.C. Skoryna, Director of Research, who had given his whole-hearted help and advice both in research and in personnal matters. I am deeply indebted to Dr. Deirdre Waldron-Edward, biochemist - in - charge, for her guidance, suggestions and constant encouragement which I received in abundance in the course of this investigation. I am obliged to Dr. D.S. Kahn, pathologist, for reviewing the histological sections.

I am extremely grateful to Mr. Michael Farrell for preparing the animals and in assisting with experimental procedures. Without his active cooperation it would have been impossible to complete this work. I am also thankful to Mr. Sergei Podymow for his help in the radioactive room and care of animals.

To Miss M. Evans and Miss D. Lang, I am greatly obliged for their help in the preparations of chemicals and specially for the

tiresome task of dissolving sodium alginate into a jelly form throughout this study. My grateful acknowledgements are due to Mrs. L. Trifonow who has helped in the histological preparations and Mrs. T. Golowanow for the help received in experimental procedures.

To Miss Eleanor Kulchycka, secretary to the department, I am greatly indebted for her pleasant and cooperative help throughout these years, for typing innumerable tables and especially for the tedious job of typing this manuscript. I am also thankful to Mrs. Isabel Griffiths, who had been keenly interested in my problems and had been helpful in more than one way. It has been a great pleasure to work with and receive helpful criticisms from the other members of the department; Dr. Franklin, Mrs. L. Woods, Dr. Y. Chun, and Dr. Shamsudduha.

My grateful acknowledgements are due to the Medical Research Council of Canada for providing me with Research Fellowships and also the necessary funds to carry out the experimental studies. Finally, I wish to state that this time spent at McGill University had given me opportunity to make many pleasant contacts and rewarding friendships which will certainly leave lasting effect on me.

TABLE OF CONTENTS

PREFACE		i
TABLE OF CONTENTS		iii
FIGURES AND TABLES		iv
CHAPTER I.	INTRODUCTION	1
CHAPTER II.	STRONTIUM AND ITS METABOLISM	3
CHAPTER III.	STRONTIUM - CALCIUM DISCRIMINATION	29
CHAPTER IV.	ABSORPTION OF CALCIUM AND STRONTIUM FROM GASTROINTESTINAL TRACT	31
CHAPTER V.	FACTORS AFFECTING ABSORPTION AND DISTRIBUTION OF STRONTIUM^{89}	38
CHAPTER VI.	INHIBITION OF STRONTIUM ABSORPTION	92
CHAPTER VII.	INHIBITION OF STRONTIUM ABSORPTION: PRACTICAL APPLICATION OF THE INHIBITORY AGENTS	138
CHAPTER VIII.	CONCLUSIONS	172
BIBLIOGRAPHY		175

FIGURES AND TABLES

FIGURES page

1. Schema of division of small intestine by ligation 43

2. Schematic drawing of a ligated intestinal segment 45

3. Sr^{89} and Ca^{45} blood levels and bone uptake after single oral dose 49

4. Bone uptake of Sr^{89} and Ca^{45} after continuous administration 55

5. CPM - in one femur at the end of five days 57

6. pH studies in rats ligated gastrointestinal segments 62

7. Influence of pH on Sr^{89} absorption from ligated intestinal segments 64

8. Influence of pH on Sr^{89} absorption - blood levels 66

9. Influence of pH on Sr^{89} absorption - uptake in femur 68

10. Influence of pH on Ca^{45} absorption from ligated intestinal segments 70

11. Influence of pH on Ca^{45} absorption - blood levels..... 72

12. Influence of pH on Ca^{45} absorption - bone uptake 74

13. Effect of pH on Sr^{89} and Ca^{45} blood levels and bone uptake 77

14. Effect of "Trisma" buffer on Sr^{89} and Ca^{45} blood levels and bone uptake in adult female rats..... 79

15. Structural formula of alginic acid 95

16. Proposed structure of strontium salt of polymannuronic acid 96

17. Polygalacturonic acid - proposed structure 98

18. Effect of addition of sodium alginate on Sr^{89} blood levels and bone uptake 102

page

19. Effect of addition of sodium alginate on Ca^{45} blood levels and bone uptake 104

20. Effect of time of administration of sodium alginate on Sr^{89} bone uptake 108

21. Effect of age of animal on Sr^{89} uptake 110

22. Comparative effects of different macromolecular substances on Sr^{89} and Ca^{45} uptake in femur following orogastric administration 120

23. Effect of sodium alginate on absorption of Sr^{89} and Ca^{45} from the ligated intestinal segments 123

24. Effect of sodium alginate on blood levels of Sr^{89} and Ca^{45} 125

25. Effect of sodium alginate on bone uptake of Sr^{89} and Ca^{45}. 127

26. Effect of increasing concentrations of sodium alginate on Sr^{89} and Ca^{45} absorption and blood levels, after administration into ligated duodenal segments..... 129

27. Effect of increasing concentrations of sodium alginate on Sr^{89} and Ca^{45} uptake after injection into ligated duodenal segment 130

28. Effect of continuous administration of sodium alginate on Sr^{89} and Ca^{45} uptake in bone 143

29. Distribution of Sr^{89} and Ca^{45} following the administration of isotopes in drinking water and sodium alginate in dry form 146

30. Effect of continuous feeding of 24% sodium alginate on Sr^{89} and Ca^{45} uptake in bone 149

31. Effect of adding increasing amounts of sodium alginate on Sr^{89} and Ca^{45} uptake 152

32. Effect of Sr^{89} and alginate administered in drinking water and food 158

33. Effect of continuous administration of polygalacturonic acid on Sr^{89} and Ca^{45} uptake in bone 161

34. Effect of continuous feeding of alginate on weight..... 163

TABLES page

I	Sr^{89} and Ca^{45} blood levels and bone uptake after single oral dose	48
II	Bone uptake of Sr^{89} and Ca^{45} after continuous administration in drinking water. 0.2 μc per day	52
III	Bone uptake of Sr^{89} and Ca^{45} after continuous administration in drinking water. 5 μc per day	53
IV	Bone uptake of Sr^{89} and Ca^{45} after continuous administration in food. 5 μc per day	54
V	Bone uptake of Sr^{89} after continuous administration. 25 and 50 microcuries per day	56
VI	pH studies in rat's ligated gastrointestinal segments	61
VII	Influence of pH on Sr^{89} absorption from rat's ligated intestinal segments	63
VIII	Influence of pH on Sr^{89} absorption - blood levels	65
IX	Influence of pH on Sr^{89} absorption - uptake in femur	67
X	Influence of pH on Ca^{45} absorption from rat's ligated intestinal segments	69
XI	Influence of pH on Ca^{45} absorption - blood levels	71
XII	Influence of pH on Ca^{45} absorption - bone uptake	73
XIII	Effect of pH on Sr^{89} and Ca^{45} blood levels and bone uptake	76
XIV	Effect of pH on Sr^{89} and Ca^{45} blood levels and uptake by bone in adult female rats	78
XV	Effect of ligation of common bile duct on Sr^{89} and Ca^{45} absorption from duodenal segment	82
XVI	Absorption of Ca^{45} from duodenal segment after ligation of bile duct	83

		page
XVII	Calcium^{45} absorption from duodenal segment after ligation of bile duct	84
XVIII	Effect of single dose of sodium alginate on Sr^{89} and Ca^{45} levels in blood	101
XIX	Effect of single dose of sodium alginate on Sr^{89} and Ca^{45} uptake by bone	103
XX	Effect of time of administration of sodium alginate on Sr^{89} uptake by bone	107
XXI	Effect of age of animal on Sr^{89} uptake	109
XXII	Effect of single dose of polygalacturonic acid on Sr^{89} and Ca^{45} levels in blood	112
XXIII	Effect of single dose of polygalacturonic acid on Sr^{89} and Ca^{45} uptake by bone	113
XXIV	Effect of single dose of carrageenin on Sr^{89} and Ca^{45} levels in blood	114
XXV	Effect of single dose of carrageenin on Sr^{89} and Ca^{45} uptake in bone	115
XXVI	Effect of single dose of carbo resin on Sr^{89} and Ca^{45} levels in bood.....	116
XXVII	Effect of single dose of carbo resin on Sr^{89} and Ca^{45} uptake in bone	117
XXVIII	Effect of single dose of Rexyn 101 (Na^{+}) on Sr^{89} and Ca^{45} levels in blood	118
XXIX	Effect of single dose of Rexyn 101 (Na^{+}) on Sr^{89} and Ca^{45} uptake by bone	119
XXX	Influence of sodium alginate on Sr^{89} and Ca^{45} absorption from rat's ligated intestinal segments ..	122
XXXI	Influence of sodium alginate on Sr^{89} and Ca^{45} levels in rat's blood	124
XXXII	Influence of sodium alginate on Sr^{89} and Ca^{45} uptake in rat's femur	126

		page
XXXIII	Effect of increasing concentrations of sodium alginate on Sr^{89} and Ca^{45} absorption	131
XXXIV	Effect of increasing concentrations of sodium alginate on Sr^{89} and Ca^{45} - levels in blood	132
XXXV	Effect of increasing concentrations of sodium alginate on Sr^{89} and Ca^{45} - uptake in bone	133
XXXVI	Effect of continuous administration of sodium alginate in drinking water on Sr^{89} and Ca^{45} uptake..	142
XXXVII	Effect of feeding alginate mixed with lab chow on Sr^{89} and Ca^{45} levels in blood and bone uptake	145
XXXVIII	Effect of continuous feeding with sodium alginate on Sr^{89} and Ca^{45} uptake.....	148
XXXIX	Effect of increasing concentrations of sodium alginate on Ca^{45} and Sr^{89} uptake	151
XXXX	Effect of administration of different doses of sodium alginate on Sr^{89} uptake by bone. 0.2 μc Sr^{89} per day	154
XXXXI	Effect of administration of different doses of sodium alginate on Sr^{89} uptake. Five μc Sr^{89} per day	155
XXXXII	Effect of administration of different doses of sodium alginate on Sr^{89} uptake. Twenty μc Sr^{89} per day	156
XXXXIII	Effect of administration of different doses of sodium alginate on Sr^{89} uptake. Fifty μc Sr^{89} per day	157
XXXXIV	Effect of continuous administration of polygalacturonic acid on Sr^{89} and Ca^{45} uptake in bone	160

CHAPTER I

INTRODUCTION

Strontium^{90} originating from nuclear detonations has now been disseminated throughout the globe and measurable quantities of this radionuclide are present in practically all surface soil, food and human bone. The biological chain of radiostrontium has been extensively investigated in the last two decades. These have shown that strontium is similar but not identical with calcium in its metabolic behaviour in mammals. Intensive studies have been done to establish the quantitative relationships between strontium and calcium in biological systems and to determine the physio - logical processes responsible for their contribution to the differential behaviour of the two elements. The major differences in strontium and calcium metabolism are found to be in those processes in which there is passage of ions across membranes under metabolic control. There is general agreement that gastrointestinal absorption of strontium is of prime importance in the ultimate body burden of this radionuclide.

Earlier work done in the field of intestinal absorption of Ca^{45} and Sr^{89} in this laboratory has revealed that there are significant differences between the two cations and that the minor differences in pH values of different regions of gastrointestinal tract may have some influence on the rate of their transport. This necessitated further studies in the role of H^+ ion concentration on absorption of Ca^{45} and Sr^{89}. An attempt was made to alter the pH in each segment of the intestine, within the physiological range, to determine whether it is possible to alter the absorption rate by the simple method of introducing buffered solutions containing the radioisotope.

The effect of chronic low-level doses of Sr^{90} in human subjects, especially in children, and the methods of preventing this damage have received wide attention. Several procedures have been explored in order to reduce Sr^{90} deposition in the body and to remove the already deposited radionuclide. To reduce deposition the usual method of approach is by reducing Sr^{90}/Ca ratio in the diet either by supplementation with stable calcium and strontium; or by removing Sr^{90} from milk by means of ion exchange resins. To remove Sr^{90} previously deposited in the bones, injections of chelating agents have been given such as EDTA. Substances with biological action such as parathormone have also been used. However, none of these methods effectively reduced the radiostrontium deposited in the bone without adversely affecting the calcium homeostasis.

The problem of Strontium^{90} is one of chronic nature. In fallout debris from thermonuclear tests or war, the radiostrontium contamination of the environment would likely to continue for a number of decades and would have extensive geographical distribution. In these circumstances the most useful method of Sr^{90} decontamination would be to block its gastro - intestinal absorption. A substance was sought which could bind Sr^{++} in the gut. Such a substance should be non-absorbable, should not alter calcium homeostasis, should be non-toxic, easily available, palatable and capable of being administered for prolonged periods of time to both man and cattle, even to the entire life span.

The in vivo binding properties of three naturally occurring macro - molecular acidic polysaccharides and two synthetic ion exchange resins were investigated. Of these, sodium alginate has been most extensively studied because of its high ability to prevent radiostrontium absorption and because it satisfies the aforementioned criteria.

CHAPTER II

STRONTIUM AND ITS METABOLISM

Introduction.

Incorporation of radiostrontium in the skeleton.

Excretion of strontium.

The effects of radiostrontium on living tissues.

The reduction of the body burden of radiostrontium.

CHAPTER II

STRONTIUM AND ITS METABOLISM

Section 1. Introduction.

A large number of radioactive isotopes are produced in nuclear explosions; many of these are of short half-life while others are of little biological significance. Some of these isotopes are produced at low yield and hence are not of serious concern. Isotopes of atomic weight in the region of 90 and 140 are produced most abundantly. In this group are included two isotopes of long half-life, namely, caesium^{137}, an alkali metal with a long half-life of 33 years and which behaves in biological systems in a similar fashion to potassium, and the alkaline earth metal, strontium^{90}. Strontium is chemically very closely related to calcium and follows much the same metabolic cycle. Once lodged in the skeleton, strontium is only slowly replaced by calcium.

Fallout reaches the earths surface by a slow process of sedimentation and ultimately by entrainment or solution in rainwater. Although it can be readily detected at all parts of the earth's surface, the highest levels of strontium^{90} are found in rainwater between the latitudes of 30° and 60° N, which happens to include the most densly populated areas. Computation of the world wide deposition of this nuclide has been carried out by a number of investigators over the past decade. These have shown a several fold increase of Sr^{90} deposition since the inception of thermonuclear explosion.

One megaton of fission is generally considered to yield about 0.1 megacuries of Strontium^{90} (Lindell, 1961) (1). It has been calculated that in thermonuclear reactors, the fission yield of Sr^{90} from slow - neutron fission of U^{235} ranges from 5.4 to 5.8 per cent (Martell, 1959) (2). Therefore, it is evident that industrial plants utilising thermo-nuclear reactors are also a potential source of Strontium^{90} contamina - tion in the environment.

Radiostrontium reaches plants either from soil through the plant roots, or by direct deposition on the aerial surface of the plant. Although there is good evidence that soil retention is an important factor, direct deposition is probably of greater significance. The relative position may change in course of time and varies from country to country. In Japan in 1962 it was observed that in rice plants 40 per cent of the Sr^{90} contamination was from the aerial parts of the plant and the rest from soil (Ichikawa et al) (3). Eighty eight samples tested from 18 states in U.S.A. showed that Sr^{90} levels varied widely between samples within any particular state (Olson, 1962) (4). There is a species difference in the absorption by plants. Legumes take up more strontium than grasses by 3 to 6 times (Vase and Koontz, 1954) (5).

Dairy cattle fed on pasture plants contaminated by radioactive strontium are one of the chief sources of contamination for human subjects.

At each stage in the food chain the nutritional processes of the plant or animal tend to discriminate against Strontium^{90} in favour of

its chemical analogue, calcium. Thus the ratio of radiostrontium to calcium will decrease as the two move together up the chain. At the first stage, the strontium-calcium ratio in the plant varies not only with the ratio in the soil but also with the character of the soil and ground cover, the method of cultivation and type of plant. For example, in a soil matted with roots the radiostrontium coming down from above will not be so quickly diluted with calcium and so will be absorbed by a plant in a higher ratio to calcium.

According to Comar et al (6) the animal metabolism rigorously regulates the strontium-calcium ratio. Animals preferentially incorporate calcium into tissue. In the human body the ratio diminishes further. Where people live on a cereal diet and get most of their calcium from plants, without the intervening discrimination by animal metabolism the drop of strontium-calcium ratio is smaller. In the jungle of the upper Amazon, a few isolated groups of Indians were found to eat a diet containing 6 times more strontium^{90} than the average diet of North America (Kulp, 1961) (7).

Inhalation of air-suspended particles may be another source of radiostrontium. Bair and Smith (1963) (8) investigated the role of the lung in affecting the translocation of inhaled Sr^{90} by exposing a dog to Sr^{90} SO_4 aerosol. About one-half of the Sr^{90} - Y^{90} deposited was rapidly transferred to the bone, and only 2% was retained in the lung one week after exposure. Fifteen percent was cleared via the

trachea, probably by the ciliary processes. But it is unlikely that air-borne particles as a source of contamination are of major impor - tance. Food and drinking water most probably represent the major source of radiostrontium in human bone.

Section 2. Incorporation of radiostrontium in the skeleton.

The disappearance of radioisotope from the circulatory system into which it is injected is a complex function resulting from the simulta - neous operation of several physiological processes. Fixation by bone tissue plays a very important role in the rapid removal of the cation of such elements as calcium, strontium, barium, and radium from the blood. Calcium and strontium in plasma are considered to be present in three forms: protein - bound, complexed and free (9, 10). Calcium and strontium ions are freely and rapidly exchanged between these two forms. The interaction of Ca^{++}, Mg^{++}, and Sr^{++} ions with native and chemically modified human albumin are similar (11), but the relative binding affinity to native albumin decreases in the order: $Ca^{++} > Mg^{++} > Sr^{++}$ ions; modified albumin binds Ca^{++} more than Mg^{++} and Sr^{++} ions. The binding to native, esterified and acetylated albumins is governed mainly by the electrostatic attraction to non-specific sites, with native albumin, specific site binding has also been found.

Radioactive strontium can be detected in all tissues within minutes of either parenteral or per oral administration. For a few hours it circu- lates in the blood and then accumulates in the skeleton. Administration

of parathyroid extract increases the urinary excretion of Ca and Sr as well as increases the deposition of these minerals in kidney tissue (12). Cortisone prevents accumulation of Sr and Ca in the kidney tissue but does not influence the urinary excretion or diuresis.

Strontium traverses the placentral membrane passing from the faetal blood to the maternal blood, and in the reverse direction (13, 14).

The distribution and relative retention of radioactive strontium by the experimental animals have been extensively studied. Pecher (1941) (15) has noted that Calcium^{45} and Strontium^{89}, following intravenous administration, selectively concentrate in bone and teeth. Fifty eight per cent of the dose of Ca^{45} and 33% of Sr^{89} were recovered in the skeletons of mice 24 hours later. After this period, the radioactivity per gram weight of the soft tissue was approximately 1/100 that of the bones. A similar conclusion was reached by Nilsson et al (1962) (16) using the method of whole body autoradiography and impulse counting after intraperitoneal injection of Sr^{90}. Soon after the injection of Sr^{90} activity was seen in all tissues, but gradually accumulated in the hard tissues simultaneously declining in the soft tissues. Within five minutes Sr^{90} activity was greatest in the bones and accumulated specifically in the growth zones. About four hours after injection and subsequently, all noticeable activity was limited to the bony tissues

of the body and a redistribution within the hard tissues could be observed in the autoradiograms.

There is unanimity of opinion that radioactive strontium is incorporated into the areas of active bone formation, such as growing metaphyseal ends of the long bones. Both studies in experimental animals and analysis of human bone samples have shown that in young subjects the relative discrimination against strontium and in favour of calcium is diminished and almost reaches unity (17, 18, 19, 20). It has been postulated that in the very young the turnover of body stores of calcium and strontium must be relatively rapid compared with that in the adults, that is, the calcium and strontium are much more labile. Kahn (1963) (21) and co-workers studying the turnover of radiostrontium in fractured femur concluded that the period of high uptake of Strontium^{89} at the fracture site corresponds to the period of active osteogenesis and the amount of the radioactivity at the fracture site varies with the amount of osteogenesis present at the time of injection. Correlating with histopathological study they noted that the persistence of this radioactivity depended on the degree of bone remodelling and reconstruction that subsequently occurs. A similar phenomenon has been observed in the uptake of radiostrontium by osteosarcomas, the distribution being highly irregular and related to the varying degree of mineralisation and degenerative changes (22).

In young growing animals tibial ends incorporate more Sr^{89} and

Ca^{45} than the incisor, while the opposite was found in older animals (Menezel, 1962) (23). Intravenous injection of Sr^{85} also showed localised high concentration in the spinal metastatic neoplasms (24). Owen et al (1957) (25) reported that following the injection of one microcurie of Sr^{89} per gram body weight to young rabbits, dose rates of the order of 200 rad per hour were measured in localised regions of the epiphyseal tissue of the tibia, representing the dose rate some twenty times greater than the calculated mean skeletal value. The preceding evidence shows that the main factor affecting the deposition of strontium is the calcium requirement of the bony tissue; that is, the degree of osteogenesis. Therefore, the greatest danger of radioactive strontium is in children.

The exact mechanism of incorporation of strontium into the bony structure is not known. The Sr^{++} is associated with bone minerals, the molecular structure is very close to that of calcium hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$. The bone salt occurs in the form of minute rods with a diameter of approximately 45Å and a length of 650 Å. Owing to the smallness of the apatite crystals, bone tissue possesses a large active surface which enables it to serve as an efficient ion exchanger. Sr^{++} may be included into the apatite lattice of the bone crystals; or it may be adsorbed to the surface of the crystals, or a combination of both these mechanisms can occur. Glas et al (1961) (26) studying Sr^{++} in bone minerals by x-ray crystallographic methods concluded that 50 per cent of Sr^{++} incorporated cannot be in the crystalline

interior, and it is likely that the surface adsorption phenomenon can totally account for the Sr^{++} fixation in bone.

Studies by Schulert and co-workers (1959) (19, 20) demonstrated that there is considerable intra-bone variations in Strontium^{90} concentrations. Bone segments analysed indicate that the epiphyses, joint areas, and bone extremities generally have higher concentrations than the midsections of the shafts. The joints average 57 per cent more than limb shafts and also rib ends average 25 per cent more than the midsections. If a threshold exists for the deleterious effects of Strontium^{90} then the highest concentration in a local area of the skeleton is of primary importance since this is the area which will first exceed the threshold. Even with a non-threshold concept dis - tribution is a consideration in evaluating hazard since certain skeletal areas are more susceptible to radiation than others. For example, leukemia is produced by bone marrow irradiation and since about 40 per cent and 25 per cent of the marrow is located in vertebrae and ribs respectively, it is calculated that the marrow is receiving twice the average radiation received by the total skeleton.

Turnover of strontium studies in man are a very complex problem. The situation may be viewed as though strontium were stored in a number of compartments with different turnover rates. The blood, soft tissues, and the rapidly exchanging and the very slowly exchanging areas of the skeleton are to be taken into consideration. The difficulty arises from the necessity of determining turnover over the span of human

lifetime. Although the biological half-life of strontium in animals has been measured by a number of investigators, the half-life of this element in man has been the subject of few studies. Cohn et al (27) has studied the turnover of strontium in adult patients using Sr^{85} and whole body counting. The turnover rates in these patients varied with concurrent pathological conditions such as hypoparathyroidism, hypopituiturism and osteoporosis. In the control group the mean biological half-life was 843 days. By comparison, the mean biological half-life for a group of rats (studied over eight month period) and a group of monkeys (studied over a four - year period) was 980 days (28). The similarity between these two widely different species led the authors of that study to suggest that their figure might also represent the biological half-life of strontium in man.

Section 3. Excretion of strontium.

The deleterious effects of Strontium^{90} in the bone has focused the attention on the mode of excretion of this element from the body. In 1939 MaCance and Widdowson (29) studied the fate of strontium after intravenous administration to normal persons. Over 90% of the strontium was found to be excreted through the kidneys.

The excretion of calcium and strontium was studied simultaneously in adult human beings by the balance radiostrontium and radiocalcium techniques by Laszlo et al (30, 31, 32). In individuals kept on a constant low calcium diets, the isotope is promptly excreted by the

kidney, as the dose of Sr^{85} is absorbed. The excretion of radio - strontium in the urine increases, reaches a maximum by the fourth hour and declines thereafter with time. The main pathway of excretion is via the kidney; approximately half the absorbed dose is excreted by this route. Approximately 10% of the absorbed dose is lost through the intestine as the endogenous faecal strontium. The effect of high calcium intake was studied (approximately 10 times the low-calcium uptake); no significant differences in Sr^{85} excretion was noted. The calculations of renal clearances of calcium and strontium have revealed that although there are individual variations, the amount of plasma cleared of strontium was 3-5 times higher than that of calcium.

Studies carried out with orally and intravenously administered (30, 33) strontium revealed wide variation in the urinary excretion of strontium and calcium. In goats the urinary excretion of Sr^{90} following I.V. dose was 63% and only 6% when administered orally (33). A high excretion of calcium was usually accompanied by a high excre - tion of radiostrontium, but the correlation is not perfect (30). The ratio of strontium to calcium clearance was approximately 5:1 in patients with low urinary calcium excretion and 3:1 in those with high excretion; suggesting that only a limited amount of radiostrontium could be cleared by increasing the urinary calcium excretion.

It is possible that as much or even more strontium may be lost through the intestine if the urinary calcium and strontium excretions

are extremely low (34) as in cases of calcium conservation in hypo - calcemia. Singer et al (35) has studied the excretion into isolated segments of gastrointestinal tract after intravenous administration of Ca^{45} and Sr^{89} into dogs. They noted that both isotopes were excreted in all parts of the intestine; about 20% more of Sr^{89} than Ca^{45}. About 75% of excreted Sr^{89} and 50% of Ca^{45} were found in the small intestine. In the large intestine values were 20% for strontium and 40% for calcium. It was estimated that 6 - 9% of the calcium and strontium excreted was derived from bile; by comparison the amount of biliary strontium was almost twice that of biliary calcium. Tracer studies with radioactive calcium and strontium on rats prepared with an artificial gall-bladder type fistula showed that between 4 - 5% of an injected dose of strontium and calcium is eliminated with bile (36).

The influence of hormones on strontium metabolism is not definitely established. Strontium, which has no accepted physio - logical function, is usually considered together with its chemical analogue, calcium, which is known to be essential for bone and soft tissue. Tweedy in 1945 (37) demonstrated that one injection of 500 U.S.P. units of parathyroid extract usually produced no effect upon the retention of the radiostrontium in the femur or soft tissues; and no change in the excretion of the isotope was observed. However, the injection of 500 units of parathyroid extract 24 hours earlier, and

another 500 units of extract with strontium injection produced the following effects: decreased retention of radiostrontium in the femur; decreased faecal excretion and increased urinary excretion of strontium with marked retention of the isotope in the kidneys. Similar effects were noted by Bacon et al (12). However, when a low calcium diet was given to the animals receiving parathyroid extract an increase in radioactivity by the epiphyseal ends of bones were noted by Goel et al (1958) (38). An increased concentration of isotope in liver, spleen, heart, skeletal muscle, lung and skin were also observed by them. This may be due to secondary deposition of strontium from circulating blood, produced by the parathyroid extract.

In milk secretion, the mammary gland apparently discriminates against strontium and in favour of calcium. Comar et al (39) found that in goat's milk the Sr/Ca ratio ranged from 0.36 to 0.48. In milk from dairy cattle the ratio is reduced about eight fold over that found in the ingested food (40); in human milk the discrimination is further accentuated (41).

Section 4. The effects of radiostrontium on living tissues.

The special hazard of Sr^{90} resides in the fact that it shares with radium and other isotopes of the alkaline earth series the ability to be deposited in the skeletal system, and as seen in the preceding sections once deposited in the bone it is only very slowly replaced by

calcium. There are certain common features in the response of tissues to irradiation. These occur following electromagnetic as well as corpuscular radiation, irrespective of whether the source of irradiation is within or without the body.

Less than a year after Roentgen (1895) reported "a new form of radiation", Stevens (1896) (42) described radiation dermatitis and epilation. Walsh in 1897 (43) reported the untoward effects of acute radiation illness. Shortly thereafter other investigators noted similar reactions. Following these observations and the in - creasing use of x-irradiation in clinical medicine numerous animal experiments were conducted on the biological effects of ionising radiation. Though these studies varied greatly in the methods of exposure and dosage, many fundamental data were obtained. Among these were the selective action of radiation on cellular constituents and different types of cells; the effects on differentiation, mitotic activity and radiosensitivity, the relationship of intensity and duration of exposure and the latency period of exposure reaction.

Although it is generally true that radiosensitivity is related to growth rate, it is clear that the relationship is not linear. In the case of Strontium^{90} because of its mechanism of incorporation in active growth zones of the skeleton, the danger is greatest in growing animals and children. In general, the haemopoietic and germinal tissues are the most sensitive. Thus the effects of radioactive strontium deposited in the bone have to be considered within these wide limits.

Although there is no doubt that ionising radiation may cause cancer in man, it has not been possible to estimate exactly the potential danger of an increase in the level of environmental radioactive strontium. The steady background dose rate of ionising radiation from cosmic rays and the naturally-occurring radioactivity in the earth's crust cannot be reduced and may be assumed to be the cause of a certain very low incidence of cancer in the human population. If this rate is raised by a given increment, an increase in cancer incidence is to be expected. Successive increments would thus be increasingly dangerous.

The maximum permissible dose of Strontium^{90} has been calculated as 1 μc per whole body (43, 44), and the maximum permissible intake as 3 μc. The maximum permissible concentrations in air and water are 2×10^{-11} μc/ml and 8×10^{-8} μc/ml respectively. Other investigators (46, 47) have calculated that 0.1 μc Sr^{90} body burden is a more realistic estimate of maximum permissible for the whole body. Björnerstedt (46) calculated that skeletal tissue receives an average dose of 2.6 rem per year from a total body burden of 1 μc of homogeneously distributed Sr^{90} in an approximately 7000 gm of skeletal tissue per standard man. One thousand rem is considered significant in the production of damage and accordingly this level could be reached in ten years in localised areas of the skeleton.

Whatever opinion one may hold as to the eventual equilibrium of

Sr^{90} level in the human skeleton, there is no experimental means of assessing possible injury, at these low levels. In discussing the probability of bone sarcoma as a result of Sr^{90} deposition, Bugher and Mead (48) made the following assumptions. There is close parallelism between Sr^{90} and radium in the type of hazard and in quantitative aspects of dosimetry. The probability of onset of sarcoma is linearly related to the total radiation dose. There is no known threshold below which the beta radiation from $Sr^{90} - Y^{90}$ is incapable of producing sarcoma. They believed that the majority, if not all sarcomas of bone are caused by radiation either natural or man made. On the basis of these assumptions, detailed calculations have been made leading to the conclusion that minute amounts of Sr^{90} accumulating over several years would cause an appreciable increase in the frequency of death from bone sarcoma.

The late effects of radioactive strontium on bone, the dose - response relationship and the effects of modifying factors have been extensively investigated in our laboratories (49, 50, 51, 52). The rat was selected as the experimental animal in these studies, because the growth apparatus persists long into the adult life in many bones. Sr^{89} was administered intraperitoneally at intervals at a dose of 4.4 µc per gram body weight. After the minimum latent period for tumour production, the following basic tissue changes were observed in the bone: I. disturbance of osteogenesis; II. fibrosis of the marrow;

III. cellular proliferation; and IV. tumour formation. These changes occurred to the greatest extent in the metaphyseal regions, which represent the area of maximum bone formation.

The greatest fibrotic changes replacing normal haemopoietic marrow occurred in the metaphysis. Fibrosis was on the whole of a relatively acellular type with abundant reticulum fibres and only minimal collagen formation. In the metaphyseal area a marked decrease in the number of trabecula was observed indicating a depression of osteogenesis. In some animals a deeply basophilic fiber bone was present in this region, indicating abnormal osteogenesis, as this type of bone is normally not found in the growing or adult animal. Changes secondary to decreased mechanical stability such as healing of microfractures, endosteal and periosteal new bone formation were also found. Whether fibrosis and osteogenesis are interrelated, is not known. It is possible that fibrosis observed in these animals was related to the primary effect of irradiation; on the other hand it may represent merely a repair tissue compensating further lack of bony trabecula.

In all animals in this study foci of cellular proliferation were present in the metaphysis as well as in proximity to endosteal and periosteal surface. These foci exhibited a considerable variation in size. Some consisted only of a few cells and others were in large groups; large hyperchromatic nuclei and mitotic figures were almost uniformly present. In connection with some larger collections of cells

evidence of invasion and destruction of normal structure was found, indicating that these cells were truly neoplastic.

Tumours demonstrable grossly or by x-ray were found in 89% of animals surviving the minimal latent period, while on microscopic examination neoplastic changes were found in all such animals. The distribution of gross tumour occurred in the following order of frequency: upper end of tibio-fibula, vertebrae, humerus, femur, and sacrum. Histologically, tumours were osteogenic sarcomas, chondrosarcomas and fibrosarcomas. These showed varying degrees of differentiation.

After administration of 2 μc per gram body weight or higher doses, malignant bone tumours were found in some rats which survived one year or longer. When 5 μc per gram body weight was administered as a single injection all animals died in the immediate post injection period. Doses of 2 - 3 μc per gram body weight given in multiple injections did not produce tumours during the same period of time. That is, the rise in radioactivity following repeated injections did not exhibit a linear pattern. A possible explanation is that a higher concentration occurs in bone which is held for a short period before slow elimination takes place following a single dose. The same total dose administered in repeated injections over a longer period of time would not accumulate to the same level as the single dose.

The group of animals receiving higher doses also exhibited chronic

radiation sickness such as loss of weight, loss of hair, epistaxis, malaise and finally tumour, or death.

Similar results were observed by the administration of radio-strontium in the experimental animals by other investigators (53, 54, 55, 56, 57). Henricson et al (58) studied the effect of Sr^{90} on mouse testis. A quantitative effect on the testicular epithelium was obvious in 5 to 35 days after I.P. or I.V. injection. The type B spermatogonium did not seem to have ceased but to some extent was retarded, while type-A spermatogonium was severely affected. Qualitative changes in the first meiotic division were noticed during the damage period, and signs of abnormal distribution of chromosomes in the anaphase appeared during the recovery period.

Budko et al (59) administered a mixture of Sr^{89} and Sr^{90} in different doses up to 1500 µc either as a single orogastric administration or continuously in drinking water to rats resulting in acute radiation sickness. Panmyelophthisis with sudden and marked haemorrhagic syndrome were noticed. During the subacute form of the sickness, a depression of the medullary hemopoiesis with marked pathologic regeneration of the reticulo-endothelial stroma, intensive ectopic splenic hemopoiesis, degenerative changes of the parenchymatous organs, gingivitis, otitis and ulcerative enteritis were observed. In chronic radiation syndrome, a compensatory aplastic anaemia with pronounced pathological regeneration of the reticulo-endothelium of

the bone marrow, leukemia and sarcomatous lesions were demonstrated.

The effect of radiation from Sr^{90} that is acquired by eating contaminated food continuously for a long period of time has been investigated by Finkel and co-workers (60). Two groups of mice were maintained on a special diet containing Sr^{90}; one group was started at 110 to 250 days old and the other was exposed to Sr^{90} from con - ception. Levels of dietary Sr^{90} as high as 10 μc/gm Ca did not adversely affect conception, parturition nor lactation. The median survival time was reduced. The median life span of mice exposed to 10 μc Sr^{90}/gm Ca from conception was reduced by about 41 per cent. A decrease in survival time was also apparent among the mice reared on 5.0, 2.5, and 1.0 μc Sr^{90}/gm Ca. Besides an increase over the normal incidence of tumours of the blood forming tissues, there have been epidermoid carcinomas, osteolytic tumours of the skeleton, and osteogenic sarcomas.

All evidence suggests that irrespective of its route of entry radiostrontium is capable of producing neoplastic lesions and appre- ciably affects the life span of the organism.

Section 5. The reduction of the body burden of radiostrontium.

Several different approaches have been made to the problem of reducing the body burden of radiostrontium. Many attempts have been made to remove radiostrontium already deposited in the bones. Other workers have sought to reduce the amount of radioisotopes available

for deposition either by decreasing the specific activity of ingested Sr^{90} (by the addition of carrier Sr) or by increasing the intake of minerals essential for bone development. A further and more practicable approach has been to reduce the amount of radioisotope in food. In particular the treatment of milk has received much attention.

It has been noted by a number of investigators that there exists a correlation between urinary excretion of calcium and strontium. Laszlo and Spencer (31, 61) have given intravenous calcium gluconate and ammonium chloride orally (as diuretic), and by the combination of calcium by either route in conjunction with ammonium chloride. Although the urinary Sr^{85} excretion increased concomittantly with that of calcium the reduction of body burden of Sr^{85} was insignificant. Carlquist and Nelson (62) gave intraperitoneal injections of calcium chloride and calcium lactate and noted that there is significant increase in the retention of Sr^{90} with an increased calcium dose.

Magnesium ion is a potent hypercalciuric agent. Since strontium resembles calcium in its affinity for bone and in its elimination from the body, magnesium was tested for its ability to increase the elimination of radioactive strontium (63). Magnesium was effective in increasing the elimination of Sr^{89} by rats when given parenterally; however on oral administration of magnesium, a decrease in faecal Sr^{89} was noted. Using the technique of peritoneal lavage Talmage (64) showed that magnesium has no direct action on bone, but acts on the

kidney to increase urinary calcium.

Zander - Principati and Kuzma (65) suggested that as zirconium can compete with strontium in many chemical reactions, zirconium salts may displace strontium in biological systems. They injected zirconium citrate intraperitoneally and observed that the incidence of tumour due to Sr^{90} in the treated animals were considerably lower. On the other hand Schubert (66) observed no specific effect on thorium or strontium metabolism by zirconium citrate other than that associated with the citrate ion alone.

The effects of various endocrine secretions in stimulating radio-strontium elimination from the body were investigated by a number of research workers (67, 38, 50). Growth hormones, thyroid extract, parathyroid extract and cortisone were used in these studies. No significant change either in uptake of strontium or in tumour incidence was noted. Moreover, the use of hormones in excess quantities derange the normal endocrine balance of the body and thereby produce various undesirable effects. The use of growth hormones in children is contraindicated as a variety of clinical problems would certainly result.

It has been shown that the ratio of uptake of Ca^{45} to Sr^{85} by bone from serum or serum ultrafiltrate is lower than from synthetic ultrafiltrates, and it has been suggested that this lowering of ratio is due to the presence of serum proteins and natural chelating agents (68). Therefore, it seemed possible that strong chelating

agents could be used for removal of bone-seeking radioisotopes. The effect of strong chelating agents in buffered solutions on the relative uptake of Ca^{45} and Sr^{85} by defatted bone were studied by Samachson (68). Ethylenediaminetetraacetic acid and cyclohexane - diaminetetraacetic acid, decreased the ratio of Ca^{45}/Sr^{85} uptake considerably in the presence of calcium, calcium plus strontium or strontium carrier. Citrate and adenosine phosphate had similar but weaker effects. No effect was shown by glucose, lactate, gluconate, bicarbonate, bicarbonate plus phosphate, glutamate, aspartate, borate, glycerophosphate, lysine and glutathione. These and several other complexing reagents, antibiotics like tetracycline were used in order to reduce the body burden of radiostrontium (69, 70, 71, 72, 73, 74, 75, 76). These studies were carried out both in animals and in man. Though they had varying degrees of efficiency on radio-strontium elimination, their action was insufficient to reduce effectively the body burden of this nuclide. Moreover, they pro - duced a hypocalcaemic effect if given in doses sufficient to reduce the radiostrontium content of bone, because almost all the known chelating agents combine with calcium more stably than with strontium. Calculations based on mass action equations led Schubert (77) to conclude that chelating agents such as EDTA and similar polyamino agents are impractical in removing strontium from bone. To be useful, the binding affinity of Sr^{++} would need to be at least from 10^3-10^4

times stronger than that of Ca^{++}.

Effects of Carrier Strontium

Harrison et al (78) administered doses of carrier strontium with radioactive strontium to rats; skeletal retention and excretion were measured 24 hours later. They found that the amount of Strontium^{89} absorbed from the alimentary tract was approximately proportional to the dose but the administration of carrier strontium had little effect on the uptake of Sr^{89}. While Hegsted and Bresnahan (79) found that the addition of 0.01% of stable strontium to the diet, sufficient to decrease the specific activity of the dietary strontium to about one-fourth that in the basal diet, actually resulted in a slight but consistent increase in deposition of Sr^{89}. Addition of greater amounts of carrier Sr resulted in decreased deposition of Sr^{89}, but this was no more effective than similar quantities of calcium. Calcium lactate or inactive strontium chloride effected an increase in Sr^{90} and Ca^{45} excretion in fowl (80).

A number of investigators have used increasing amounts of calcium and phosphate in the diet in order to increase gastrointestinal discrimination against strontium. MacDonald (81) and co-workers reported on the effect of a series of common food materials, on Sr^{90} absorption and uptake. They suggested that the potential hazards from ingestion of foods contaminated with radiostrontium are somewhat diminished in proportion to the calcium and phosphate concentration

existing in the foods, naturally or by enrichment.

Elevated dietary calcium levels with or without increased phosphorous levels produced an almost proportionate reduction in the body burden of dietary radiostrontium in immature rats. (Wasserman and Comar) (82). In mature rats elevation of both calcium and phosphorus levels in the diet was necessary to have even a slight reduction of radiostrontium uptake. On the other hand, Van Putten (83) noted reduction of radiostrontium retention in mice fed with phosphorus deficient diet. Harrison (84) observed that by doubling the oral intake of calcium, skeletal retention of Sr^{89} was decreased in immature rats but not in adult rats.

Other dietary substances and complexing reagents such as tannin, citrate, phytate, and sodium ethylene diaminetetraacetate were without effect in reducing the body burden of continuously ingested radiostrontium (85). Wasserman (85) demonstrated that the presence of two to four per cent of tannin significantly increased the Sr/Ca ratio in femur. A similar effect was observed with a liquid milk diet containing sodium EDTA . This may be due to the fact that both these substances bind calcium ion to a somewhat greater degree than strontium ion.

MacDonald et al (86) studied the effect of oral administration of various agents such as $MgSO_2$, Na_2SO_4, ammonium salt of amido - phosphate, cation exchange resins, calcium phytate, pectin, bran cereal, castor oil, etc. and failed to observe any significant

reduction of Sr^{++} accumulation in the skeleton. The use of ion exchange resins for early treatment of radioactive contamination in the digestive tract was also investigated by Michon and Guilloux (87). All the cationic resins used were effective, especially the sulfonic resins administered in the ammonium form. However, repeated administration of the resins produced considerable reduction in the speed of digestive transit and a marked decrease in the efficiency of the treatment developed.

As the removal of Sr^{90} from bones appeared to be so difficult, a new approach was necessary. Several investigators (88, 89, 90, 91, 92, 93) successfully removed radioactive strontium from liquid milk with the use of ion exchange resins. Several methods have been proposed. Migicovsky (88) submitted a procedure which was later improved by Edmondson et al (91). This method is based on the use of a bed of resin (Dowex 50 W - X 12) with a mixed solution of calcium, potassium, sodium and magnesium ions. In pilot plant experiments this process proved capable of removing ninety per cent of the radiostrontium in milk. The C form of resin was more effective in the removal of isotope than Na form (92). However, these methods are not only laborious and expensive but also produce some undesirable changes in the nutritional value of the milk. All plant materials as well as meat derived from the areas of contamination would also contain radioactive strontium and would present much greater difficulties in Sr^{90} decontamination by this method.

CHAPTER III

STRONTIUM - CALCIUM DISCRIMINATION

CHAPTER III

STRONTIUM - CALCIUM DISCRIMINATION

All secretory and excretory processes which have been studied in man and animals are able to discriminate between calcium and strontium. Thus the kidney clears plasma of strontium at a rate 3.5 times that of calcium (94, 95, 96, 97). There is also small but preferential endogenous excretion of strontium over calcium into the gastrointestinal tract (35, 98). On the other hand, calcium is preferentially transported to milk and the strontium/calcium ratio of milk averages one tenth that of mother's diet (39, 40, 41). An adult man is capable of absorbing twice as much calcium as strontium from dietary sources. From such data, it can be seen that there is an overall biological discrimination against strontium. This physio - logical behaviour has been summarised by the term "Observed Ratio" which represents the discrimination and is defined as:

$$\text{OR Sample/Percursor} = \frac{\text{Sr/Ca in sample}}{\text{Sr/Ca in precursor}} \quad (39)$$

The term "Discrimination factor" is used to sum the contribution of each of the physiological processes to the overall discrimination

$$\text{OR} = (\text{DF absorption})\ (\text{DF milk})\ (\text{DF urinary})\ \text{etc.}$$

The OR body/diet has been determined in many animals and ranges generally between 0.2 and 0.5. In humans and rats the OR falls with age; in beagles

however, although absorption of each element falls rapidly with age, the OR remains constant (99).

The OR for each physiological process has been determined in many cases. The values indicate that absorptive discrimination makes a much greater contribution than any of the other processes.

In the problem of reducing the body burden of radioactive strontium, it seems obvious that an attempt should be made to interfere with the uptake of the radionuclide through the absorptive phase.

The investigations to be described in this thesis have followed two main pathways; these are, firstly, a study of the factors influencing absorption of strontium from the gastrointestinal tract and secondly, a search for materials which would reduce or inhibit absorption.

CHAPTER IV

ABSORPTION OF CALCIUM AND STRONTIUM FROM GASTROINTESTINAL TRACT

Introduction.

Factors affecting absorption.

The role of pH in the absorption of calcium and strontium.

CHAPTER IV

ABSORPTION OF CALCIUM AND STRONTIUM FROM GASTROINTESTINAL TRACT

Most of our present knowledge of strontium absorption has been deduced by comparison with calcium absorption. The intestine plays a much greater role in the homeostasis of calcium than other tissue electrolytes such as sodium and potassium (100). Strontium, on the other hand, is a physiologically non-essential element, and may not follow the same mechanism of gastrointestinal absorption.

Absorption is a complex process which depends on simple diffusion, osmotic and hydrostatic pressure differences on both sides of the mucosal wall (101, 102, 103, 104) and also on more elaborate chemical mechanisms. The penetration of membranes through pores in the cell wall has also been postulated. However, although such pores could account for the rapid transit of small, hydrophilic non-electrolytes such as glucose and urea, and for the passage of electrolytes, depending on their charge density and size, no pores have so far been actually demonstrated. The lipid layer of cell membranes may assist in the penetration of hydro - phobic molecules by solution.

In general however, nutritionally essential substances are transported across the mucosal wall by "active transport" or by "facilitated diffusion". The former brings about net transfer against an electrochemical potential gradient and is an energy requiring process. A "facilitated diffusion" mechanism makes possible a rapid transit of

solute but does not require energy nor can bring about net transport against a potential gradient. Wasserman (40) using surviving isolated intestinal segments observed that in the duodenal segment Ca^{45}, but not Sr^{85}, was transported against a concentration gradient from mucosa to serosa. On incubation of segments at 0°, under N_2 or in the presence of respiratory inhibitors "active transport" of calcium was eliminated, but strontium was not affected, indicating that calcium is probably absorbed by an "active transport" process.

Factors Affecting Absorption

The role of hormones on the rate of absorption from the gastrointestinal tract is far from clear. The early literature presents no clear evidence on the effect of parathyroid on the intestinal absorption of calcium. More recently Talmage and Elliott (105) reported that parathyroidectomy resulted in reduced radioactive calcium absorption from ligated rat's intestine in vivo. Rasmussen (106) noted a decreased Ca^{45} accumulation on the serosal sides of sacs prepared from duodenum of parathyroidectomised rats. On the other hand, Gran (107) failed to observe lowered calcium absorption in parathyroidectomised rats. Wasserman and Comar (108) reported no significant change of Ca^{45} or Sr^{85} absorption in parathyroidectomised young rats. Cramer (109, 110) and co-workers noted that administration of parathyroid extract in dogs causes increased absorption of dietary calcium from the gastrointestinal tract. They also reported significant reduction in the calcium absorption rate 24 hours or

more after thyroparathyroidectomy and a restoration of the normal calcium absorption rate within one day after administration of parathyroid extract.

A state of deficiency usually increases the rate of absorption. Thus calcium is absorbed more readily by individuals suffering from calcium deficiency. The apparent absorption of calcium in adults is 20% or less of the daily intake of this element (111). On the contrary, in children for whom calcium demand is high, the absorption rate reaches a much higher degree of efficiency. Strontium being a physiologically non-essential element is unlikely to be influenced by this factor. However, it seems likely that in the case of calcium deficiency, the chemical analogue strontium may be absorbed more readily. Similar examples of increased absorptive efficiency are demonstrated in patients on salt restricted diets, when sodium practically disappears from the contents of the terminal ileum, suggesting a sodium conserving action by the upper intestine. With the use of radioactive iron it has been demonstrated that increased gastrointestinal absorption takes place if normal human subjects are suddenly exposed to high altitudes of about 15000 feet(112), which stimulate a polycythemia and consequently an increased demand for iron (112).

The influence of Vitamin D on absorption and bone uptake of both calcium and strontium has been studied extensively, but is not clearly understood. Vitamin D markedly increases calcium absorption from the large intestine and the distal ends of the small intestine in rats, but has little effect on absorption from the proximal end of the small intestine. It has been suggested that Vitamin D increases the

efficiency of calcium absorption only under conditions in which intestinal calcium is poorly soluble (114, 115). Lengemann (116) noted that the amount of calcium and strontium absorbed was increased by the presence of Vitamin D, but the Sr/Ca ratio remained the same, showing that the effect was on the mechanism or mechanisms involved in absorbing both alkaline earth elements. Schachter et al (117) placed Vitamin D_3 directly into loops of rat's duodenum in Vitamin D deficient animals. Subsequently there was a marked increase in the transport of calcium by slices of the duodenum in vitro. Under similar conditions, the same dose of Vitamin D_3 given intravenously or placed in a jejunal loop had little or no effect on the duodenal tissue. This evidence suggests that Vitamin D acts directly on the small intestine without prior mediation in another organ.

Wasserman et al (118) observed that certain amino-acids such as lysine, arginine, and also lactose and other organic compounds increase the absorption of Ca^{45} and Sr^{89} in the rat. The effect of lactose was not inhibited by metabolic inhibitors such as phlorrhizin (119). Excess of magnesium or phytic acid is said to interfere with the absorption of calcium.

The various anatomical regions of the gastrointestinal tract show different rates of absorption for different metabolites. In the case of iron, Brown (120) noted that it was taken up equally well by all the segments in vitro; however in vivo studies showed a distinct gradient, with the highest rate in the duodenal region and progressively decreasing towards the distal part of the small intestine. Scholar and

Code (121) using heavy water as tracer noted that water is ten times more rapidly absorbed from the small intestine than from the stomach. Benson and co-workers (122) by using radioactive iodine^{131} labelled olive oil found that optimal absorption of fat occurs in the third quarter of the small intestine. Kremen et al (123) demonstrated that in dogs the proximal 50 to 70 percent of the small intestine could be removed with no apparent ill effect on fat absorption. Studies in experimental animals and in human subjects have demonstrated that the proximal bowel in general and duodenum in particular has the maximum absorptive capacity (120, 124, 125, 126, 127).

Lengemann and Comar (128) had shown that in rats maximum rate of absorption takes place from the duodenal and proximal jejunal regions. Others have also confirmed similar results (40). On the other hand, Cramer and Copp found that although the highest initial rate occurred in the duodenum, the greatest total absorption took place in the ileum (129).

An in vivo technique developed by Skoryna and Dukay (130) in which the small intestine was divided into nine segments of equal length, has been utilized by Dukay to study the absorption of Ca^{45} in detail. The radioisotope was injected into the ligated segment which remained in situ in the abdominal cavity, with vascular and lymphatic system intact, throughout the experiment. Dukay showed that Ca^{45} had a maximum rate of absorption from the proximal part of small intestine. In thirty minutes as much as 65% of the total dose was absorbed from the duodenum whereas in the distal half of the small intestine less than 10% was absorbed in

that time. Within sixty minutes the absorption from the duodenum was increased to 79% while it was almost complete (97%) in 120 minutes. Dukay further noted that ligation of common bile duct reduces the absorption of a slightly acid solution containing Ca^{45} from the duodenal segment. However, if Ca^{45} is administered in a solution of neutral pH or if the test solution is kept in the duodenal segment for over sixty minutes the percentage absorption is unaffected by the ligation of common bile duct. These results suggested that absorption of calcium takes place only from a neutral milieu. When the common bile duct was ligated, the supply of alkali to neutralize the acid solution of radioisotope in the duodenum was cut off.

The Role of pH in the Absorption of Calcium and Strontium

The precise effect of variations in H^+ ion concentration on the absorption of calcium is not known. Mraz (131) observed very little change in the rate of absorption in rats when he perfused the entire small intestine with unbuffered solutions of various pHs in range of 5 to 11. In the range of pHs 2 to 4 absorption of Sr^{85} increased but Ca^{45} did not change. There was a sharp drop of Ca^{45} absorption at the extreme pHs 1 and 12, probably as a result of damage to the mucosal wall. The solutions used in these experiments were mainly outside of the physiological range of pH found in the intestine. Moreover as the solutions were unbuffered, continuous secretory and excretory activities of the intestinal mucosa would alter the pH of the perfusate.

Dukay (130) studied the effect of introducing buffered solutions of Ca^{45} at different pHs (in the range 4 - 8) on the rate of absorption from duodenal segments. The common bile duct was ligated beforehand in order to eliminate the neutralising effect of bile. At all pHs tested, except for that in the physiological range, that is, at a pH~7.0, the rate of absorption was markedly reduced. In a previous investigation (132), the present author measured the pH obtaining in different regions of the small intestine and compared them with variations in the rate of absorption of Sr^{89}. It was observed that as the pH increased the rate of absorption decreased. By introducing a solution of sufficient buffer capacity, it was found possible to stabilize the pH at a predetermined value in each ligated intestinal segment. In this way the influence of small changes in pH on the rate of absorption of strontium could be observed. In the preliminary investigation (132) the absorption rate of strontium was decreased in the duodenum by decreasing the pH and conversely the rate for more distal segments was increased. Further studies on the influence of pH on the absorption of both strontium and calcium will be described in a subsequent chapter.

Although the rate of absorption differs from segment to segment, there appears to be no limit to the total amount of calcium or strontium absorbed, provided that the time of contact is sufficiently long. From this it must be assumed that the mechanism of absorption of strontium from each segment must be the same, and that variations in absorption rate depend on environmental conditions in the lumen.

CHAPTER V

FACTORS AFFECTING ABSORPTION AND DISTRIBUTION OF STRONTIUM^{89}

Introduction.

Materials.

Methods.

Radioactivity assay.

Sr^{89} absorption and uptake after a single oral dose.

Ca^{45} absorption and uptake after a single oral dose.

Sr^{89} and Ca^{45} uptake after continuous administration.

Effect of change in pH on Sr^{89} and Ca^{45} uptake from ligated intestinal segments.

Effect of pH on Sr^{89} and Ca^{45} uptake after single orogastric administration.

Effect of pH on Sr^{89} and Ca^{45} uptake by adult female rats.

Effect of bile on Sr^{89} and Ca^{45} absorption.

Discussion.

CHAPTER V

FACTORS AFFECTING ABSORPTION AND DISTRIBUTION OF STRONTIUM^{89}

Although the mechanism of absorption can be studied by employing in vitro techniques, the physiological environment of normal intestinal absorption is appreciably altered, and the results obtained cannot strictly be compared to the natural process. The most physiological method of studying the intestinal absorption is the "material balance" or "physiological disposition" study, whereby the substance of interest is fed by mouth and its appearance or that of its by-product is measured in blood, urine, tissues or stool. In some experiments it is necessary to give the entire dose at once; frequently this entails intestinal intubation under light anaesthesia. However, in order to determine the rate of absorption from different regions of the intestine, surgically prepared intestinal segments, remaining with the vascular and lymphatic supply undisturbed are essential. The availability of radioactive isotopes has considerably improved the accuracy of these in vivo studies.

The experimental period chosen for absorption to take place in all the experiments with intestinal segments was limited to thirty minutes; previous work had shown that over this period the rate of absorption for both calcium and strontium was maximal (130, 132). To establish a norm for studies on pH changes and also for the efficiency of binding agents (to be described in the following chapter) the

absorption pattern of both Ca^{45} and Sr^{89} from nine segments of the small intestine were studied, with simultaneous studies on the blood levels and bone uptake.

Blood levels and bone uptake at various intervals after intubation of solution of radioisotopes were estimated. The distribution of Sr^{89} in bone after continuous administration of the isotope in drinking water and mixed with food was also studied.

Role of pH in absorption

As discussed in the preceding chapter, earlier investigations suggested that the pH obtaining in the lumen of the intestine had a role definite in the rate of absorption of both calcium and strontium. To extend these studies, the effect on absorption of increasing the pH in all regions of the intestine was investigated by injecting the isotopes in an alkaline buffer. Tris(hydroxymethyl)aminomethane was chosen as buffer in this pH range as it is known to be non-toxic and forms soluble salts with strontium and calcium. A phosphate buffer, used to reduce the pH to slightly more than 6.0 in the second experiment of this series, forms soluble calcium and strontium salts at this pH.

The effect on absorption and bone uptake of oral dosing with buffered solutions of radioisotopes was compared in young and mature adult rats. The young rats were six weeks old and weighed 100 to 120 grams. Adult female rats (300 - 350 grams) who had had several litters previously were chosen for comparison, assuming that these animals may be in a state of negative calcium balance.

Materials

Rats of Royal Victoria Hospital strain of either sex were used. These rats were bred in the Department of Experimental Surgery, McGill University. The exact origin of this strain remains unknown, but it is believed that these rats are the offspring of Wistar and Norwegian wild rats. The colony has been thoroughly inbred by con - tinuous brother - sister matings since 1933. Animals aged about six weeks old, weighing 100 to 120 grams, were used throughout the experiments with the exception of a few adult rats of 300 to 350 grams which were studied to demonstrate the effect of age on strontium absorption.

Radioactive Strontium^{89} and Calcium^{45} were supplied by the Atomic Energy of Canada Limited, Ottawa. Both the isotopes were in the form of chlorides in aqueous solutions of HCl. Strontium^{89} was supplied with a high specific activity, and no significant amount of inactive salt. Calcium^{45} contained an appreciable amount of inactive $CaCl_2$. Previous studies had demonstrated that addition of inactive strontium chloride produces a significant reduction in the absorption of radio-strontium and also more consistent results were obtained (132).

Methods

Three in vivo techniques were used in this investigation.

1. Intubation.

The animals were fasted for 24 hours and then anaesthetised under light ether anaesthesia. The test solution was introduced into the stomach through an orogastric tube, Fr. 8 size red rubber catheter.

Groups of animals were sacrificed at different time intervals, one ml. of blood and one femur were assayed for radioactivity. Animals kept for longer than a six hour period were given food and water ad libitum.

2. Feeding and drinking ad libitum.

The test materials were either dissolved in drinking water or mixed with food. At regular intervals, groups of animals were sacrificed and one femur assayed for radioactivity. In this method the test solution or food was discontinued 24 hours prior to sacrifice and animals were allowed to have their regular food and water. This procedure was adopted because studies with the intubation method demonstrated that in about 24 hours time the amount of radioisotope deposited in the bone and that circulating in blood reached a fairly stable equilibrium. Radioactive strontium or calcium uptake in one femur was considered as a reliable index of the total uptake by the skeleton of these isotopes. This has been proved previously by a number of investigators (75, 79, 86, 133).

3. Ligated intestinal segments.

This technique involving an operative procedure has been developed and extensively used in this laboratory by Skoryna and Dukay (130). The animals were fasted for 24 hours and then anaesthetised with nembutal. The anterior abdominal wall was shaved and laparotomy performed. The stomach was ligated with silk at the oesophagus and pylorus and was designated as segment No. I. The total length of small intestine was divided into nine segments of approxi - mately 5 cms each. The duodenal segment was numbered II and the rest

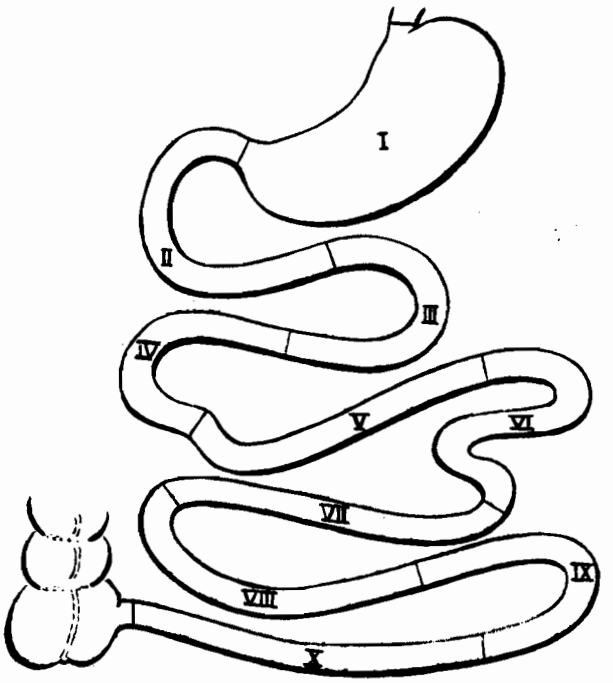


Fig. 1 Schema of division of small intestine by ligation into nine segments (II - X). The stomach is segment I.

in serial sequence, the terminal ileal segment being number I. A schematic diagram showing the segments is given in Fig. 1. Each segment was ligated at both ends without disturbing the vascular or lymphatic supply. A diagram showing a typical ligated intestinal segment is given in Fig. 2. Only one segment was used, in each rat. Test solutions were introduced into the lumen of the intestinal segment by means of a disposable tuberculin syringe with a 27G needle. A standard volume of 0.5 ml was used in all the experiments, as this amount was found to be the optimal fluid capacity of a 5 cms segment of the small intestine. Any increase in volume gives an undue distention of the segment which may alter the absorption pattern. It was noted that a tiny drop of fluid escaped at the moment when the needle was withdrawn from the intestinal wall. This was demonstrated by using methylene blue solution as well as with radioactive solutions. Since this loss of fluid into the peritoneal cavity was constant and uniform, it was considered that the effect of leakage can be discounted. Throughout the experiments, the animals were kept alive at room temperature and under laboratory conditions. After thirty minutes the animals were sacrificed. The ligated segment was then stripped of its mesentry, resected with ligatures intact and analysed. When radio - active isotope solutions were used, simultaneously one ml. of blood and one femur were removed to assay the radioactivity.

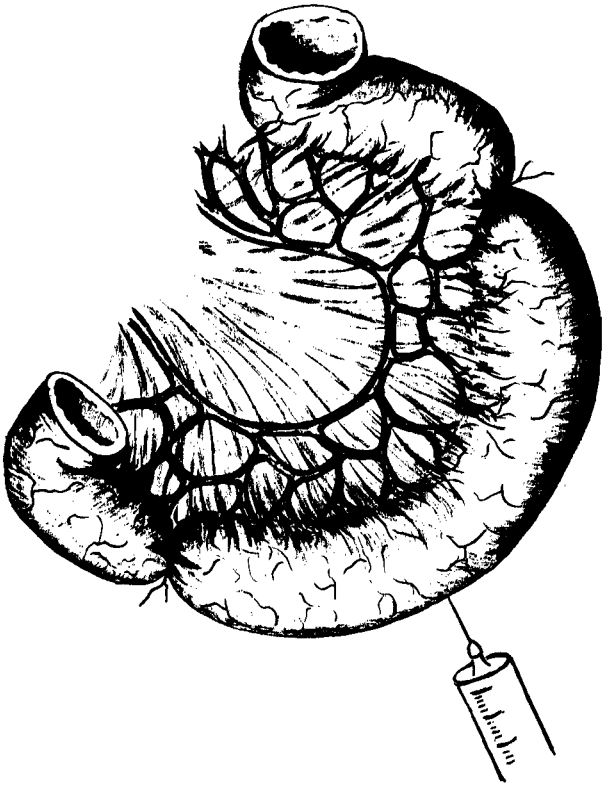


Fig. 2 Schematic drawing of a ligated intestinal segment. The segment was left in continuity with the remaining small bowel.

Radioactivity assay

The tissues removed for radioactivity determinations were treated in the following manner: one ml. of blood was collected into a tube containing 2 ml. of sodium citrate solution. The excised intestinal segments and femur were placed in 3 ml. and 2 ml. respectively of 38% HCl for 24 hours, by which time they were completely dissolved in the acid. The acidic solutions were neutralised and suitably diluted. One ml. aliquot samples were dried in an oven and counted.

In the early part of this study counting was carried out in a shielded Manual Changing Chamber, using End - Window Geiger tubes having a thin window thickness of 1.9 mg/cm^2 (Tracer Laboratory Inc., Boston). An electric circuit with Dekatron type of Scaler (Measurement Engineering Limited, Ontario) was used to register the counts. Only one series of experiments using ligated segments was done using this equipment. In all the other experiments, radioactivity was measured in a Nuclear - Chicago Model D 47 Gas flow detector with a micromil window with C 110 B Automatic Sample Changer; 181 B decade scaler and C 111 D printing timer. A constant flow (50 cc/minute) of 1.3% Butane and 98.7% helium gas mixture was maintained during detection and was operated in the Geiger region. The background count was less than 16 c/m.

Each sample was counted to give a minimum of 5,000 counts. A constant geometrical set up was used in all measurements. All the counts were corrected for self absorption.

EXPERIMENTS AND RESULTS

Sr^{89} absorption and uptake after a single oral dose

Strontium^{89} was diluted with inactive strontium chloride solution to give a final concentration of 0.9 μM and 10 μc activity per ml. The animals (100 - 120 gram rats) which had fasted for the preceding 24 hours, were given one ml. of this radioactive test solution through an orogastric tube under light ether anaesthesia. Groups of six animals were sacrificed at intervals of 2, 4, 6, 24 and 72 hours after dosing. Those animals kept for longer than 6 hours were allowed food and water ad libitum. One ml. of blood and one femur were removed for radio - active assay.

The resulting distribution of Sr^{89} in blood and bone is shown in Fig. 3 and Table I. The blood level of Sr^{89} was at a maximum at about the 2 hours period; thereafter, it declined rapidly. In 24 hours, Sr^{89} blood level was less than 6% of the 2 hour level. By 72 hours no significant amount of radioactivity was detected in the blood. Sr^{89} uptake by bone reached a peak by 6 hours; thereafter, it declined and by 72 hours it had reached a stable level. It can be assumed from these results that most of the gastrointestinal absorption of Sr^{89} was complete by 2 to 4 hours, and that the maximum rate of absorption would have been reached before 2 hours, as shown by the Sr^{89} level in blood. From the steep increase in bone Sr^{89} from the second hour to the sixth hour, it is evident that most of the radiostrontium is deposited in

TABLE I

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ or 10 microcuries of Ca^{45} in 1.1 μM of inactive $CaCl_2$ was administered through orogastric tube.

	BLOOD		BONE		
Time	Sr^{89}	Ca^{45}	Sr^{89}	Ca^{45}	O. R.
2 hours	10006 (±2506)	8288 (±2084)	60206 (±12592)	103433 (±32544)	0.58
4 hours	4153 (±664)	4625 (±696)	88398 (±12923)	144041 (±32185)	0.62
6 hours	2685 (±983)	2837 (±941)	95826 (±28312)	120508 (±44173)	0.80
24 hours	569 (±188)	1495 (±296)	74501 (±11192)	141283 (±18792)	0.53
72 hours	----	----	65503 (±19618)	132600 (±15281)	0.49

Each figure represents mean counts per minute and standard deviation for studies in six animals.

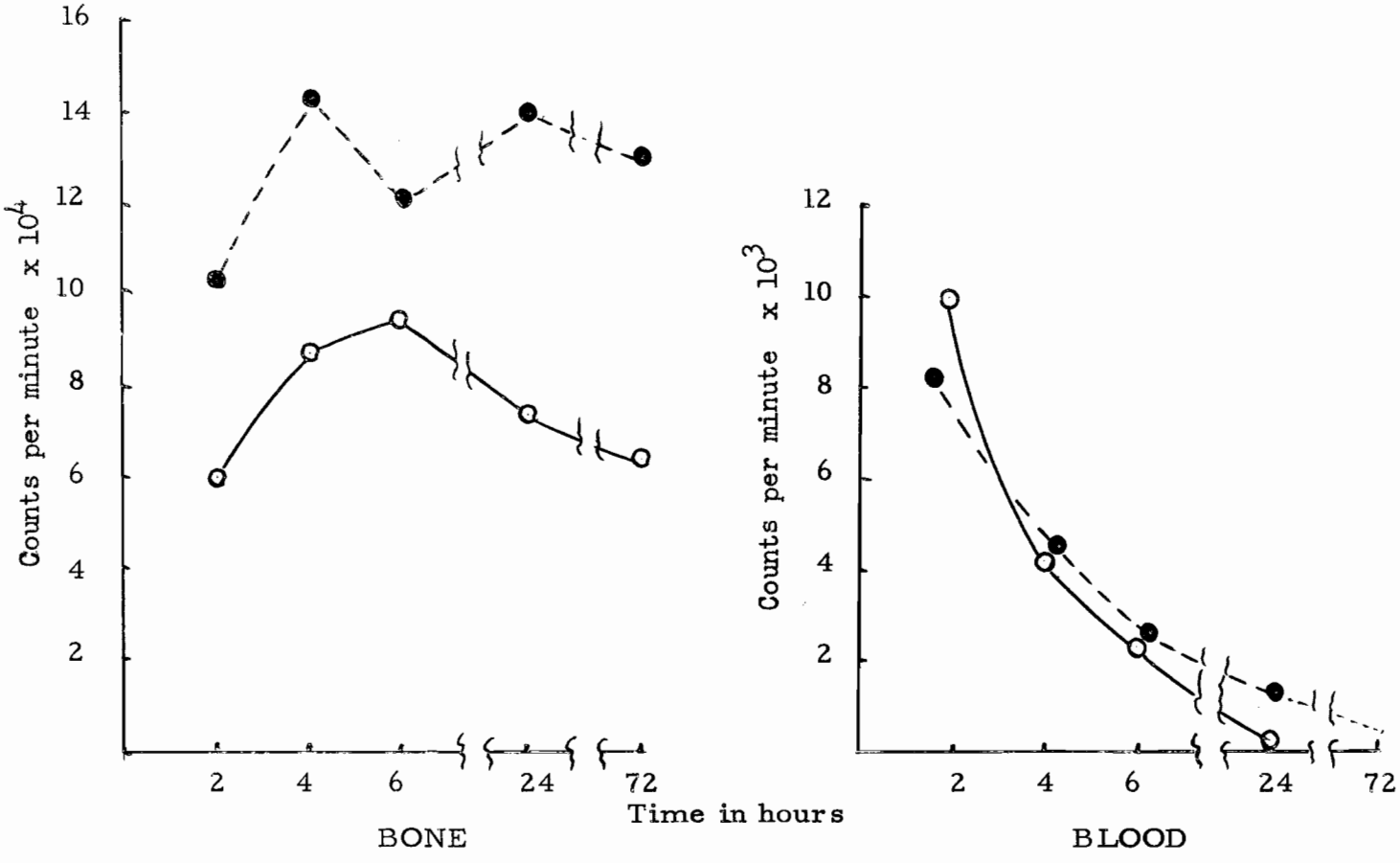


Fig. 3 Sr^{89} and Ca^{45} blood levels and bone uptake after single oral dose.

the bony tissue and perhaps only a very small percentage is eliminated during this period. In the next 18 hours, Sr^{89} level in blood and bone decreases, as the body eliminates these ions. In the following 48 hours the rate of decline of the bone Sr^{89} is very slow, probably indicating that most of the remaining Sr^{89} present in bony tissue is more stably incorporated; there is no measurable exchange of Sr^{89} from bone to circulating blood in this period of time.

Ca^{45} absorption and uptake after a single oral dose.

Ca^{45} was diluted with inactive $CaCl_2$ solution to give a final concentration of 1.1 μM and 10 μc activity per ml. Intubation and assay for radioactivity in blood samples and in femurs were carried out in groups of rats exactly as in the experiments using radio - strontium.

The blood level of Ca^{45} after absorption of the test dose declined more slowly than Sr^{89} and some activity remained in the blood for more than 24 hours (Fig. 3 and Table I). Bone uptake of Ca^{45} increased more rapidly than Sr^{89}; a maximum was reached after 4 hours, after which there was no further change in the amount of Ca^{45} deposited in the bones in the period investigated.

Sr^{89} and Ca^{45} uptake after continuous administration

Rats aged six weeks old were given different doses of Sr^{89} or Ca^{45} in their drinking water or mixed with standard purina chow and given ad libitum. The lowest dose used contained 0.01 μc of one of the isotopes

per ml. of drinking water. On an average they imbibed 20 to 25 ml. of water a day, thus had an average intake of 0.2 to 0.25 μc per day. These rats were kept for 55 days and at regular intervals of 5 days, 6 rats were sacrificed and one femur assayed for radioactivity. In the subsequent experiment the dose was increased to 5 μc per day; 0.25 μc per ml. of drinking water or 0.5 μc per gram of chow were given. In the food the isotope was mixed with boiled corn starch and then thoroughly mixed with purina chow in order to ensure a uniform distribution of the radioactive Sr^{89} or Ca^{45}. At 5 days interval 12 animals were sacrificed and one femur assayed for radioactivity.

Two higher doses of Sr^{89}, 25 μc and 50 μc per day were given as described above for 5 days; radioactivity was assayed at the end of this period.

The results of this study are given in Tables II, III, IV, and V and Figs. 4 and 5. In all the groups of animals irrespective of the dose used and the time interval at which radioactivity were assayed Ca^{45} had a greater uptake than Sr^{89}. Between the 15th and the 20th days Sr^{89} had reached a steady equilibrium. Whether the isotopes were administered in food or drinking water, the observed ratio, as plotted in Fig. 4, was more or less constant. The ratio was maintained at exactly the same level when the dose of radioactive Sr^{89} and Ca^{45} were increased. The counts per minute in one femur measured at the end of 5 days in animals receiving Sr^{89} doses in

TABLE II

BONE UPTAKE OF Sr^{89} and Ca^{45} AFTER CONTINUOUS ADMINISTRATION IN DRINKING WATER. 0.2 μc PER DAY.

The drinking water contained 0.01 μc Sr^{89} or Ca^{45} per ml. One femur was assayed for radioactivity.

DAYS	STRONTIUM ^{89}	CALCIUM ^{45}	O.R.
1	785 ± 184	2,541 ± 824	0.30
5	2,971 ± 546	7,950 ± 1812	0.37
10	5,270 ± 1073	14,850 ± 2850	0.35
15	6,194 ± 3017	26,108 ± 1644	0.23
20	8,421 ± 1567	26,660 ± 4617	0.31
25	8,128 ± 1478	30,585 ± 4759	0.26
30	8,583 ± 1366	50,516 ± 5532	0.16
35	10,002 ± 2643	46,858 ± 10,209	0.21
40	9,909 ± 1702	46,982 ± 2699	0.21
45	8,091 ± 1815	28,950 ± 15110	0.27
50	7,133 ± 735	39,416 ± 6438	0.18
55	11,424 ± 2015	53,450 ± 8726	0.21

Each figure represents mean counts per minute and standard deviation for studies in six animals.

TABLE III

BONE UPTAKE OF Sr^{89} and Ca^{45} AFTER CONTINUOUS ADMINISTRATION IN DRINKING WATER. 5 μc PER DAY.

The drinking water contained 0.25 μc Sr^{89} or Ca^{45} per ml. One femur was assayed for radioactivity.

DAYS	STRONTIUM ^{89}	CALCIUM^{45}	O.R.
5	48,548 ± 12,534	205,358 ± 74,626	0.23
10	88,410 ± 33,050	515,555 ± 104,200	0.17
15	114,791 ± 41,921	686,680 ± 124,700	0.16
20	151,930 ± 48,223	664,565 ± 208,700	0.22
25	201,791 ± 31,668	856,080 ± 192,500	0.23
30	205,916 ± 63,064	904,390 ± 99,813	0.22

Each figure represents mean counts per minute and standard deviation for studies in twelve animals.

TABLE IV

BONE UPTAKE OF Sr^{89} and Ca^{45} AFTER CONTINUOUS ADMINISTRATION IN FOOD.
5 μc PER DAY.

The food contained 0.5 μc of Sr^{89} or Ca^{45} per gram. One femur was assayed for radioactivity.

DAYS	STRONTIUM ^{89}	CALCIUM ^{45}	O.R.
5	44,605 ± 11621	145,350 ± 27238	0.30
10	72,219 ± 14166	308,227 ± 52389	0.23
15	102,129 ± 29422	462,504 ± 63043	0.22
20	117,776 ± 24735	451,325 ± 62568	0.26

Each figure represents mean counts per minute and standard deviation for studies in twelve animals.

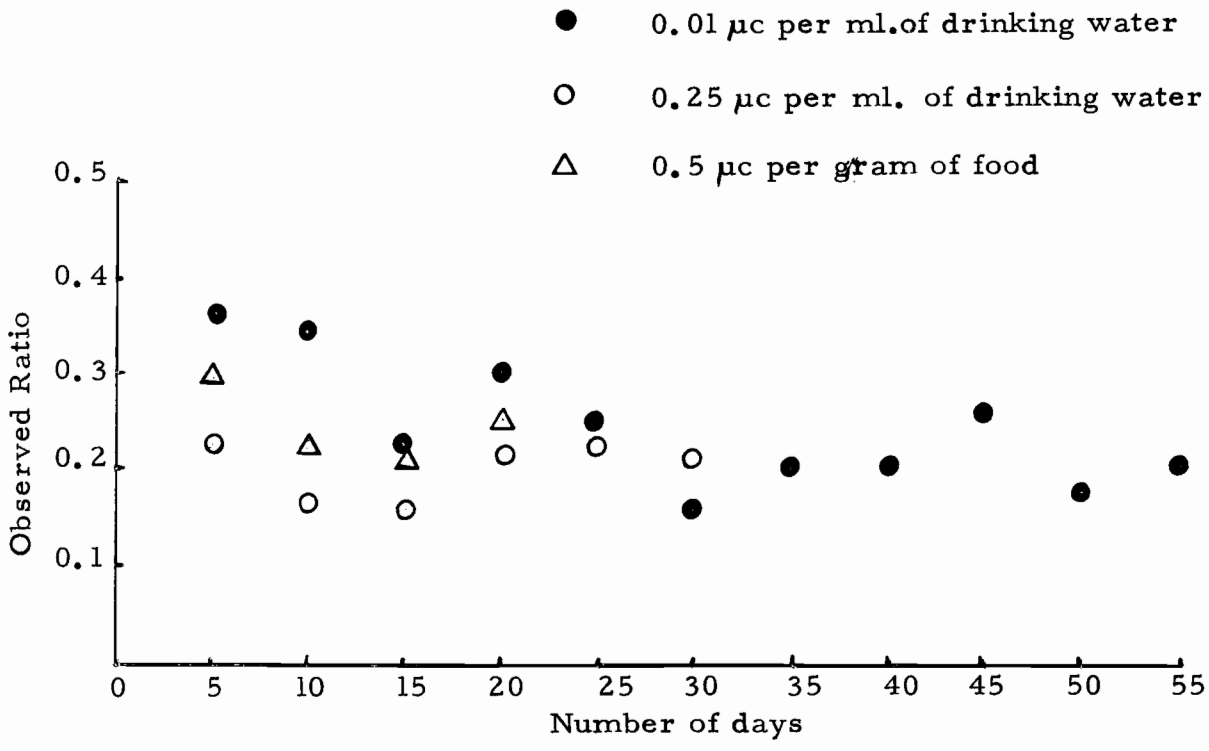


Fig. 4 Bone Uptake of Sr^{89} and Ca^{45} after continuous Administration.

TABLE V

BONE UPTAKE OF STRONTIUM^{89} AFTER CONTINUOUS ADMINISTRATION. 25 μc PER DAY.

The drinking water contained 1 μc Sr^{89} per ml. or 2 μc Sr^{89} per gm. of food. One femur assayed for radioactivity.

In Drinking Water	In Food
265,410 ± 61,102	215,439 ± 45,066

50 microcuries per day

The drinking water contained 2.5 μc Sr^{89} per ml. or 5 μc Sr^{89} per gm. of food. One femur assayed for radioactivity.

In Drinking Water	In Food
584,401 ± 163,100	370,470 ± 103,700

Each figure represents mean counts per minute and standard deviation for studies in twelve animals.

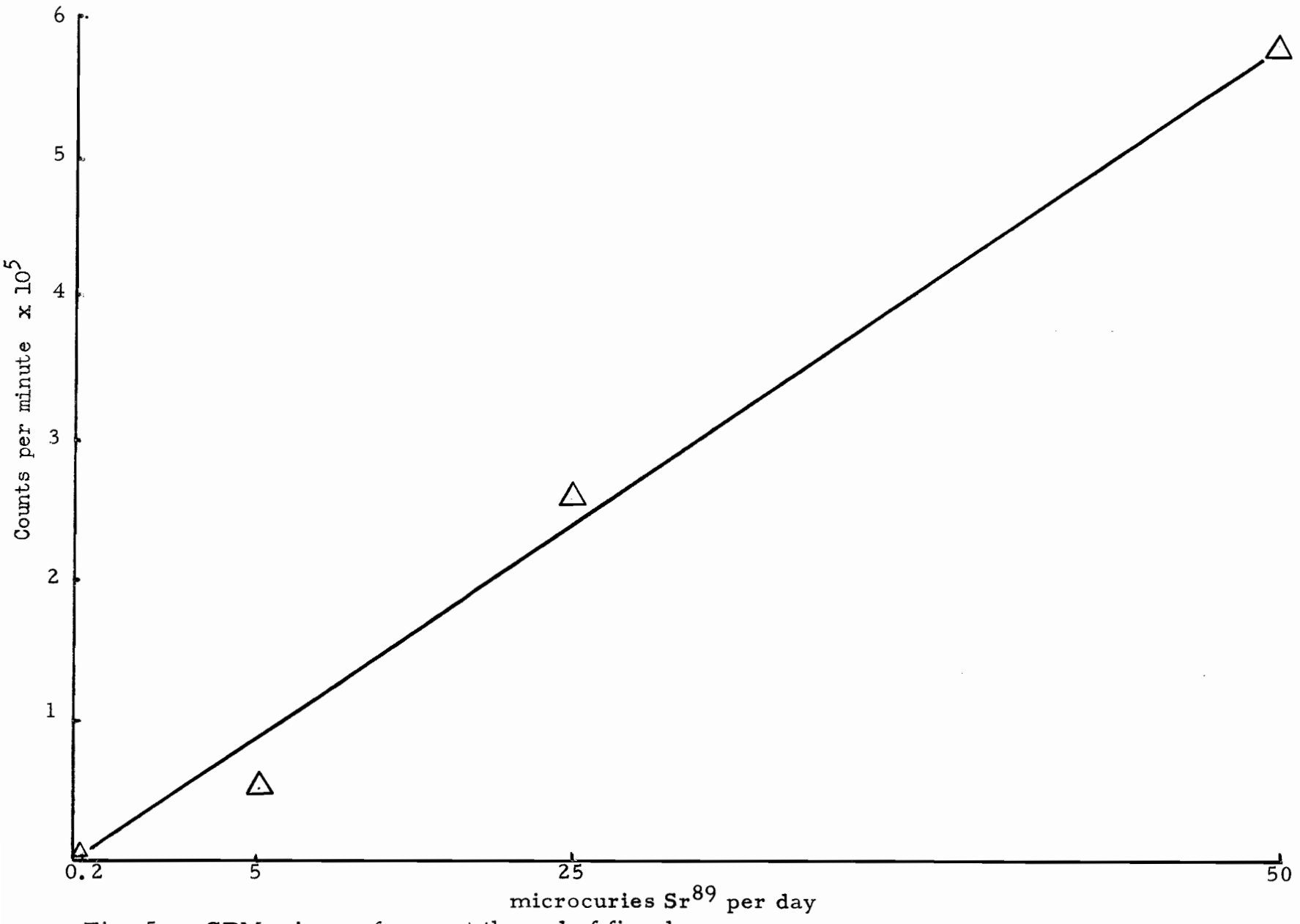


Fig. 5 CPM - in one femur at the end of five days.

drinking water is shown in Fig. 5. From this it appears that uptake of Sr^{89} increases linearly with the dose of radionuclide.

Effect of changes in pH on Sr^{89} and Ca^{45} uptake from ligated intestinal segments.

In order to understand the role of H^+ ion concentration it is necessary to know the normal pH in different sections of the intestinal tract. A preliminary study was undertaken to measure the normal pH in saline washings of the intestinal tract of rats.

The rats were prepared with ligated intestinal segments as described previously. 0.5 ml. of isotonic saline was injected into each of the segments. At the end of thirty minutes the segment was resected and the contents of the intestinal lumen collected in a small vessel. The pH was determined using a glass electrode and a Beckman pH meter. Thirty rats were studied for each segment.

The above procedure was repeated exactly with two buffering agents, one at an alkaline pH and the other at an acidic pH: "Trisma" (Trishydroxy methyl aminomethane), ionic strength 0.1, at pH 8.5 at 25°C; and phosphate buffer with pH 5.75 and 0.2 ionic strength were used after considerable experimentation with different concentrations of buffer. Due to the continuous secretion and excretion of different ions by the intestinal mucosa and the digestive process that take place in the lumen of intestine, any buffer solution will be considerably altered in pH in a very short period of time unless they have a high buffer capacity. Higher concentrations of buffer produced distension

of the segments. "Trisma" was chosen as buffer in the pH range of 8.5 as it is known to be non - toxic and forms soluble salts of strontium and calcium. The phosphate buffer used gives a pH slightly above 6.0 in the lumen of intestine and also forms soluble salts of strontium and calcium at this pH. 0.5 ml of the buffering solution was introduced into the ligated intestinal segments and the pH determined at the end of thirty minutes. Fifteen animals were used for each segment and for each solution.

The results are given in Table VI and Fig. 6. The saline washings of the stomach were acid with a mean pH of 2.35 ± 0.89. The duodenal segment was alkaline, 7.75 ± 0.37, while the following segment was slightly acid, 6.85 ± 0.31; thereafter in the subsequent segments the pH was progressively more alkaline, the terminal ileal segment having a pH of 8.79 ± 0.56. The differences in pH between the segments are statistically significant.

By the use of "Trisma" buffer at pH 8.5, the pH of the stomach was maintained on the alkaline side for the period under investigation. Throughout the small intestine a more or less uniform pH of slightly above eight was maintained. The very small standard deviation indicates that the buffering capacity of the solution was sufficient to maintain a constant pH in the lumen. Likewise with phosphate buffer an acidic pH just below 6.5 was maintained more or less uniformly throughout the small intestine. Here again the consistency of readings in any given segment is remarkable as can be seen from the small standard deviation.

With the use of these buffering agents for thirty minutes, no

histologically detectable lesion was demonstrated in the intestinal mucosa.

Influence of buffering agents on Sr^{89} and Ca^{45} absorption

Strontium^{89}, 10 μc in 0.9 μM of inactive $SrCl_2$ or 10 μc Ca^{45} in 1.1 μM of inactive $CaCl_2$ per one ml. of the buffering solution were used to study the absorption of these cations from the intestinal tract. 0.5 ml of these solutions were injected into each intestinal segment with either "Trisma" or phosphate buffer in the concentrations described above. At the end of thirty minutes the residual radio - activity in the segment and in one ml. of blood and in one femur were assayed. Twelve animals were used for each segment and for each solution. An equal number of animals were used for control studies, with the isotopes dissolved in isotonic saline.

The results of these studies are given in Tables VII, VIII, IX, X, XI, and XII and in Figs. 7, 8, 9, 10, 11 and 12. Absorption was estimated as the difference between the total radioactivity administered and the radioactivity remaining in the lumen plus that of the intestinal wall. Absorption of strontium and calcium took place mainly from the duodenum and the adjacent segment. In the control animals 68% of Ca^{45} and 38% of Sr^{89} were absorbed from the duodenum. In the segment immediately following the duodenum absorption of both ions declined. In the subsequent segments absorption of Ca^{45} dropped by about one-third and Sr^{89} dropped by one half; this decline continued slowly towards the distal ileum. From the tables and figures it can be seen that blood levels and bone uptake corresponded to the amount of isotope absorbed from each segment.

TABLE VI

PH STUDIES IN RAT'S LIGATED GASTROINTESTINAL SEGMENTS

Segments	with isotonic saline	with "Trisma" buffer pH 8.5 and 0.1 ionic strength	with phosphate buffer of pH 5.75 & 0.2 ionic strength
I	* 2.35 ± 0.89	** 7.79 ± 0.37	** 3.31 ± 0.77
II	7.75 ± 0.37	8.14 ± 0.09	6.49 ± 0.19
III	6.85 ± 0.31	8.13 ± 0.09	6.00 ± 0.05
IV	7.18 ± 0.38	8.14 ± 0.08	6.01 ± 0.03
V	7.15 ± 0.44	8.10 ± 0.09	5.98 ± 0.07
VI	7.63 ± 0.05	8.23 ± 0.09	6.12 ± 0.07
VII	7.89 ± 0.07	8.27 ± 0.06	6.13 ± 0.08
VIII	8.1 ± 0.05	8.35 ± 0.07	6.16 ± 0.17
IX	8.56 ± 0.02	8.34 ± 0.10	6.19 ± 0.09
X	8.79 ± 0.07	8.28 ± 0.08	6.28 ± 0.11

* Each figure represents mean pH values and standard deviation for studies in thirty animals.

** Each figure represents mean pH values and standard deviation for studies in fifteen animals.

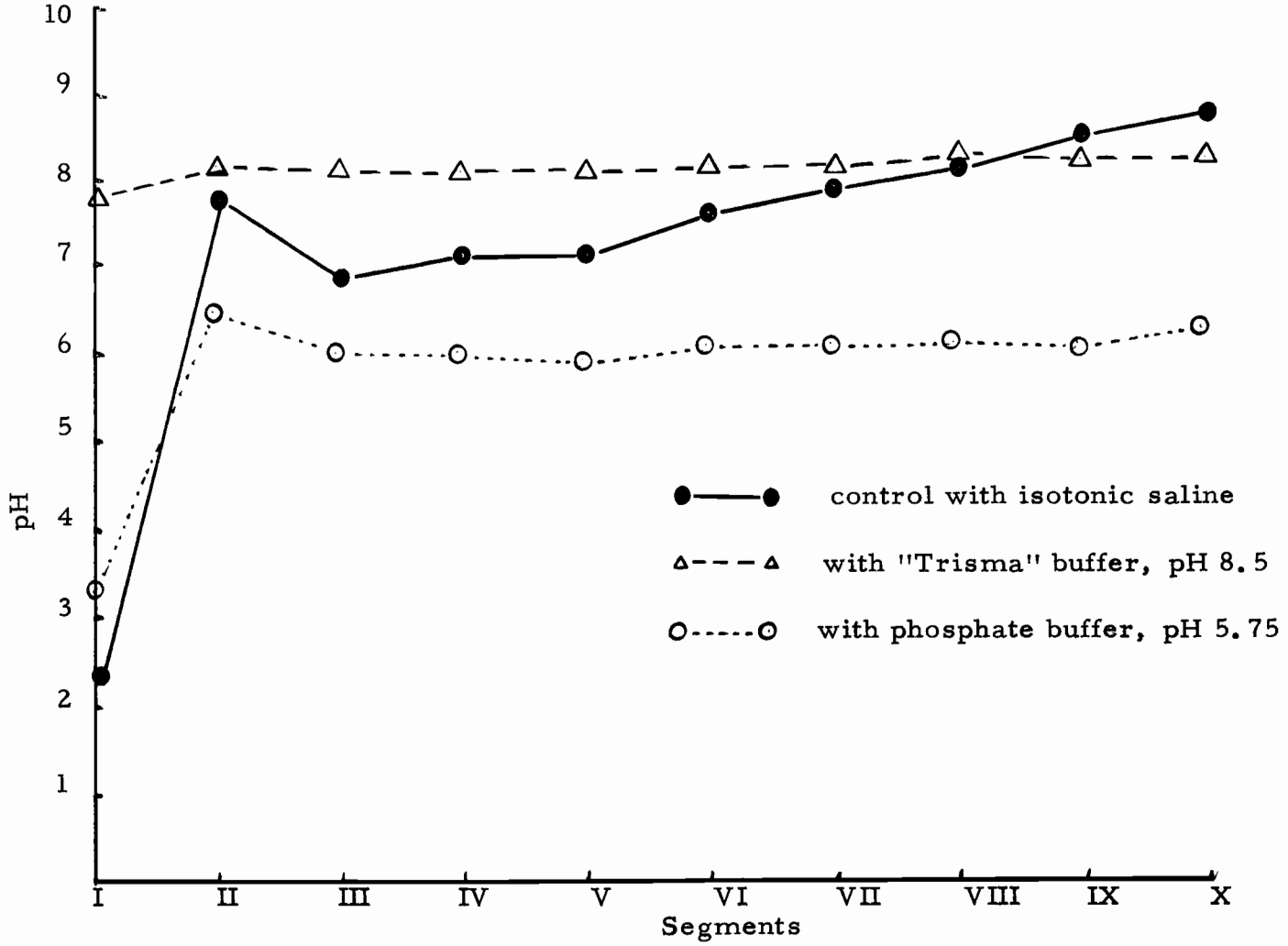


Fig. 6 pH studies in rats ligated gastrointestinal segments.

TABLE VII

INFLUENCE OF pH ON Sr^{89} ABSORPTION FROM RAT'S LIGATED INTESTINAL SEGMENTS. PERCENTAGE OF ABSORPTION AT THE END OF THIRTY MINUTES.

Segment	Control	with "Trisma" buffer pH 8.5 and 0.1 ionic strength	with phosphate buffer pH 5.75, 0.2 ionic strength
I	-----	-------	------
II	38.6 ± 8.6	39.8 ± 10.2	29.9 ± 13.0
III	25.7 ± 9.5	27.3 ± 13.1	16.1 ± 6.0
IV	17.1 ± 4.0	21.0 ± 7.1	11.3 ± 5.4
V	23.9 ± 8.3	25.1 ± 9.6	10.6 ± 4.2
VI	16.6 ± 8.3	20.8 ± 8.5	9.7 ± 3.3
VII	14.9 ± 5.5	24.1 ± 7.0	9.4 ± 3.3
VIII	15.4 ± 3.4	22.6 ± 9.4	11.1 ± 3.1
IX	15.7 ± 3.8	20.7 ± 9.0	9.4 ± 3.4
X	15.2 ± 4.1	14.5 ± 6.1	10.1 ± 3.4

Each figure represents mean percentage of absorption and standard deviation of studies in twelve animals.

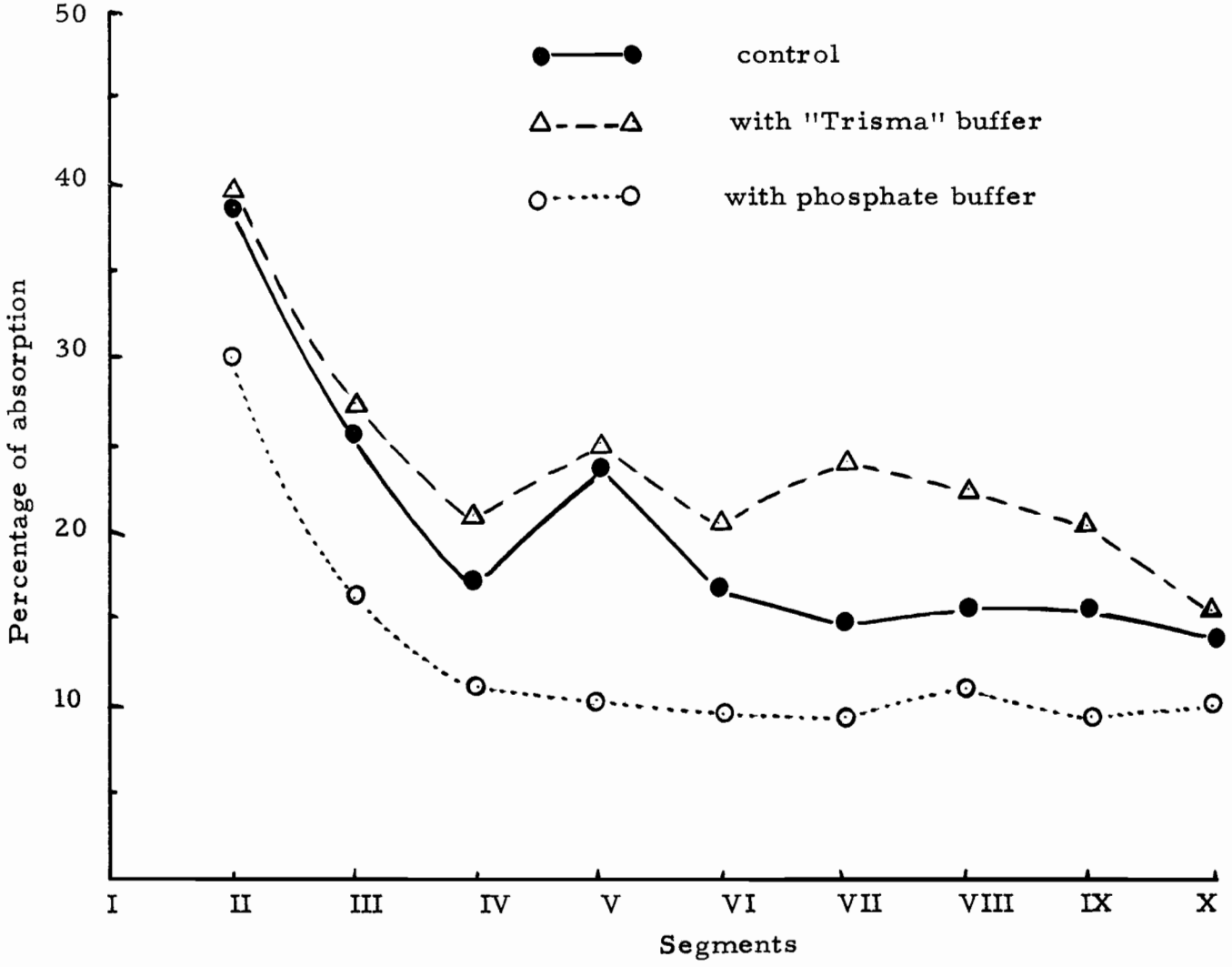


Fig. 7 Influence of pH on Sr^{89} absorption from ligated intestinal segments.

T A B L E VIII

INFLUENCE OF pH ON Sr^{89} ABSORPTION FROM RAT'S LIGATED INTESTINAL SEGMENTS RADIOACTIVITY IN ONE ML. OF BLOOD MEASURED AT THE END OF THIRTY MINUTES

Segment	Control	with "Trisma" buffer pH 8.5 and 0.1 ionic strength	with phosphate buffer pH 5.75 and 0.2 ionic strength
I	------	---------	-------
II	16142 ± 3309	16711 ± 5087	11804 ± 2052
III	8184 ± 2676	8332 ± 3312	3578 ± 968
IV	4500 ± 1158	8505 ± 3125	2093 ± 446
V	5090 ± 1275	9642 ± 3072	2014 ± 396
VI	4229 ± 1066	6385 ± 1376	2446 ± 469
VII	5053 ± 1554	7094 ± 1565	2512 ± 465
VIII	4256 ± 690	6353 ± 2038	2155 ± 442
IX	3715 ± 940	5830 ± 1174	2042 ± 490
X	2910 ± 590	4051 ± 1002	2277 ± 426

Each figure represents mean counts per minute and standard deviation of studies in twelve animals.

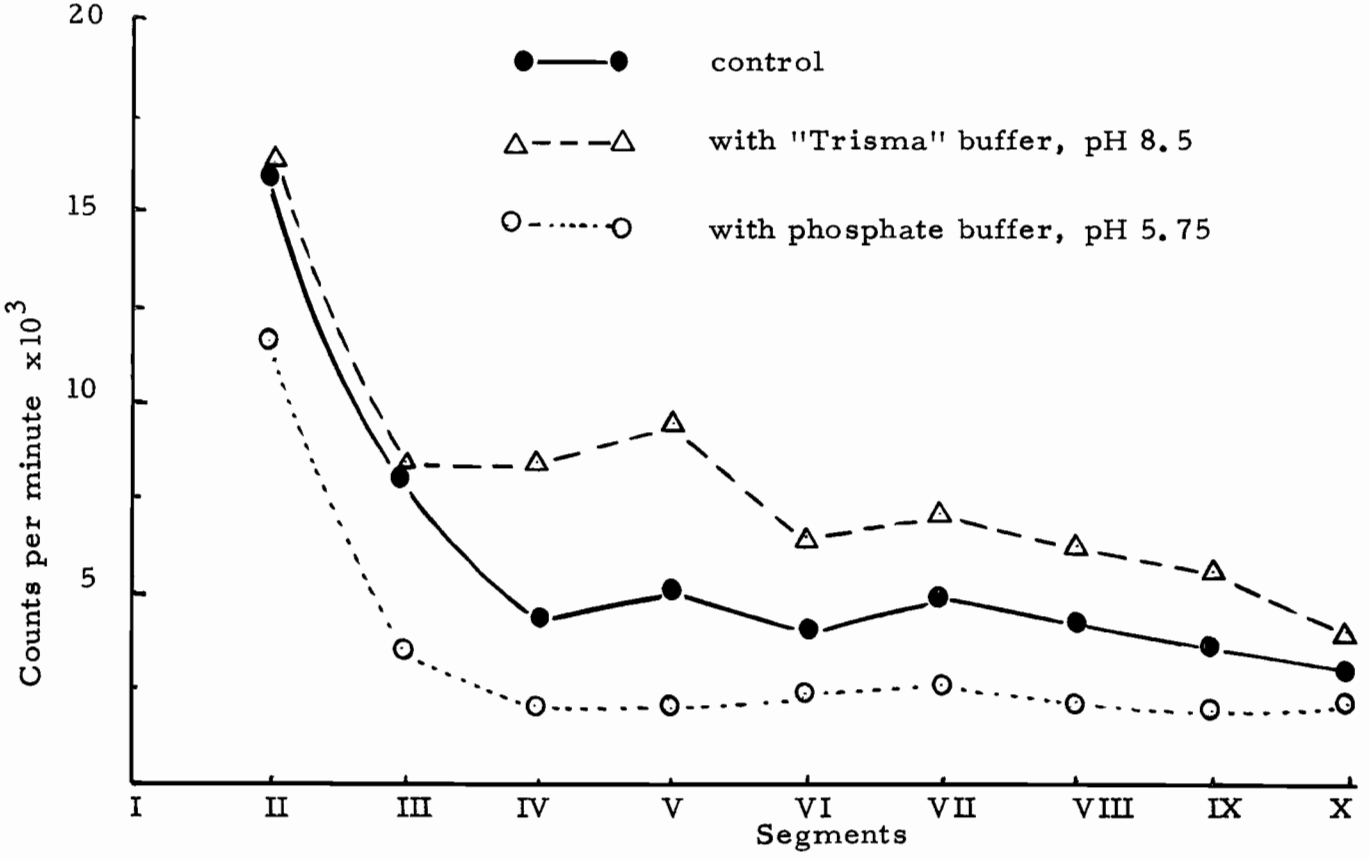


Fig. 8 Influence of pH on Sr^{89} absorption from ligated intestinal segments. Radioactivity in one ml. of blood measured at the end of 30 minutes.

TABLE IX

INFLUENCE OF pH ON Sr^{89} ABSORPTION FROM RAT'S LIGATED INTESTINAL SEGMENTS RADIOACTIVITY IN ONE FEMUR ASSAYED AT THE END OF THIRTY MINUTES

Segment	Control	with "Trisma" buffer pH 8.5 and 0.1 ionic strength	with phosphate buffer pH 5.75 and 0.2 ionic strength
I	----	--------	-------
II	31051 ± 14099	31822 ± 10903	24372 ± 6878
III	9658 ± 2451	14249 ± 4540	6690 ± 1948
IV	6488 ± 2305	9594 ± 3411	4527 ± 1252
V	7251 ± 1834	18140 ± 4481	3535 ± 670
VI	7909 ± 3325	8295 ± 1171	2428 ± 547
VII	9669 ± 3941	8792 ± 2543	2036 ± 493
VIII	7125 ± 2480	9053 ± 1460	2117 ± 485
IX	9967 ± 2925	8255 ± 1923	2155 ± 567
X	8248 ± 1832	8653 ± 1811	2315 ± 699

Each figure represents mean counts per minute and standard deviation of studies in twelve animals.

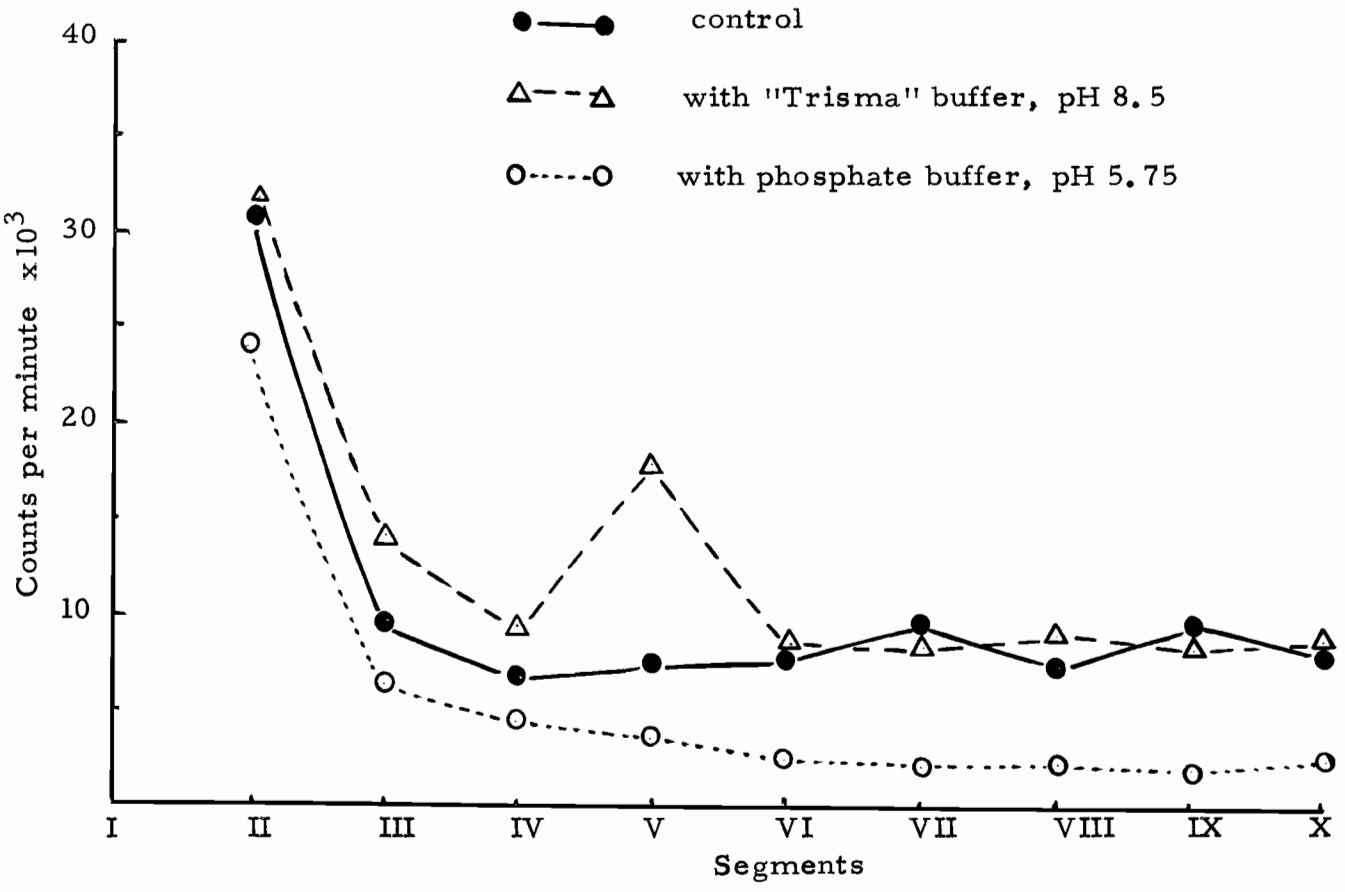


Fig. 9 Influence of pH on Sr^{89} absorption from ligated intestinal segments. Radioactivity in one femur assayed at the end of 30 minutes.

TABLE X

INFLUENCE OF pH ON Ca^{45} ABSORPTION FROM RAT'S LIGATED INTESTINAL SEGMENTS. PERCENTAGE OF ABSORPTION AT THE END OF THIRTY MINUTES.

Segment	Control	with "Trisma" buffer pH 8.5 and 0.1 ionic strength	with phosphate buffer pH 5.75 and 0.2 ionic strength
I	------	--------	--------
II	68.4 ± 9.6	65.3 ± 12.8	71.9 ± 14.1
III	60.0 ± 4.3	48.7 ± 13.7	51.0 ± 9.2
IV	26.9 ± 6.5	40.5 ± 12.8	29.4 ± 8.7
V	20.8 ± 5.6	31.5 ± 8.7	28.4 ± 11.7
VI	23.5 ± 6.2	43.3 ± 13.1	29.2 ± 5.3
VII	23.7 ± 6.0	33.7 ± 7.1	33.8 ± 6.2
VIII	20.0 ± 3.6	33.1 ± 9.1	37.4 ± 9.5
IX	17.9 ± 5.6	25.3 ± 6.9	16.0 ± 6.4
X	17.7 ± 4.4	21.5 ± 11.7	14.1 ± 6.1

Each figure represents mean percentage of absorption and standard deviation of studies in twelve animals.

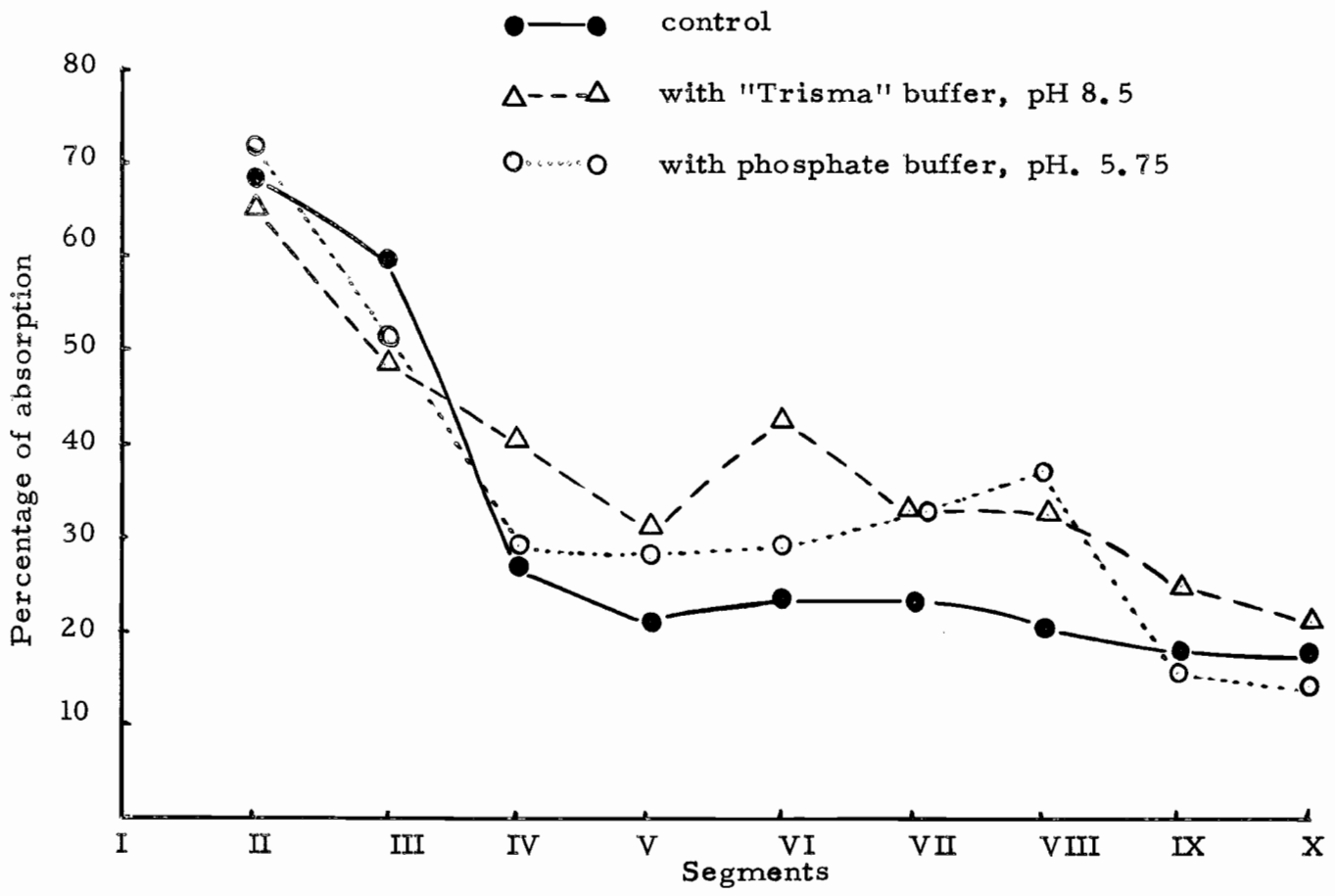


Fig. 10 Influence of pH on Ca^{45} absorption from ligated intestinal segments.

TABLE XI

INFLUENCE OF pH ON Ca^{45} ABSORPTION FROM RAT'S LIGATED INTESTINAL SEGMENTS. RADIOACTIVITY IN ONE ML. OF BLOOD MEASURED AT THE END OF 30 MINUTES.

Segment	Control	with "Trisma" buffer pH 8.5 and 0.1 ionic strength	with phosphate buffer pH 5.75 and 0.2 ionic strength
I	------	-------	--------
II	20392 ± 6585	13904 ± 2773	14816 ± 2582
III	10100 ± 2847	8596 ± 3606	8031 ± 1782
IV	6998 ± 2246	6700 ± 2382	3517 ± 1614
V	4474 ± 2093	6249 ± 1318	1679 ± 553
VI	4770 ± 1760	6757 ± 2003	1556 ± 415
VII	3677 ± 1561	5203 ± 1542	1777 ± 628
VIII	3678 ± 915	6047 ± 2249	1920 ± 535
IX	3047 ± 915	5207 ± 2415	1393 ± 622
X	3160 ± 1193	3172 ± 1346	1335 ± 316

Each figure represents mean counts per minute and standard deviation of studies in twelve animals.

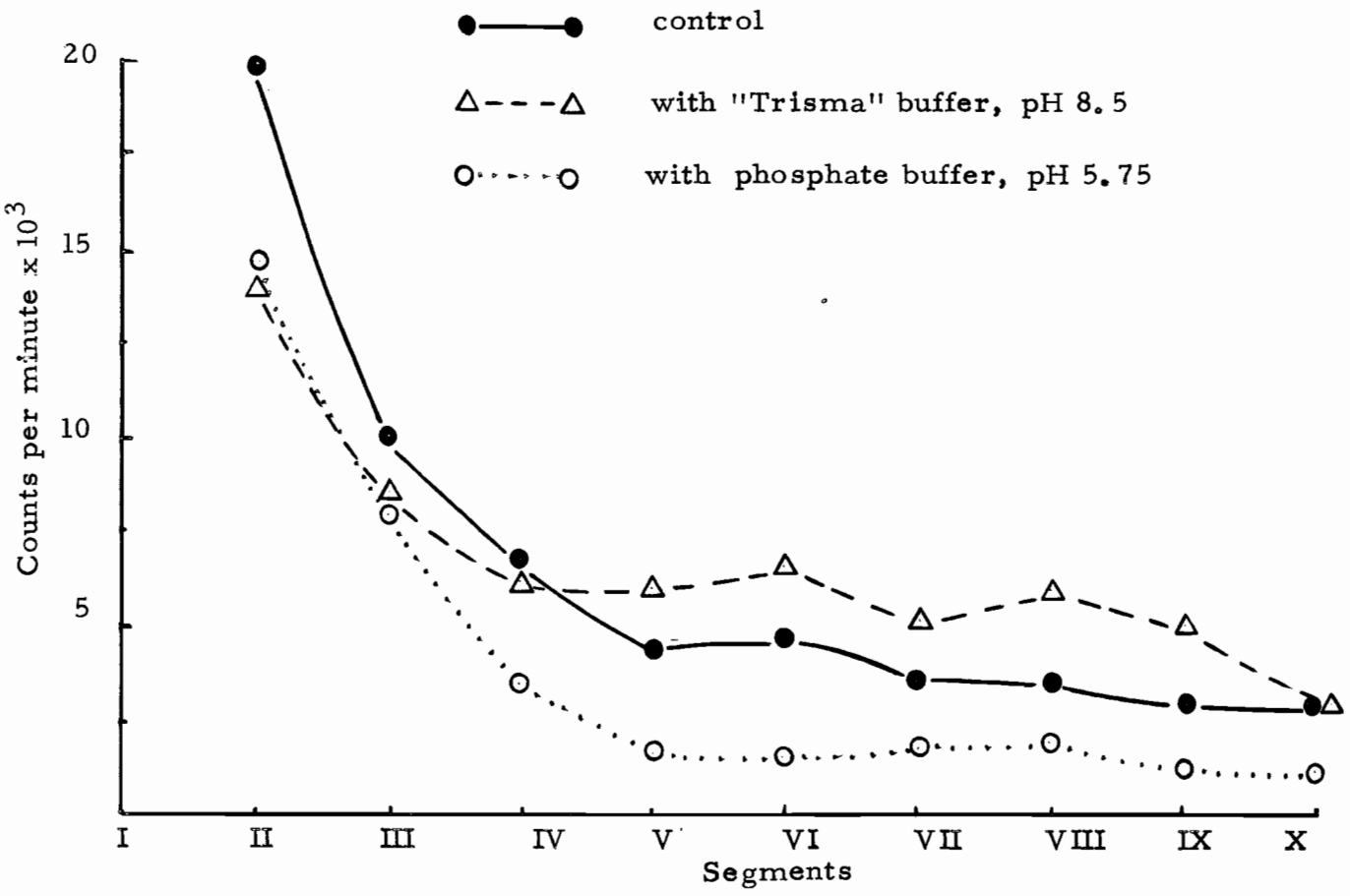


Fig. 11 Influence of pH on Ca^{45} absorption from ligated intestinal segments. Radioactivity in one ml. of blood measured at the end of 30 minutes.

T A B L E XII

INFLUENCE OF pH ON Ca^{45} ABSORPTION FROM RAT'S LIGATED INTESTINAL SEGMENTS RADIOACTIVITY IN ONE FEMUR ASSAYED AT THE END OF THIRTY MINUTES

Segment	Control	with "Trisma" buffer pH 8.5 and 0.1 ionic strength	with phosphate pH 5.75 and 0.2 ionic strength
I	------	------	------
II	38110 ± 13249	25201 ± 9354	38625 ± 6934
III	17722 ± 4951	12038 ± 5943	13250 ± 5585
IV	8350 ± 5238	9762 ± 2582	8062 ± 2999
V	8452 ± 2795	16204 ± 3373	2837 ± 744
VI	8232 ± 2033	10895 ± 4246	2828 ± 902
VII	7442 ± 2399	8826 ± 3251	3303 ± 863
VIII	6479 ± 2616	7163 ± 1859	3072 ± 820
IX	5625 ± 2054	5945 ± 2533	1921 ± 692
X	4982 ± 1848	3781 ± 1622	1803 ± 655

Each figure represents mean counts per minute and standard deviation of studies in twelve animals.

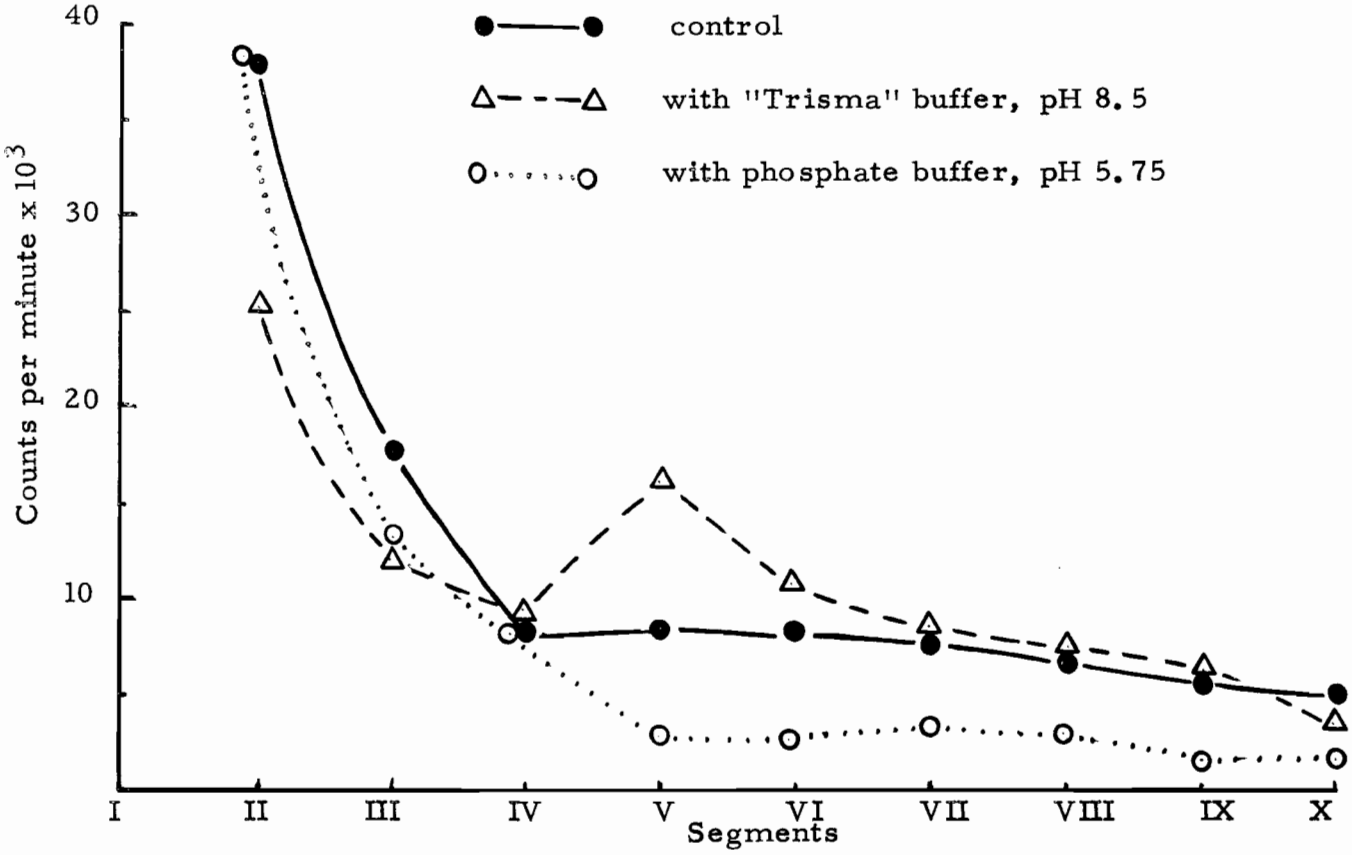


Fig. 12 Influence of pH on Ca^{45} absorption from ligated intestinal segments. Radioactivity in one femur assayed at the end of 30 minutes.

No significant change in absorption of Sr^{89} or Ca^{45} from the duodenum or terminal ileum occurred when the pH was increased. However there was a small but definite increase in the percentage of both ions absorbed from the distal jejunum and proximal ileum under these condi - tions. The blood levels and bone uptake of the isotope correlated with those of the absorption.

By lowering the pH with phosphate buffer the rate of Sr^{89} absorption was reduced from all segments; this finding correlated well with those of blood level and bone uptake. Decreasing the luminal pH appears to have an enhancing effect on Ca^{45} absorption from mid intestinal segments; however both the blood level and bone uptake declined from the mid jejunal to the terminal ileal segment (Tables X, XI, and XII and Figures 10, 11 and 12). This discrepancy may be due to experimental error, but possibly reflects a more profound alteration in calcium metabolism.

Effect of pH on Sr^{89} and Ca^{45} uptake after single orogastric administration

One ml. of Sr^{89} or Ca^{45} in the concentrations described in the previous experiments, dissolved in "Trisma" pH 8.5 or phosphate buffer pH 5.75 were given through an orogastric tube to rats weighing 100 to 120 grams. At the end of two hours animals were sacrificed and one ml. of blood and one femur were assayed for radioactivity.

The results of this experiment are given in Table XIII and Fig. 13. It can be seen that the "Trisma" buffer has enhanced the blood level and bone uptake of Sr^{89} to nearly 250% of the control. On the other hand, though the Ca^{45} level in blood was increased with the use of "Trisma"

TABLE XIII

EFFECT OF pH ON Sr^{89} AND Ca^{45} BLOOD LEVELS AND UPTAKE BY BONE

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ or ten microcuries of Ca^{45} in 1.1 μM of inactive $CaCl_2$ in one ml of test buffer were administered through orogastric tube to 100 - 120 gram rats. One ml. of blood and one femur assayed for radioactivity at the end of 2 hours.

	Strontium^{89}	Expt. / Control %	Calcium^{45}	Expt. / Control %
BLOOD				
Control	6,178 ± 1871		7,008 ± 1130	
Trisma buffer	14,663 ± 6202	237%	12,969 ± 1724	185%
Phosphate buffer	3,209 ± 1378	52%	5,242 ± 1379	75%
BONE				
Control	55,145 ± 18727		121,091 ± 29653	
Trisma buffer	142,609 ± 23730	258%	130,050 ± 18098	107%
Phosphate buffer	32,412 ± 10973	59%	98,175 ± 31503	81%

Each figure represents mean counts per minute and standard deviation for studies in six animals.

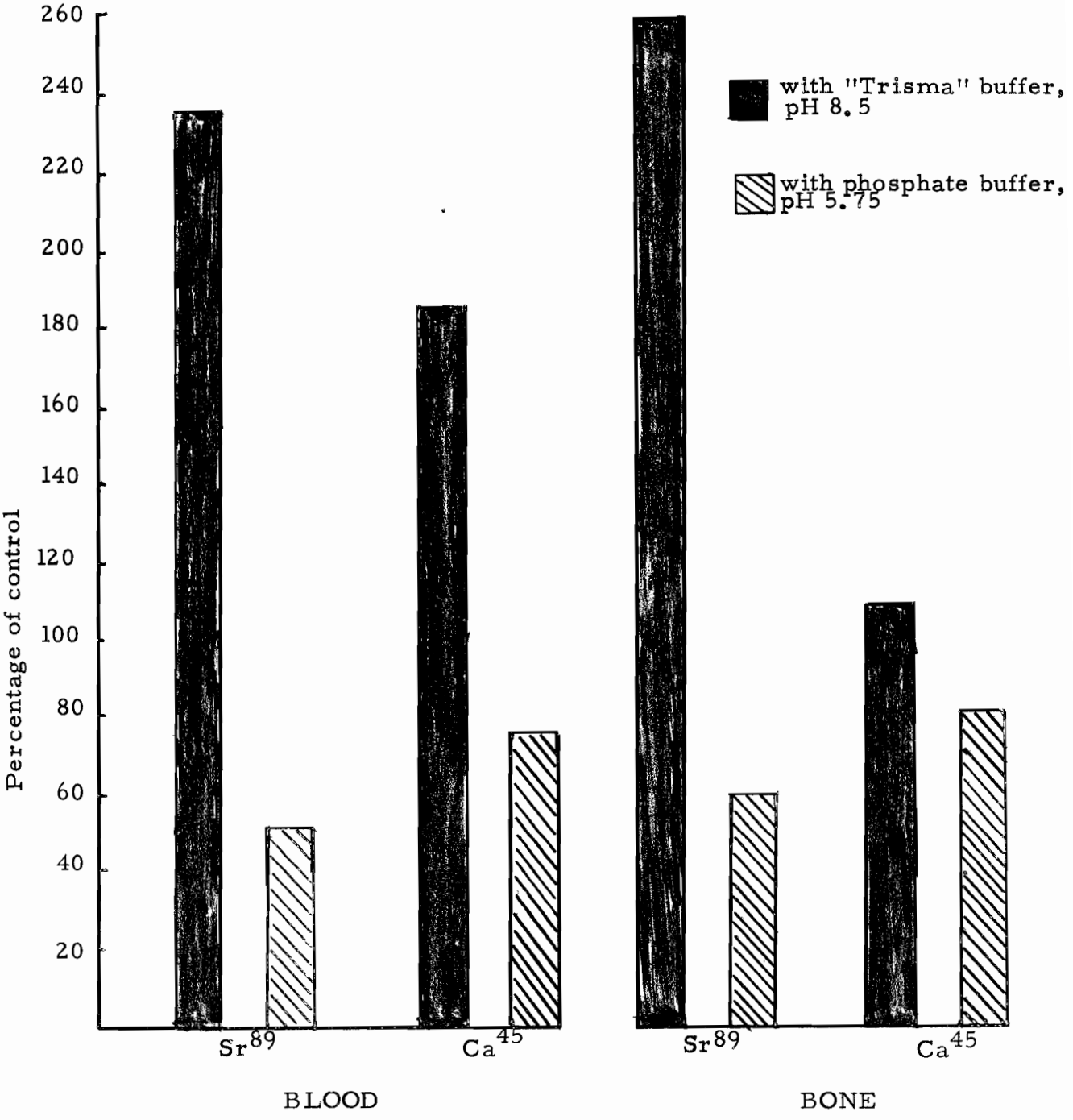


Fig. 13 Effect of pH on Sr^{89} and Ca^{45} blood levels and bone uptake after single orogastric administration, shown as percentage of control.

TABLE XIV

EFFECT OF pH ON Sr^{89} AND Ca^{45} BLOOD LEVELS AND UPTAKE BY BONE IN ADULT FEMALE RATS

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ or 10 μc of Ca^{45} in 1.1 μM of $CaCl_2$ in one ml. of Trisma buffer per 100 gm weight were administered through orogastric tube to adult rats weighing 300 - 350 gm. One ml of blood and one femur assayed for radioactivity at the end of 2 hours.

	BLOOD			
	Strontium^{89}	Expt.% / Control	Calcium^{45}	Expt.% / Control
Control	7,577 ± 2151		7,906 ± 1078	
Trisma buffer	24,876± 8897	328%	14,675 ± 3990	186%
	BONE			
Control	24,158 ± 3540		26,783 ± 5452	
Trisma buffer	72,550 ± 41542	300%	46,533 ± 16542	174%

Each figure represents mean counts per minute and standard deviation for studies in six animals.

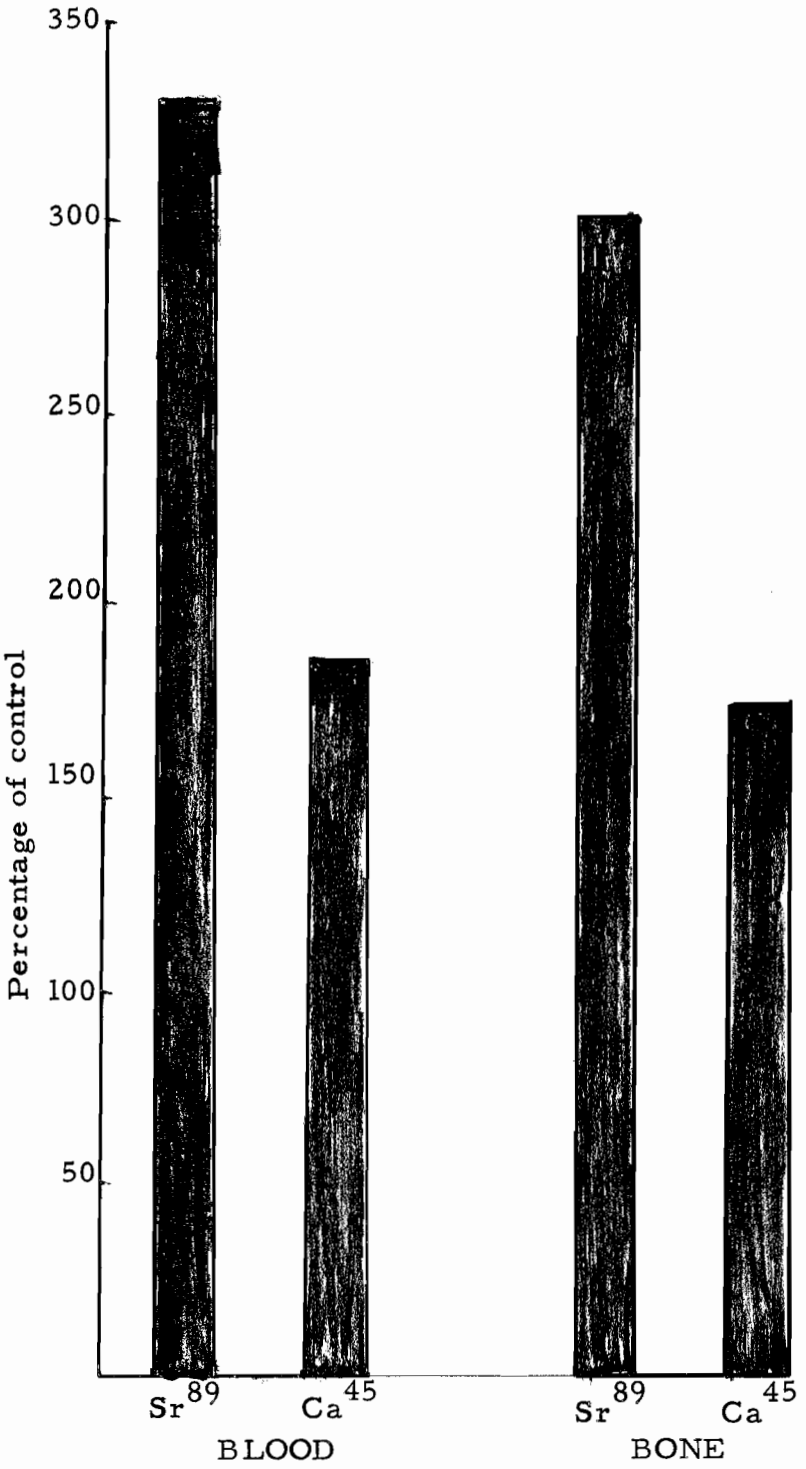


Fig. 14 Effect of "Trisma" buffer on Sr^{89} and Ca^{45} blood levels and bone uptake in adult female rats; shown as percentage of control

buffer to 185% of control, the bone uptake was very little more than that of the controls.

The acid phosphate buffer decreased by approximately one half both blood levels and bone uptake of Sr^{89}. The blood levels and bone uptake of Ca^{45} were also reduced but to a much lesser extent (Table XIII and Fig. 13). These differences in the behaviour of the two isotopes under the same conditions extend the observations made in the previous experiments with ligated segments and suggest that they reflect the real differences in metabolism between the essential and the non-essential mineral.

Effect of pH on Sr^{89} and Ca^{45} uptake by adult female rats.

Adult female rats weighing 300 to 350 grams which had had litters previously, were used in this study assuming that they were in a relative calcium deficiency state due to repeated pregnancies. They were dosed with 10 microcuries of Sr^{89} in 0.9 μM $SrCl_2$ or 10 μc of Ca^{45} in 1.1 μM $CaCl_2$ in one ml. "Trisma", pH 8.5 per 100 gm. One ml. of blood and one femur were assayed for radioactivity at the end of 2 hours.

The results of this study are given in Table XIV and Fig. 14. In the control animals both Sr^{89} and Ca^{45} uptake by bone was less than that by young growing animals. "Trisma" buffer considerably increased the blood levels and bone uptake of both the isotopes. The Sr^{89} level in blood and bone was increased by over 300% of the control.

Ca^{45} level in blood and bone was increased by 186% and 174% respectively.

Effect of bile on Sr^{89} and Ca^{45} absorption

The previous studies by Dukay (130) had shown that Ca^{45} absorption from duodenal segment is reduced when common bile duct is ligated. This study was repeated using Sr^{89} and Ca^{45}. Two microcuries of Sr^{89} in 0.45 μM of inactive $SrCl_2$ or 2 μc of Ca^{45} in 0.5 μM of inactive $CaCl_2$ in 0.5 ml were injected into ligated duodenal segment of rats in which the common bile duct was also ligated. At the end of thirty minutes the percentage of absorption was assayed.

The percentage of absorption of Sr^{89} or Ca^{45} was not altered by ligating the common bile duct (Table XV).

As the above studies did not reveal any difference in the rate of absorption of Ca^{45} after ligation of the common bile duct, it was repeated using solutions with different pH values, different concentrations of inactive $CaCl_2$, and furthermore, after ligating the bile duct 24 hours prior to absorption studies. The results of these studies are given in Tables XVI and XVII.

No change in rate of absorption was noted with the use of solutions with different pHs. However ligation of the common bile duct 24 hours prior to absorption study produced slight reduction in the percentage of absorption. A five fold increase of inactive $CaCl_2$ has produced about

TABLE XV

EFFECT OF LIGATION OF COMMON BILE DUCT ON Sr^{89} AND Ca^{45} ABSORPTION FROM DUODENAL SEGMENT

Strontium^{89}		Calcium^{45}	
Control	Ligated Common Bile Duct	Control	Ligated Common Bile Duct
38.2 ± 7.1	33.8 ± 11.0	71.2 ± 6.3	70.1 ± 7.1

Each figure represents mean percentage of absorption and standard deviation for studies in fifteen animals.

TABLE XVI

ABSORPTION OF Ca^{45} FROM DUODENAL SEGMENT OF RAT'S GASTROINTESTINAL TRACT AFTER LIGATION OF BILE DUCT

Two microcuries of Ca^{45} with 0.5 μM of inactive $CaCl_2$ in 0.5 ml. of solution with different pH's was injected into the ligated duodenal segment. After thirty minutes percentage of absorption assayed.

Control Open Bile Duct pH 3.3	Ligated Bile Duct pH 3.3	Ligated Bile Duct pH 2.1	Bile Duct Ligated 24 hrs earlier; pH 3.3
71.2% (±6.3)	70.1% (±7.1)	71.6% (±5.5)	52.8% (±9.9)

Each figure represents mean percentage of absorption and standard deviation for studies in fifteen animals.

TABLE XVII

CALCIUM^{45} ABSORPTION FROM DUODENAL SEGMENT OF RAT'S GASTROINTESTINAL TRACT AFTER LIGATION OF BILE DUCT

Activity measured at the end of thirty minutes.

1 μc Ca^{45} with 47.5 μgm of inactive $CaCl_2$		2 μc Ca^{45} with 80 μgm of inactive $CaCl_2$		2 μc Ca^{45} with 408 μgm of inactive $CaCl_2$	
Control	Bile Duct Ligated	Control	Bile Duct Ligated	Control	Bile Duct Ligated
77.4% (±9.0)	71.8% (±7.5)	71.2% (±6.3)	70.1% (±7.1)	37.8% (±8.2)	38.2% (±19.6)

Each figure represents mean percentage of absorption and standard deviation for studies in fifteen animals.

fifty percent reduction of absorption of radiocalcium both in control animals and in those with ligated common bile duct. This is due to the reduction of specific activity of the radiocalcium used.

DISCUSSION

Calcium^{45} uptake was found to be considerably higher than that of Strontium^{89}, continuing the observations of a number of investigators on the gastrointestinal process of discrimination against strontium and in favour of calcium (39, 128, 134, 135, 136). The "Observed Ratios" in the experiments described above were calculated from measurements in which the isotopes were given in a separate series of animals, but under identical conditions; the dose rate ratio of Sr^{89}/Ca^{45} was invariably equal to one. The O.R. values given in Fig. 4 and Tables II, III and IV are very similar to those of Comar et al (39), while the age of animals at which equilibrium was reached for Sr^{89} uptake (Fig. 4) are in agreement with those obtained by Thompson (137).

All the experimental animals were fed with standard purina chow containing 1.42% of calcium which is considered to be nutritionally adequate. Although the strontium content of this diet is not known exactly, it is expected to be very low. The apparent increase of calcium uptake (Tables II, III and IV) in bone with increased Ca^{45} dose is due to the increase of specific activity of the ingested calcium. Therefore the total calcium uptake is the same in all the experiments. Similarly the inactive strontium content of the diet can be assumed to be at a constant minimal level, then with increasing the dose of radiostrontium in the diet, it was found that there is a linear increase of Sr^{89} uptake in the femur due to the increasing specific activity of the ingested alkaline metal (Fig. 5). Thus it appears that radiostrontium uptake in bone is not

dependent upon the calcium content of the diet, even when a constant, relatively high, level of calcium is maintained. Further investigations should be carried out to determine the effect of either a low calcium diet or a diet with a very much higher calcium content.

The "Observed Ratio", though useful as an index in experimental studies, does not reveal the magnitude of the radiostrontium uptake in the bone. On a low calcium diet, the "Observed Ratio" will increase, as shown in studies by Thompson (137). While on a high calcium diet there may be corresponding reduction in the O.R.; however this does not mean that the amount of radiostrontium absorbed had been either increased or decreased. Comar et al (98) have shown that in cows and goats, high levels of stable strontium in the diet did not alter the O.R. diet/milk values of radiostrontium, thus indicating that the gastrointestinal discrimination against strontium declines with the increasing quantities of the element in the diet.

Absorption from ligated segments.

The rate of absorption of both strontium and calcium was greatest from the duodenal segment, in conformity with results of Wasserman (40) and Lengemann (116). The rate of absorption for the segments following the duodenum dropped sharply; a slow decline in rate was observed on proceeding down the bowel towards the ileum. However, the contribution of a given region of intestine to the total amount absorbed in the intact animal has to be judged from the rate of movement through that

region as well as the length of segment involved. Food materials pass most rapidly through the duodenum; this rate of transit decreases quite markedly as the material progresses towards the large intestine. As a result food spends the shortest time in the duodenum and the longest period in the ileum. Therefore, in the absorption of calcium and strontium, the distal regions of the small intestine play a much more important role in the intact animal. Recently, Cramer (138) has shown in dogs that the rate of calcium absorption per cm. length of the duodenum was almost double that in the jejunum or ileum but the estimated calcium absorption in the whole ileum exceeded that in the jejunum and that in the duodenum. This phenomenon may account for the striking increase in absorption of Sr^{89} in "Trisma" buffer in the intubation experiment: whereas the ileal segments show only a relatively small increase in the amount absorbed, the same solution, administered through an orogastric tube, causes as much as 300% increase in absorption. These experiments suggest that further study, maintaining the segments for at least two hours after injection of the isotope solution, is indicated.

Effect of pH on absorption

The experiments using buffered solutions described in this study cannot be compared with the pH studies reported by Mraz (131) and Dukay (130). Both these investigators used solutions of different pH values, but no attempt was made to buffer them, nor was any estimate made of the pH of

the intestinal luminal contents at the end of the experimental period. It does not follow, therefore, that the actual pH of the intestinal lumen was altered by their treatment, and the results of their studies do not necessarily reflect the influence of pH on absorption. As can be seen from Table VI, a relatively high buffering capacity is needed to maintain the desired pH value even in a short (5 cms.) segment of the intestine.

Absorption of strontium was not altered when the pH of the duodenum was increased slightly (±0.3 pH) with the "Trisma" buffer, while earlier work (132) had indicated that by lowering the pH (-0.2 units) a signi - ficant decrease in absorption was obtained with the same buffer. This suggests that the increased absorption of Sr^{89} obtained in the intubation experiments with "Trisma" does not occur in the duodenum; it may be assumed that the duodenum normally absorbs at its maximum capacity and that this rate cannot further be increased. Similarly, an increased pH with Trisma did not alter the absorption rate of Ca^{45} from the duodenum.

Percentage absorption of Sr^{89} and Ca^{45} was increased in most of the more distal segments when the radioisotope was introduced into the gut with Trisma, pH 8.5, confirming the earlier work (132) on the absorption of Sr^{89} in the presence of Trisma at pH 7.8. The increase in percentage absorbed in thirty minutes however was relatively small from each segment. To account for the enormous increase in absorption in the intubation experiments, one must assume that there is a steep increase in the absorption rate after thirty minutes has elapsed. Futher studies

on this problem are necessary.

Absorption of Sr^{89} was decreased from all segments of the intestine in the presence of the acidic phosphate buffer. The blood levels and bone uptake of Sr^{89} corresponded with the decreased absorption. Ca^{45} absorption on the other hand, was unaffected in most segments or was even slightly increased from segments V and VII. Blood levels and bone uptake from all segments were significantly lower when absorption took place from phosphate. These anomalous results with calcium correspond with those on the absorption of Ca^{45} in the intubation experiments, when the effect of phosphate on Ca^{45} blood levels and bone uptake is much less than on Sr^{89}.

Calcium therefore is not easily influenced by either enhancing or depressing agents. There is general agreement that calcium metabolism is under close homeostatic control and in this the intestine plays a major role. In the young growing animals there is no remarkable in - crease of calcium absorption in the presence of "Trisma" buffer or decrease with phosphate buffer. However in female rats who had several litters previously and hence may be in negative calcium balance, experiments showed a considerable increase in blood level and bone uptake of Ca^{45} (Table XIV and Fig. 14). This indicates that calcium absorption and uptake can be significantly increased in animals with negative calcium balance, in the presence of slightly alkaline buffering agents like "Trisma". Whether this mechanism is similar to those of lysine, arginine and lactose in enhancing the calcium absorption

is not known. This needs further investigation.

"Trisma" buffer was more effective in increasing strontium absorption. This may be due to the fact that strontium forms soluble salt in "Trisma" buffer at the pH used. Strontium absorption is not under homeostatic control and hence the enhancing effect of "Trisma" is not inhibited by other influences as in the case of calcium. Phosphate buffer was more effective in depressing the strontium absorption than calcium. This finding is similar to those of MacDonald et al (81). The phosphate may also have produced insoluble precipitates of strontium and calcium and thus reduced the amount of these cations absorbed.

Ligation of the common bile duct did not produce any significant change in the rate of absorption of Sr^{89} and Ca^{45} from the ligated duodenal segment.

CHAPTER VI

INHIBITION OF STRONTIUM ABSORPTION

Introduction.

The effect of single dose of sodium alginate on uptake of Sr^{89}.

Effect of single dose of sodium alginate on uptake of Ca^{45}.

Effect of time of administration of sodium alginate on Sr^{89} uptake by bone.

Effect of age of animals on blood levels and bone uptake of Sr^{89} in the presence of sodium alginate.

Comparative study with other macromolecular substances.

Effect of sodium alginate on absorption of Sr^{89} and Ca^{45} from the ligated intestinal segments.

Effect of increasing concentrations of sodium alginate on Sr^{89} and Ca^{45} uptake in bone from the ligated duodenal segments.

Discussion.

CHAPTER VI

INHIBITION OF STRONTIUM ABSORPTION

Although it is estimated that the average quantity of Sr^{90} in the human skeleton is, at present, far below the danger level there is no immediate remedy available to counteract this hazard if the level of radioactive strontium in the atmosphere increases. Man now has the capacity to create an environment, perhaps unwittingly, which might raise the radioactive content of dietary materials to a dangerously high level. This has naturally stimulated several investigators to study the possibility of decontaminating biological systems from radiostrontium.

As was described in Chapter III the physiological steps that bring about the major differences in behaviour in strontium and calcium in the biological system are found to be in gastrointestinal absorption, renal excretion, secretions of the mammary gland and placental transfer, in this order of magnitude. These are the processes in which ions pass across membranes under metabolic control. On the other hand, there seems to be no evidence of significant difference in the movement of calcium and strontium between blood and bone. Therefore any attempt to enhance the excretion of radiostrontium already deposited in the skeleton by giving diuretics, hypercalcuric agents, hormones and complexing reagents are bound to produce equal or greater effects on calcium with

the resultant danger of bone demineralisation.

The diet contains very minute quantities of strontium compared with those of calcium. Thus it would appear that the mechanism or mechanisms which provide for the gastrointestinal discrimination against strontium are quite specific and are able to distinguish traces of strontium in the presence of large amounts of calcium. It is also known that strontium is not actively transported across the intestinal mucosa, whereas calcium is actively transported (40).

These facts suggested that means should be found to prevent absorption of dietary strontium from the gastrointestinal tract. For this purpose we sought for some substance of high molecular weight which would bind strontium in the intestine and which itself is not absorbed. Such a substance should be non-toxic, palatable and easily available. In all, three naturally occurring acidic polysaccharides and two synthetic ion exchange resins were tested. Two of the naturally occurring polysaccharides, sodium alginate and carrageenin are derived from marine algae. The third, polygalacturonic acid is derived from fruit pectin.

Sodium alginate is a water soluble derivative of alginic acid which was discovered as a constituent of kelp about seventy years ago. Alginic acid and its derivatives are in general use in the food industry as emulsifying and gelling agents. The generally accepted structure of alginic acid is shown in Figure 15. It is a linear polymer of anhydro-β - D - mannuronic acid of high molecular weight. From this structure it can be seen that the 1, 4 - linkages result in each anhydro-mannuronic

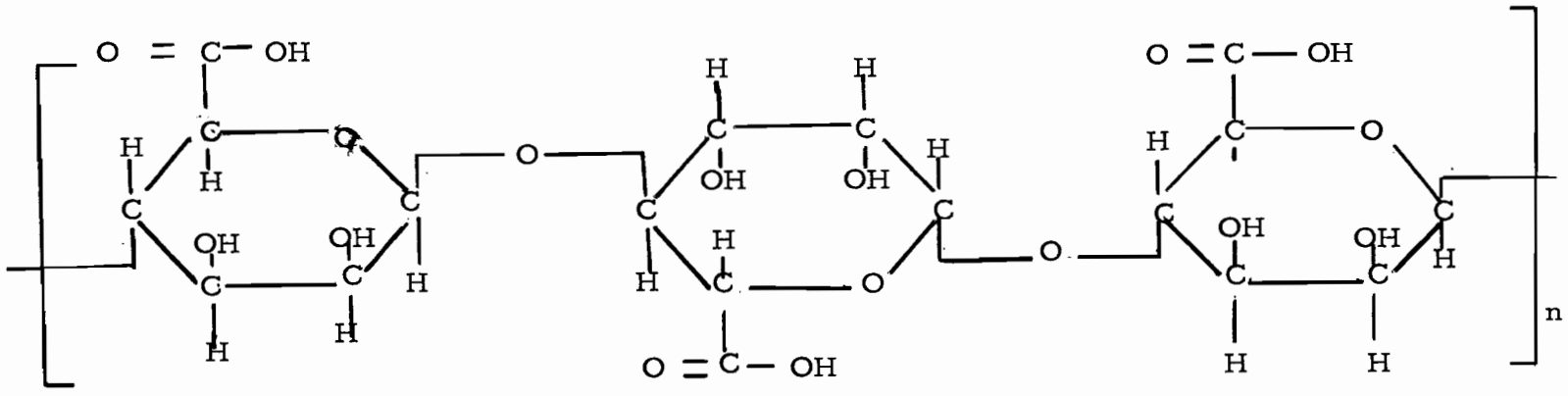


Fig. 15 Structural formula of alginic acid.

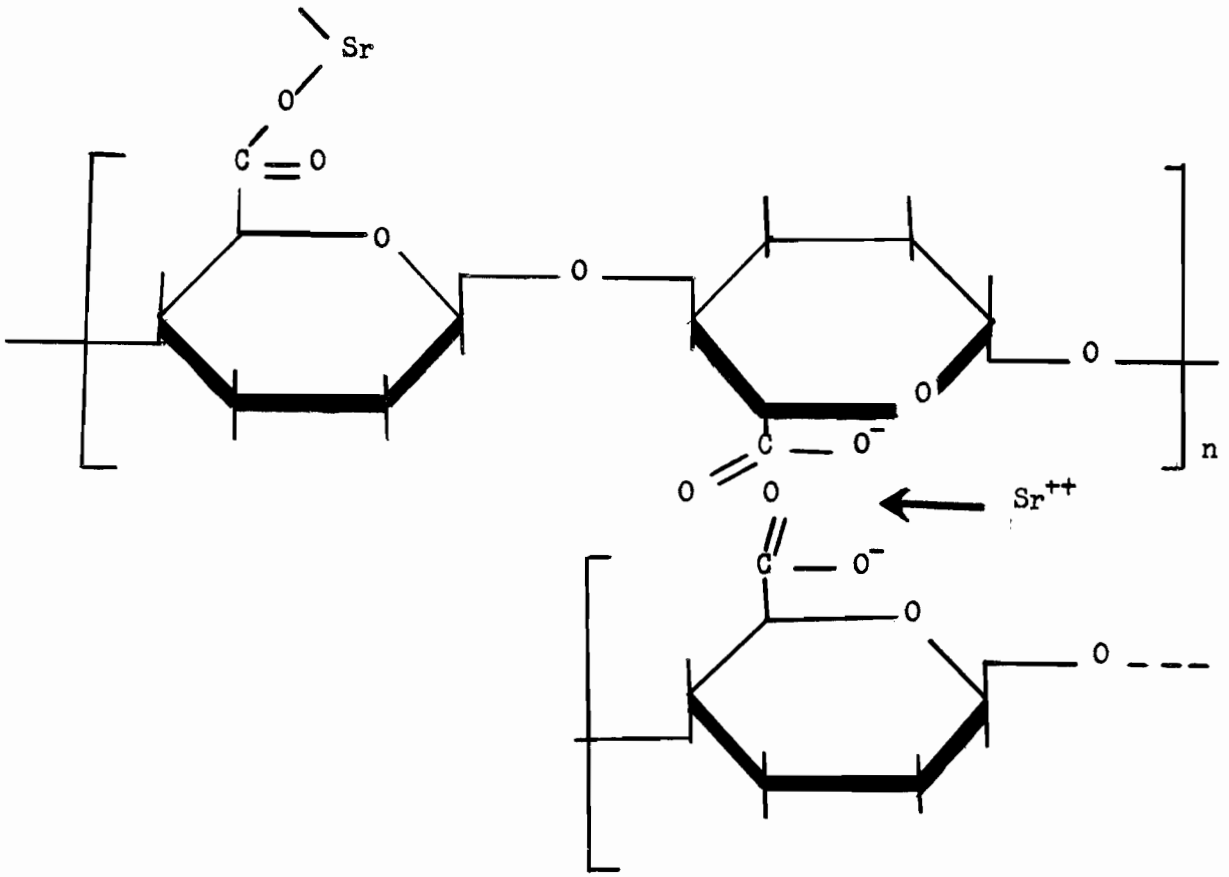


Fig. 16 Proposed structure of strontium salt of polymannuronic acid.

acid unit having one free carboxylic acid group and two free hydroxy groups while the aldehyde portion is securely blocked. In addition, commercial experience as well as laboratory results points to the ability of algin to adsorb ions aside from and beyond its carboxyl ion exchange properties (139). The proposed binding of divalent metal ions by the acidic groups is depicted in Fig. 16. Sodium alginate precipitates with calcium and strontium salts in vitro.

Sodium alginate used in this study was of standard grade and obtained from commercial source (Fisher Scientific Company Limited, Montreal).

Pectic acid is derived from fruit pectin and is also used in the food industry as gelling agent. It is a polymer composed of galacturonic acid residues and precipitates as a calcium salt in vitro. The proposed structure is given in Fig. 17.

Carrageenin is derived from the red marine algae chondrus crispus, popularly known as Irish moss. It is a polymer composed of anhydro - galactose units with sulphonic acid ester group and forms soluble calcium and strontium salts.

The two synthetic ion exchange resins used were 1. Carboresin, a product of Eli Lily and Company Limited, and 2. Rexyn 101 (Na^{-}) of Fisher Scientific Company Limited. Carboresin is a mixture of three resins: one anion and two cation exchangers. Rexyn 101 (Na^{+}) is an organic strong acid cation exchanger, sulfonated polystyrene copolymer with medium porosity.

The inhibiting activity of these polymers was tested by administering solutions or suspensions simultaneously with the radioactive isotope

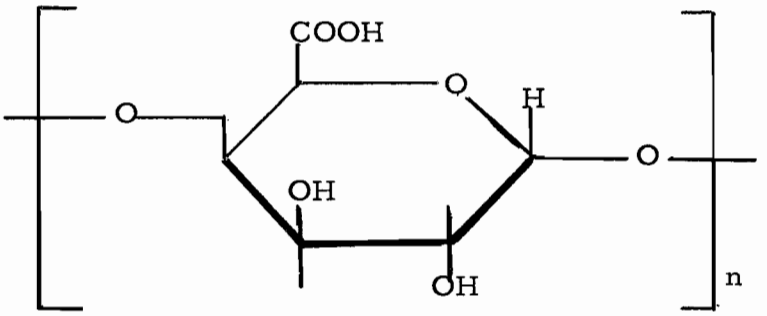


Fig. 17 Polygalacturonic acid - proposed structure.

by means of orogastric intubation. Blood levels and bone uptake were measured at various intervals after administration.

Finally, the polysaccharides were added to the diet, either in the chow, or dissolved in drinking water.

To test the possibility of applying the findings to humans, it was necessary to be able to introduce the polysaccharide into the normal diet over a prolonged period of time. Sodium alginate was mixed with the dry chow, and also dissolved in drinking water. The effect of varying the concentration of alginate in the diet on bone uptake, and the ratio of dose of Sr^{89} to bone uptake were also studied.

The effect of sodium alginate on Ca^{45} metabolism under identical conditions was investigated as it is of great importance that the skeleton should not be depleted of this element.

EXPERIMENTS AND RESULTS

The effect of single dose of sodium alginate on uptake of Sr^{89}

Strontium^{89} was diluted with inactive $SrCl_2$ solution to give a final concentration of 0.9 μM and 10 μc activity per ml. In one ml. of this solution 20 mg of sodium alginate was dissolved. The resulting solution was viscous but sufficiently fluid to pass through a Fr. No. 8 rubber catheter. High concentrations of alginate were impracticable. The animals (100 - 120 grams) which had fasted for the preceding 24 hours, were given one ml. of this radioactive test solution through an orogastric tube under light ether anaesthesia. Groups of six animals were sacrificed at intervals of 2, 4, 6, 24 and 72 hours after dosing. One ml. of blood and one femur assayed for radioactivity.

In the presence of sodium alginate there was a considerable reduction in the levels of Sr^{89} in blood and bone (Tables XVIII and XIX, Fig. 18). After 24 hours no radiostrontium was detectable in the blood, while bone uptake was reduced to 26% of the control values; this was further reduced to 23% in 72 hours.

Effect of single dose of sodium alginate on uptake of Ca^{45}

Ca^{45} was diluted with $CaCl_2$ solution to give a final concentration of 1.1 μM, 10 μc per ml. Twenty mg sodium alginate were dissolved in one ml. of this radioactive solution and administered as above. Assays

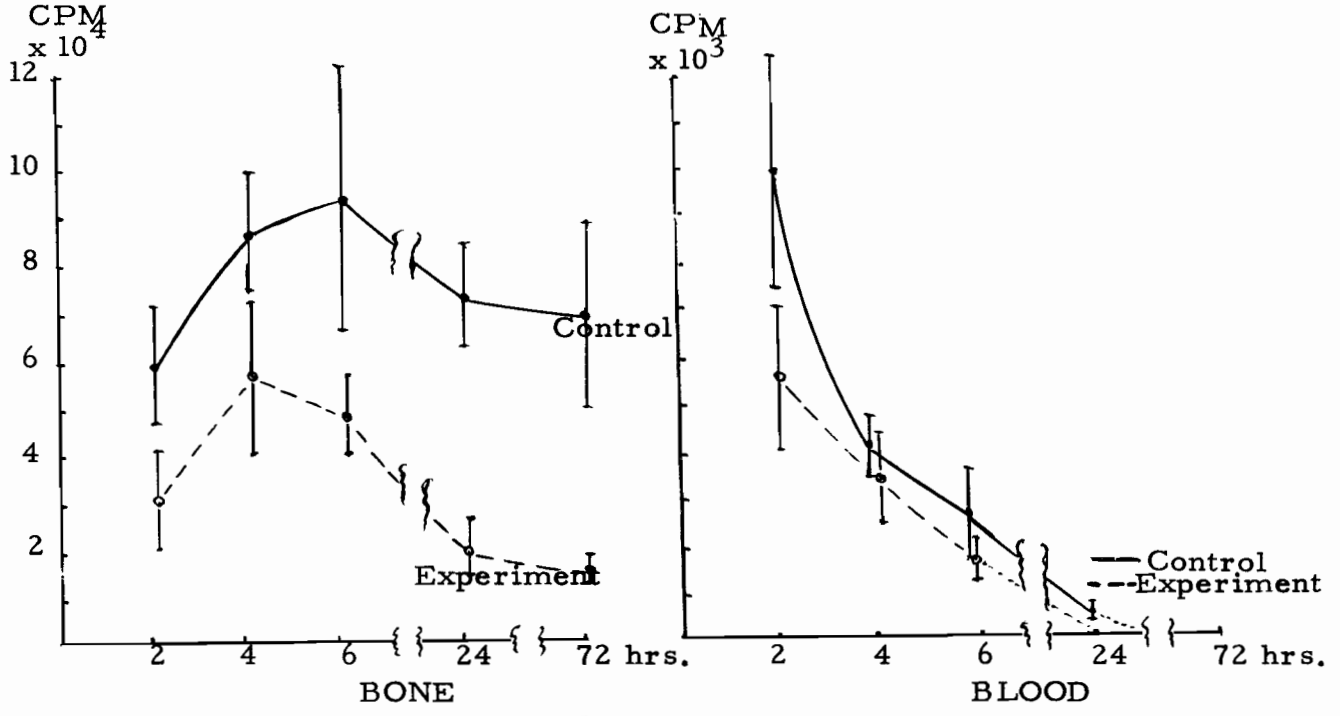
TABLE XVIII

EFFECT OF SINGLE DOSE OF SODIUM ALGINATE ON Sr^{89} AND Ca^{45} LEVELS IN BLOOD

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ or 10 μc of Ca^{45} in 1.1 μM of inactive $CaCl_2$ with 20 mg of sodium alginate in one ml. were administered through orogastric tube.

	STRONTIUM^{89}			CALCIUM^{45}		
TIME	CONTROL	EXPERIMENT	EXPT. % CONTROL	CONTROL	EXPERIMENT	EXPT. % CONTROL
2 hours	10006 ± 2506	5685 ± 1521	56	8288 ± 2084	7519 ± 748	90
4 hours	4153 ± 664	3510 ± 1076	84	4625 ± 696	3697 ± 630	79
6 hours	2685 ± 983	1755 ± 490	65	2837 ± 941	2293 ± 577	80
24 hours	569 ± 188	------	--	1495 ± 296	1552 ± 576	103
72 hours	-----	------	--	------	------	--

Each figure represents mean counts per minute and standard deviation in six animals.
Blank space indicates radiation was low to count.



89

Fig. 18 Control: distribution of Sr after orogastric intubation. Experiment: Effect of addition of sodium alginate on blood level and bone uptake. C. P. M. - counts per minute.

TABLE XIX

EFFECT OF SINGLE DOSE OF ALGINATE ON Sr^{89} AND Ca^{45} UPTAKE BY BONE

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ or 10 μc of Ca^{45} in 1.1 μM of inactive $CaCl_2$ with 20 mg of sodium alginate in one ml. were administered through orogastric tube.

TIME	STRONTIUM^{89}			CALCIUM^{45}			OBSERVED RATIO	
	CONTROL	EXPERIMENT	EXPT. % / CONTROL	CONTROL	EXPERIMENT	EXPT. % / CONTROL	CONTROL	EXPT.
2 hours	60206±12592	30039±10521	49	103433±32544	88458±16030	85	0.58	0.34
4 hours	88398±12923	56647±16711	64	144041±32185	98816±17089	68	0.62	0.57
6 hours	95826±28312	48989±8638	51	120508±44173	93958±34513	77	0.80	0.52
24 hours	74501±11192	19742±5907	26	141283±18792	77758±16813	55	0.53	0.25
72 hours	65503±19618	15602±3158	23	132600±15281	73908±10229	55	0.49	0.21

Each figure represents mean counts per minute and standard deviation for six animals.

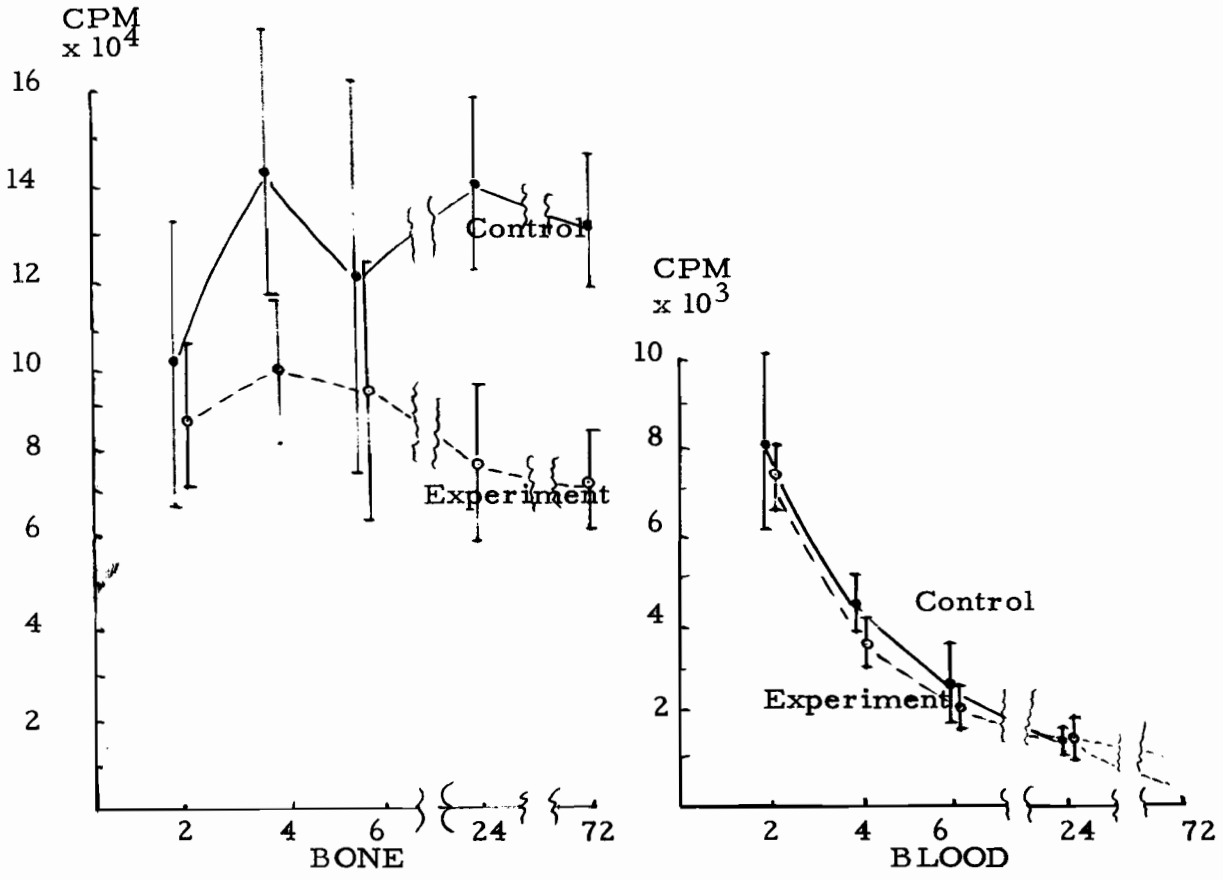


Fig.19 Control: distribution of Ca^{45} after orogastric intubation. Experiment: effect of addition of sodium alginate on blood level and bone uptake. C. P. M. - counts per minute.

for radioactivity in blood and femur were carried out in groups of rats exactly as in the experiments using radiostrontium.

In the presence of sodium alginate the Ca^{45} blood level was not significantly altered at 2 hours (Fig. 19, Tables XVIII and XIX). After 4 hours it was reduced by 20% returning to the same level as the controls thereafter. Bone uptake, however, continued to decrease slowly, reaching 55% of the control values after 72 hours.

Effect of time of administration of sodium alginate on Sr^{89} uptake by bone

To determine the binding efficiences of alginate when given at a separate time, the previous experiments were repeated exactly except that in one group of six animals sodium alginate was administered thirty minutes before the dose of Sr^{89}, and in a second group, thirty minutes after Sr^{89} administration. In the third group both Sr^{89} and sodium alginate were given simultaneously.

Animals were sacrificed at the end of 24 hours and one femur from each animal assayed for radioactivity. An equal number of control animals was studied using water instead of sodium alginate solution.

Sodium alginate, given thirty minutes before isotope dosing was more effective and reduced the femur uptake of radiostrontium to 56.1% of the control (Fig. 20, Table XX). Administration of sodium alginate thirty minutes after the dose of Sr^{89} reduced the bone uptake of radiostrontium to 67% of the control. Optimum reduction was obtained by simultaneous administration of the binding agent and radiostrontium.

The effect of age of animals on blood levels and bone uptake of Sr^{89} in the presence of sodium alginate

The uptake of calcium and strontium is known to be greater in rapidly growing skeletal tissue. The effect of sodium alginate in adults with a steady metabolic rate was studied in six rats weighing 350 to 375 grams. Solutions containing 0.9 μM $SrCl_2$, 10 μc Sr^{89} with 20 mgs. sodium alginate per millilitre were introduced by orogastric tube at the rate of 1 ml. per 100 gms. body weight. Animals were kept alive for 24 hours and allowed to have water and food ad libitum. Assay for radioactivity on one millilitre of blood and one femur was carried out using the method of preparation described previously. An equal number of control animals was studied without addition of sodium alginate.

The Sr^{89} blood level in the mature control animals was twice as much as in young growing rats (Fig. 21, Table XXI). Conversely, Sr^{89} uptake by the adult rat femur was reduced to 54% of that in a young growing rat. The effect of sodium alginate on adult rats was more pronounced; after 24 hours no radioactivity was detected in the blood, while bone uptake had been reduced to less than 15% of the control value.

Comparative study with other macromolecular substances

The effect of sodium alginate was compared with two other naturally occurring polysaccharides, pectic acid and carrageenin, and two synthetic resins, Rexyn 101 (Na) and Carboresin. Dose solutions or fine suspensions containing 0.9 μM of inactive $SrCl_2$, 10 μc Sr^{89} and 20 mgs. of the binding

TABLE XX

EFFECT OF TIME OF ADMINISTRATION OF SODIUM ALGINATE ON Sr^{89} UPTAKE BY BONE

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ in one ml. was administered through orogastric tube.

	CONTROL	EXPERIMENT	$\frac{\text{EXPT.}}{\text{CONTROL}}$ %
GROUP I	104266 ± 43856	69696 ± 15237	66.8
GROUP II	74501 ± 11192	19742 ± 5907	26.4
GROUP III	73265 ± 15127	41110 ± 8609	56.1

Each figure represents mean counts per minute and standard deviation for six animals.

GROUP I Sodium alginate administered 30 minutes after Sr^{89}

GROUP II Simultaneous administration of Sr^{89} and sodium alginate

GROUP III Sodium alginate administered 30 minutes before Sr^{89}

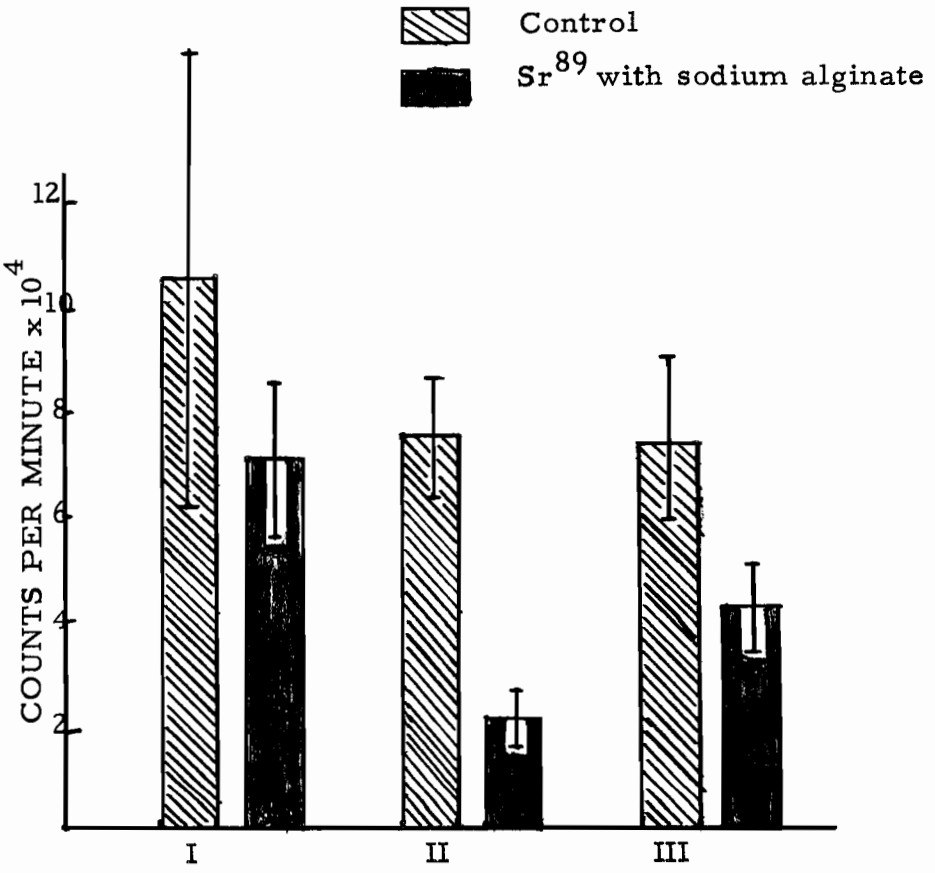


Fig. 20 Effect of time of administration of sodium alginate on Sr^{89} bone uptake following orogastric intubation; sodium alginate administered I) thirty minutes after Sr^{89}; II) simultaneously; III) thirty minutes before Sr^{89}.

TABLE XXI

EFFECT OF SODIUM ALGINATE ON Sr^{89} UPTAKE BY ADULT RATS

Ten μc of Sr^{89} in 0.9 μM of inactive $SrCl_2$ with 20 mgs. of sodium alginate in one ml. per 100 gms. weight were administered through orogastric tube to adult rats weighing 350 - 375 gms. At the end of 24 hours sacrificed, one ml. of blood and femur assayed for radioactivity.

BLOOD		BONE		
CONTROL	EXPERIMENT	CONTROL	EXPERIMENT	$\frac{\text{EXPT.}}{\text{CONTROL}}$ %
1013 ± 347	***	40172±8922	5875 ± 1665	16.5

Each figure represents mean counts per minute and standard deviation for six animals.
*** Counts not more than background.

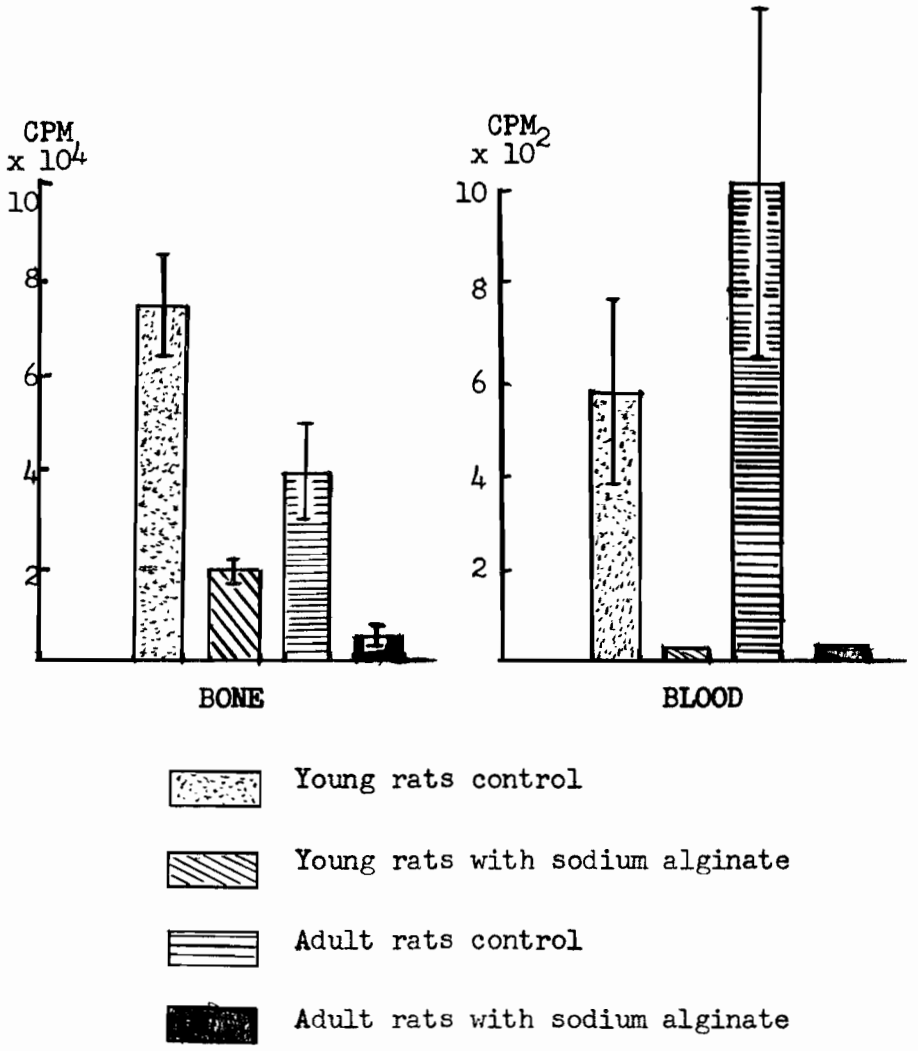


Fig. 21 Effect of age of animal on Sr^{89} uptake after orogastric intubation. C.P.M. - counts per minute.

agent in one ml. were introduced by orogastric tube to fasting animals under light ether anaesthesia. Groups of six animals were sacrificed at 2, 4, 6, and 24 hours and one ml. of blood and one femur assayed for radioactivity.

Experiments under identical conditions with an equal number of rats were carried out using 1.1 μM of inactive $CaCl_2$, 10 μc Ca^{45} mixed with 20 mg of binding agent. The results of these experiments are given in Tables XXII to XXIX. The bone uptake of Sr^{89} and Ca^{45} at the end of 24 hours after administration with different binding agents is shown in Figure 22.

Strontium ^{89} level in blood was effectively reduced by all the macromolecular binding agents with the exception of carrageenin. Blood levels of Ca^{45} were affected only to a minor degree. Bone uptake of Sr^{89} was also reduced by all the agents except carrageenin, the polyuronic acids and sulphonic acid resin being the most effective. Ca^{45} uptake was reduced to a lesser degree.

Carrageenin produced a consistent enhancing effect on Ca^{45} uptake in bone (TableXXV and Fig. 22). Whether this effect is similar to those of lysine and lactose is not known and needs further investigation.

<u>The effect of sodium alginate on Sr^{89} and Ca^{45} absorption from ligated intestinal segments.</u>

One mg sodium alginate in 0.5 ml of solution containing 2 μc Sr^{89} plus 0.45 μM of inactive $SrCl_2$ or 2 μc Ca^{45} plus 0.55 μM of inactive $CaCl_2$ was injected into each ligated intestinal segment. At the end of

TABLE XXII

EFFECT OF SINGLE DOSE OF POLYGALACTURONIC ACID ON Sr^{89} AND Ca^{45} LEVELS IN BLOOD

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ or 10 μc of Ca^{45} in 1.1μM of inactive $CaCl_2$ with 20 mg Polygalacturonic acid suspension in one ml. were administered through orogastric tube.

TIME	STRONTIUM^{89}			CALCIUM^{45}		
	CONTROL	EXPERIMENT	EXPT. % CONTROL	CONTROL	EXPERIMENT	EXPT.% CONTROL
2 hours	10,006 (±2506)	3613 (±808)	36%	8288 (±2084)	7520 (±1448)	91%
4 hours	4153 (±664)	2575 (±572)	62%	4625 (±696)	3123 (±679)	68%
6 hours	2685 (±983)	1173 (±171)	44%	2837 (±941)	2715 (±519)	96%
24 hours	569 (±188)	----	---	1495 (±296)	1282 (±149)	86%

Each figure represents mean counts per minute in one ml. of blood and standard deviation for studies in six rats.

TABLE XXIII

EFFECT OF SINGLE DOSE OF POLYGALACTURONIC ACID ON Sr^{89} AND Ca^{45} UPTAKE BY BONE

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ or 10 μc of Ca^{45} in 1.1 μM of inactive $CaCl_2$ with 20 mg of polygalacturonic acid suspension in one ml. were administered through orogastric tube.

TIME	STRONTIUM^{89}			CALCIUM^{45}			OBSERVED RATIO	
	Control	Experiment	Expt. % / Control	Control	Experiment	Expt. % / Control	Control	Experiment
2 hours	60206 (±12592)	25213 (±8590)	42%	103433 (±32544)	88775 (±33057)	86%	0.58	0.28
4 hours	88398 (±12923)	41573 (±14372)	47%	144041 (±32185)	94734 (±23160)	66%	0.62	0.43
6 hours	95826 (±28312)	29764 (±7752)	31%	120508 (±44173)	118850 (±31395)	98%	0.80	0.25
24 hours	74501 (±11192)	24741 (±6179)	33%	141283 (±18792)	97858 (±7042)	69%	0.53	0.25

Each figure represents mean counts per minute (one femur) and standard deviation for studies in six rats.

TABLE XXIV

EFFECT OF SINGLE DOSE OF CARRAGEENIN ON STRONTIUM^{89} AND CALCIUM^{45} LEVELS IN BLOOD

Ten microcuries of Strontium^{89} in 0.9 μM of inactive $SrCl_2$ or 10 microcuries of Calcium^{45} in 1.1 μM of inactive $CaCl_2$ with 20 mg of carrageenin in one ml. were administered through orogastric tube.

TIME	STRONTIUM^{89}			CALCIUM^{45}		
	Control	Experiment	$\frac{\text{Expt.}}{\text{Control}}$ %	Control	Experiment	$\frac{\text{Expt.}}{\text{Control}}$ %
2 hours	10006 (±2506)	6544 (±1296)	65%	8288 (±2084)	8074 (±789)	97%
4 hours	4153 (±664)	4090 (±814)	98%	4625 (±696)	4367 (±1109)	94%
6 hours	2685 (±983)	2398 (±907)	89%	2837 (±941)	3312 (±696)	116%
24 hours	569 (±188)	309 (±92)	54%	1495 (±296)	691 (±313)	46%

Each figure represents mean counts per minute in one ml. of blood and standard deviation for studies in six rats.

TABLE XXV

EFFECT OF SINGLE DOSE OF CARRAGEENIN ON Sr^{89} AND Ca^{45} UPTAKE IN BONE

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ or 10 μc of Ca^{45} in 1.1 μM of inactive $CaCl_2$ with 20 mg of carrageenin in one ml. were administered through orogastric tube.

TIME	STRONTIUM^{89}			CALCIUM^{45}			OBSERVED RATIO	
	Control	Experiment	Expt. % Control	Control	Experiment	Expt. % Control	Control	Experiment
2 hours	60206 (±12592)	48698 (±5189)	81%	103433 (±32544)	115983 (±14633)	112%	0.58	0.41
4 hours	88398 (±12923)	75965 (±10464)	86%	144041 (±32185)	151625 (±17201)	105%	0.62	0.50
6 hours	95826 (±28312)	66717 (±24939)	71%	120508 (±44173)	125558 (±15454)	104%	0.80	0.53
24 hours	74501 (±19618)	66717 (±11113)	89%	141283 (±18792)	193158 (±33400)	137%	0.53	0.34

Each figure represents mean counts per minute (one femur) and standard deviation for studies in six rats.

TABLE XXVI

Effect of single dose of carbo resin on Sr^{89} and Ca^{45} levels in blood

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ or 10 μc of Ca^{45} in 1.1 μM of inactive $CaCl_2$ with 20 mg of Carbo resin suspension in one ml. were administered through orogastric tube.

TIME	STRONTIUM^{89}			CALCIUM^{45}		
	Control	Experiment	$\frac{\text{Expt.}}{\text{Control}}$ %	Control	Experiment	$\frac{\text{Expt.}}{\text{Control}}$ %
2 hours	10006 (±2506)	5752 (±1545)	57%	8288 (±2084)	7208 (±2790)	87%
4 hours	4153 (±664)	2997 (±1327)	72%	4625 (±696)	3778 (±1057)	82%
6 hours	2685 (±983)	2606 (±357)	97%	2837 (±941)	2737 (±284)	96%
24 hours	569 (±188)	---	---	1495 (±296)	1864 (±280)	125%

Each figure represents mean counts per minute in one ml. of blood and standard deviation for studies in six rats.

TABLE XXVII

EFFECT OF SINGLE DOSE OF CARBO RESIN ON Sr^{89} AND Ca^{45} UPTAKE BY BONE

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ or 10 μc of Ca^{45} in 1.1 μM of inactive $CaCl_2$ with 20 mg of Carbo Resin. Suspension in one ml. were administered through orogastric tube.

TIME	STRONTIUM^{89}			CALCIUM^{45}			OBSERVED RATIO	
	Control	Experiment	Expt. % / Control	Control	Experiment	Expt. % / Control	Control	Experiment
2 hours	60206 (±12592)	42603 (±22181)	71%	103433 (±32544)	91116 (±37583)	88%	0.58	0.46
4 hours	88398 (±12923)	53779 (±29445)	61%	144041 (±32185)	146100 (±39450)	101%	0.62	0.36
6 hours	95826 (±28312)	68092 (±13202)	71%	120508 (±44173)	142116 (±22960)	118%	0.80	0.47
24 hours	74501 (±11192)	30284 (±8349)	41%	141283 (±18792)	112833 (±18851)	80%	0.53	0.26

Each figure represents mean counts per minute (one femur) and standard deviation for studies in six animals.

TABLE XXVIII

EFFECT OF SINGLE DOSE OF REXYN 101 (Na^+) ON Sr^{89} AND Ca^{45} LEVELS IN BLOOD

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ or 10 μc of Ca^{45} in 1.1 μM of inactive $CaCl_2$ with 20 mg of Rexyn suspension in one ml. were administered through orogastric tube.

TIME	STRONTIUM^{89}			CALCIUM^{45}		
	Control	Experiment	$\frac{\text{Expt.}}{\text{Control}}$ %	Control	Experiment	$\frac{\text{Expt.}}{\text{Control}}$ %
2 hours	10006 (±2506)	3552 (±721)	35%	8288 (±2084)	5969 (±1250)	72%
4 hours	4153 (±664)	2708 (±673)	65%	4625 (±696)	4445 (±817)	96%
6 hours	2685 (±983)	1773 (±744)	66%	2837 (±941)	3377 (±1095)	119%
24 hours	569 (±188)	----	---	1495 (±296)	1134 (±419)	76%

Each figure represents mean counts per minute in one ml. of blood and standard deviation for studies in six rats.

TABLE XXIX

EFFECT OF SINGLE DOSE OF REXYN 101 (Na^+) ON Sr^{89} AND Ca^{45} UPTAKE BY BONE

Ten microcuries of Sr^{89} in 0.9 μM of inactive $SrCl_2$ or 10 μc of Ca^{45} in 1.1 μM of $CaCl_2$ with 20 mg of Rexyn suspension in one ml. were administered through orogastric tube.

TIME	STRONTIUM^{89}			CALCIUM^{45}			OBSERVED RATIO	
	Control	Experiment	$\frac{\text{Expt.}}{\text{Control}}$ %	Control	Experiment	$\frac{\text{Expt.}}{\text{Control}}$ %	Control	Experiment
2 hours	60206 (±12592)	18416 (±5326)	31%	103433 (±32544)	45850 (±6442)	44%	0.58	0.40
4 hours	88398 (±12923)	35625 (±9451)	40%	144041 (±32185)	103791 (±26431)	72%	0.62	0.34
6 hours	95826 (±28312)	42059 (±13531)	44%	120508 (±44173)	121083 (±33305)	100%	0.80	0.34
24 hours	74501 (±11192)	18512 (±4042)	25%	141283 (±18792)	80450 (±17503)	57%	0.53	0.23

Each figure represents mean counts per minute (one femur) and standard deviation for studies in six rats.

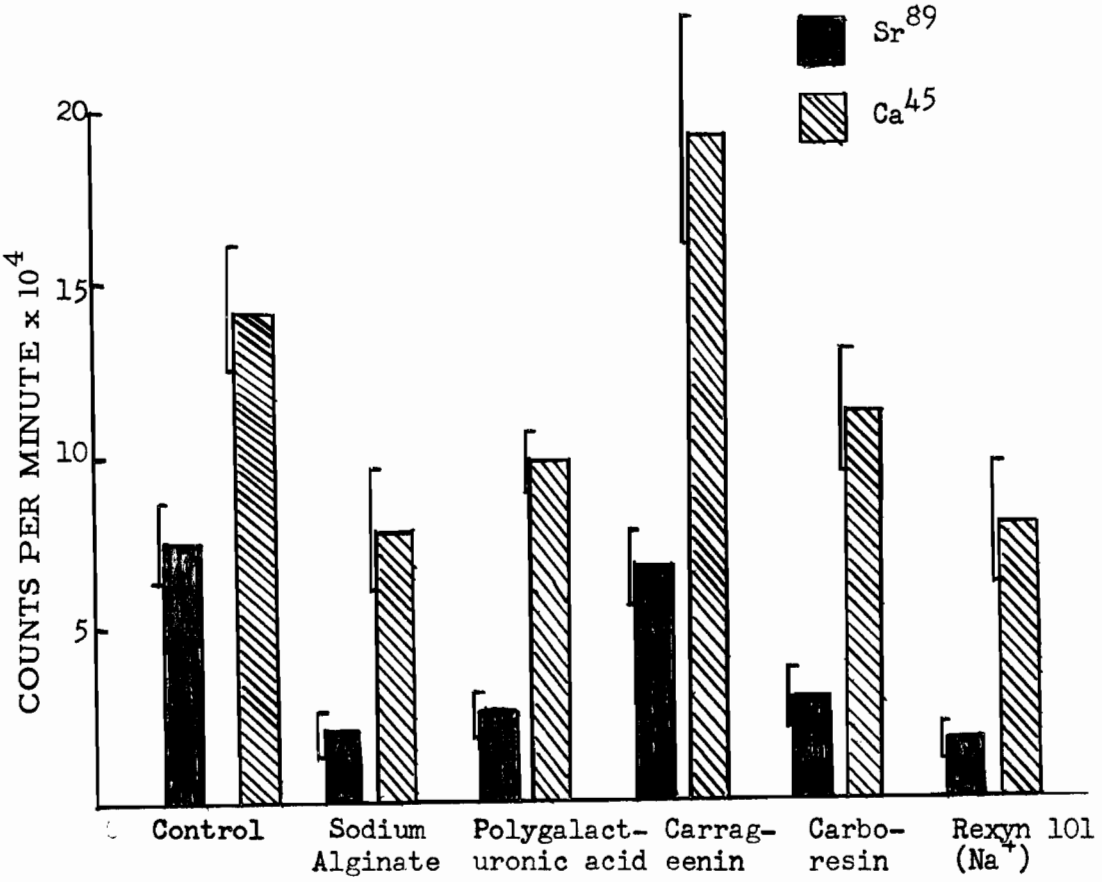


Fig. 22 Comparative effects of different macromolecular substances on Sr^{89} and Ca^{45} uptake in femur following orogastric intubation.

thirty minutes the remaining radioactivity in the segment, in one ml. of blood and one femur was assayed as described previously.

The results of this study are given in Tables XXX, XXXI and XXXII and Figures 23, 24, and 25. Sodium alginate administered in quantities somewhat in excess of that required for two acid residues to bind one atom of the divalent metal effectively reduced the absorption of strontium from all intestinal segments by as much as 50 to 80% of the control values. Reduction in blood level and in bone uptake followed the same pattern. No significant reduction in the absorption of calcium was observed in the concentrations used and these results were confirmed by the unchanged calcium blood levels and bone uptake.

Effect of increasing concentrations of sodium alginate on Sr^{89} and Ca^{45} absorption from ligated duodenal segments.

Solutions containing increasing concentrations of sodium alginate, 2, 4, 8, 12 and 16 mg per ml. (10 to 80 micromoles), with 10 μc Sr^{89} plus 0.9 μM $SrCl_2$ or 10 μc Ca^{45} plus 1.1 μM $CaCl_2$ were prepared. 0.5ml of these solutions were injected into ligated duodenal segments of rats weighing 100 to 120 grams. At the end of thirty minutes the radio - activity in one ml. of blood, one femur and that remaining in the segment was assayed.

At a rough approximation 2 mgs. sodium alginate contributes 10 micromoles of free carboxyl groups; as discussed in the introduction

TABLE XXX

INFLUENCE OF SODIUM ALGINATE ON Sr^{89} AND Ca^{45} ABSORPTION FROM RAT'S INTESTINAL TRACT

(Test solutions were administered into the ligated intestinal segments)

Segment	2 μc Sr^{89} with 0.45 μM of inactive $SrCl_2$ for 30 mins.		2 μc Ca^{45} with 0.55 μM of inactive $CaCl_2$ for 30 minutes	
	Control	with 1.5 μM of sodium alginate	Control	with 1.5 μM of sodium alginate
I	10.0 ± 4.95	2.3 ± 2.19	4.1 ± 3.6	4.4 ± 2.89
II	33.46± 5.89	15.2 ± 4.17	66.2 ± 11.53	55.1 ± 18.02
III	18.3 ± 7.11	1.7 ± 3.24	20.3 ± 10.1	15.9 ± 4.76
IV	16.7 ± 7.61	4.3 ± 5.00	19.2 ± 5.65	19.7 ± 5.56
V	10.5 ± 5.91	3.4 ± 4.85	18.3 ± 8.12	16.2 ± 5.05
VI	9.7 ± 5.1	5.5 ± 4.40	12.9 ± 5.91	10.7 ± 6.85
VII	10.4 ± 6.96	1.5 ± 2.32	20.9 ± 6.92	22.8 ± 7.07
VIII	11.2 ± 5.59	4.0 ± 3.01	13.5 ± 5.29	14.6 ± 3.60
IX	10.4 ± 5.66	1.6 ± 1.48	16.6 ± 6.77	20.9 ± 8.02
X	11.8 ± 4.52	2.9 ± 1.61	16.1 ± 5.74	16.8 ± 5.19

Each figure represents the mean percentage of absorption and standard deviation of studies in twelve rats.

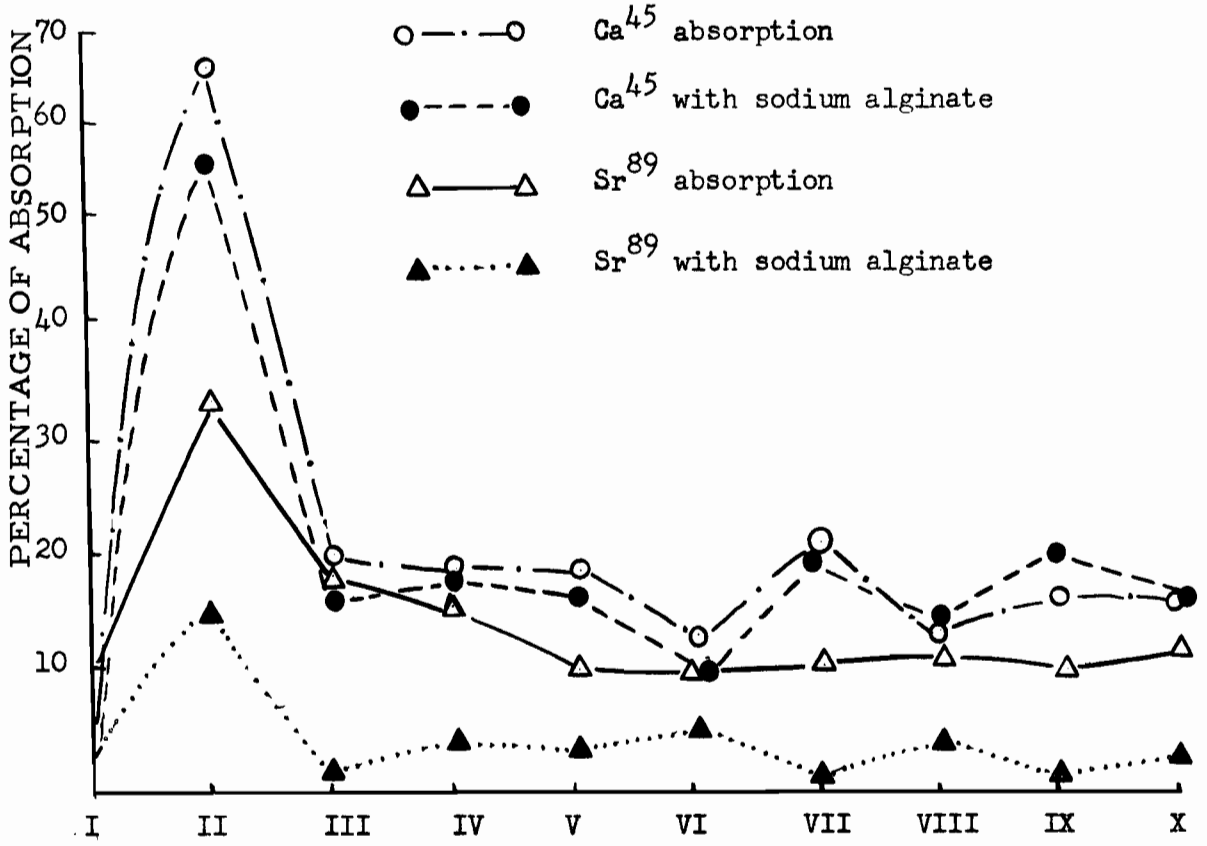


Fig. 23 Effect of sodium alginate on absorption of Sr^{89} and Ca^{45} from the ligated intestinal segments.

TABLE XXXI

INFLUENCE OF SODIUM ALGINATE ON Sr^{89} AND Ca^{45} LEVELS IN RAT'S BLOOD

(Test solutions were administered into the ligated intestinal segments)

Segment	2 μc of Sr^{89} with 0.45 μM of inactive $SrCl_2$ for 30 mins.		2 μc Ca^{45} with 0.55 μM of inactive $CaCl_2$ for 30 minutes	
	Control	with 1.5 μM of sodium alginate	Control	with 1.5 μM of sodium alginate
I	86 ± 32	30 ± 21	----------	----------
II	3133 ± 1328	1476 ± 409	2351 ± 436	2187 ± 770
III	1504 ± 781	255 ± 46	856 ± 226	651 ± 280
IV	548 ± 195	293 ± 79	612 ± 156	624 ± 200
V	497 ± 116	265 ± 71	553 ± 195	504 ± 139
VI	578 ± 159	232 ± 74	492 ± 119	431 ± 114
VII	862 ± 594	246 ± 58	465 ± 131	541 ± 141
VIII	624 ± 250	214 ± 44	534 ± 185	551 ± 163
IX	748 ± 258	240 ± 81	578 ± 163	556 ± 139
X	805 ± 375	278 - 108	551 ± 146	495 ± 165

Each figure represents the mean counts per minute and standard deviation for studies in twelve rats.

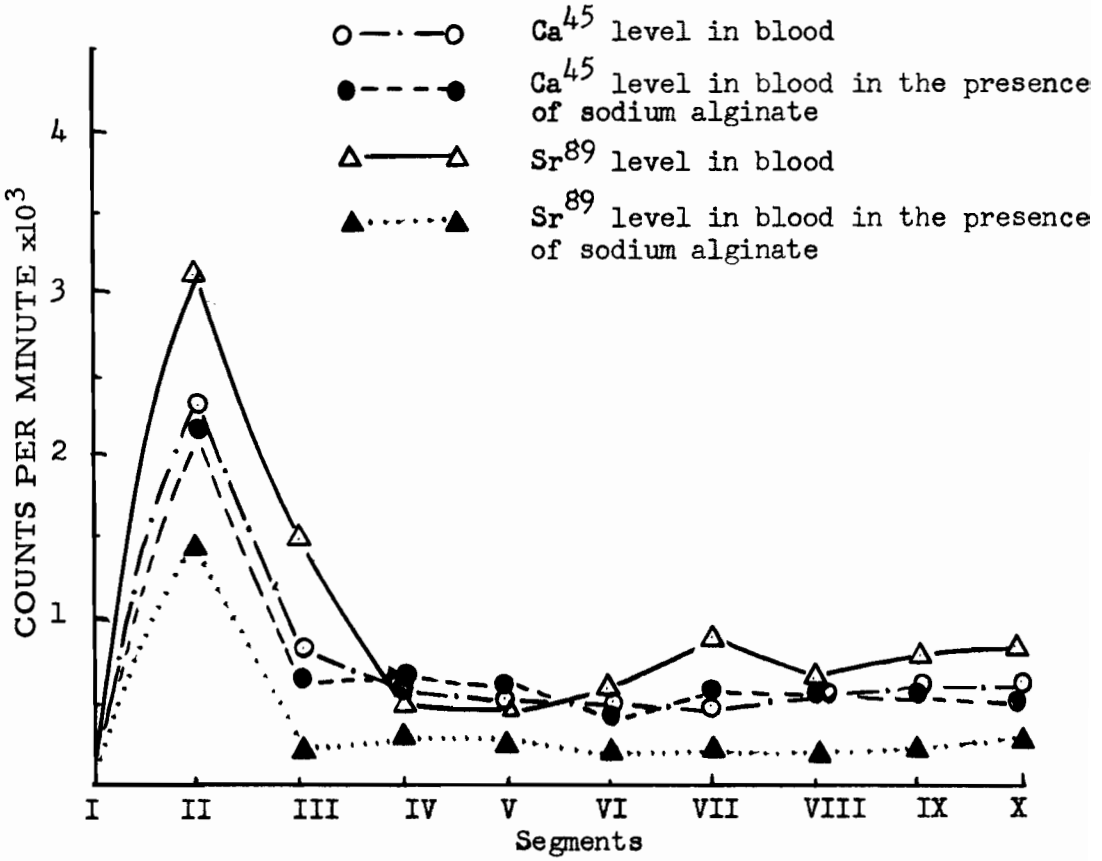


Fig. 24 Effect of sodium alginate on blood level of Sr^{89} and Ca^{45} following injection into the ligated intestinal segments.

TABLE XXXII

INFLUENCE OF SODIUM ALGINATE ON Sr^{89} AND Ca^{45} UPTAKE BY RATS FEMUR

(Test solutions were administered into the ligated intestinal segments)

Segment	2 μc Sr^{89} with 0.45 μM of inactive $SrCl_2$ for 30 mins.		2 μc Ca^{45} with 0.55 μM of inactive $CaCl_2$ for 30 minutes	
	Control	with 1.5 μM of sodium alginate	Control	with 1.5 μM of sodium alginate
I	164 ± 81	86 ± 38	-------	---------
II	4180 ± 1865	2095 ± 570	2333 ± 1123	1714 ± 980
III	2625 ± 1034	593 ± 189	1185 ± 285	1090 ± 214
IV	692 ± 186	403 ± 120	1228 ± 190	1090 ± 290
V	888 ± 529	379 ± 117	814 ± 214	771 ± 142
VI	826 ± 324	421 ± 124	833 ± 176	661 ± 119
VII	1353 ± 473	510 ± 164	723 ± 180	780 ± 242
VIII	859 ± 289	452 ± 156	652 ± 133	766 ± 180
IX	1457 ± 506	449 ± 79	685 ± 138	771 ± 157
X	1326 ± 551	460 ± 130	714 ± 119	804 ± 228

Each figure represents the mean counts per minute and standard deviation in twelve rats.

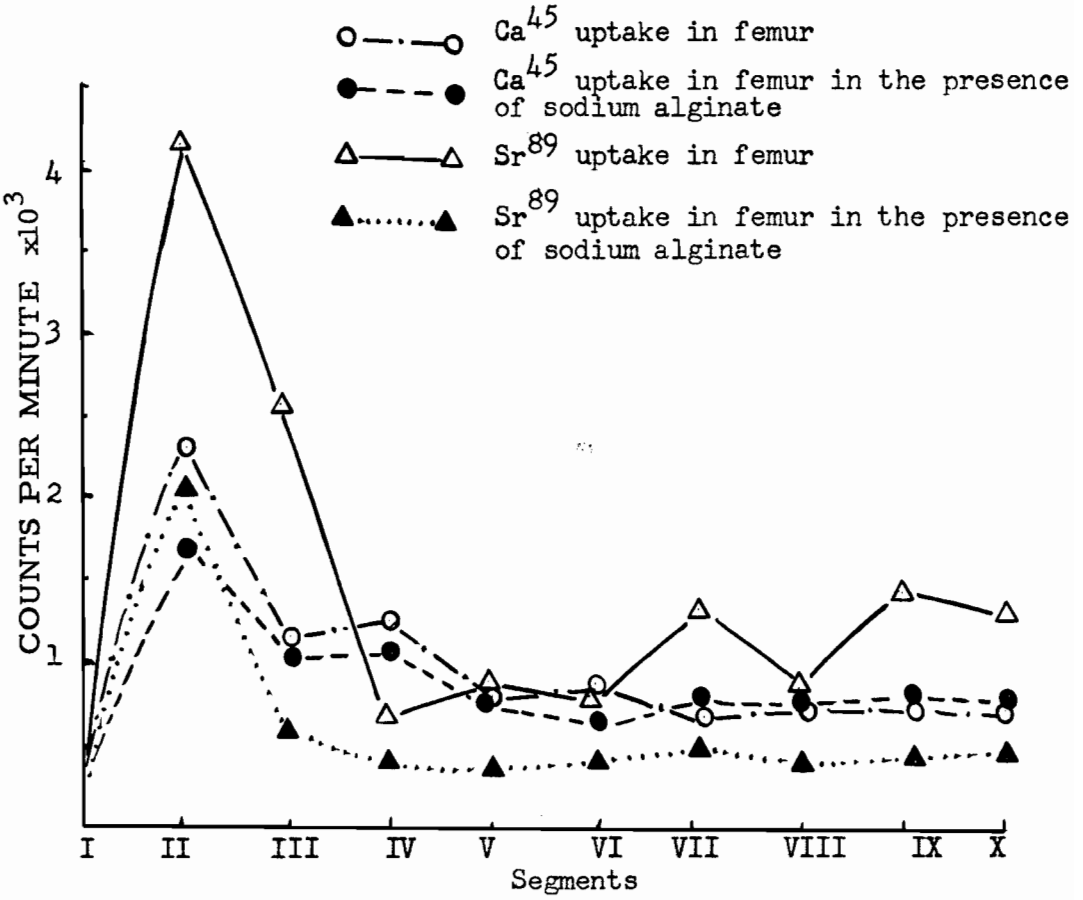


Fig. 25 Effect of sodium alginate on bone uptake of Sr^{89} and Ca^{45} following injection into the ligated intestinal segments.

it has been assumed that 2 carboxyl groups are required to combine with the alkaline earth metal. Absorption of Sr^{89} from the duodenal segment was reduced by approximately fifty percent when injected simultaneously with alginate in the molar proportion 1:10. On decreasing the molar ratio to 1:80, percentage absorption decreases proportionately with the logarithm of dose of alginate in mgs. (Figures 26 and 27, Tables XXXIII, XXXIV, and XXXV). Blood levels and bone uptake, expressed as counts per minute (CPM x 10^3) reflect this rate of absorption, showing a linear relationship with the logarithm of the dose.

Calcium on the other hand, showed no appreciable change in percentage of absorption until the proportion of Ca^{++} to alginate was to 1:40. Blood levels of Ca^{45} also remained unchanged from normal values, until the dose of alginate was increased to this figure. Bone uptake of calcium however showed a slow but definite decline at all dosages of alginate administered, indicating that some binding of Ca^{++} in the intestine must have taken place, even at the lower rates of dosage.

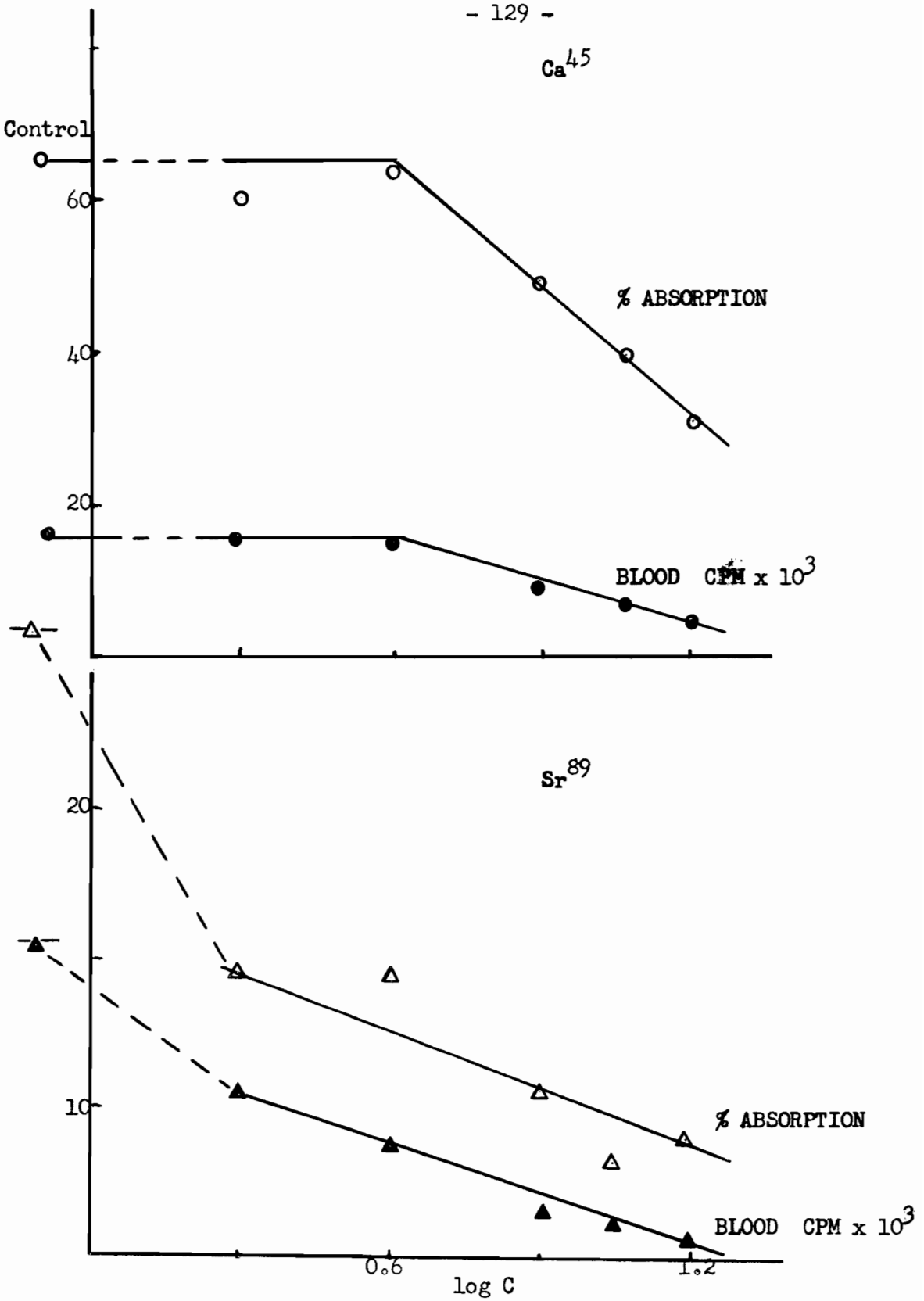


Fig. 26 Effect of increasing concentrations of sodium alginate on Sr^{89} and Ca^{45} absorption and blood levels, after administration into ligated duodenal segment.

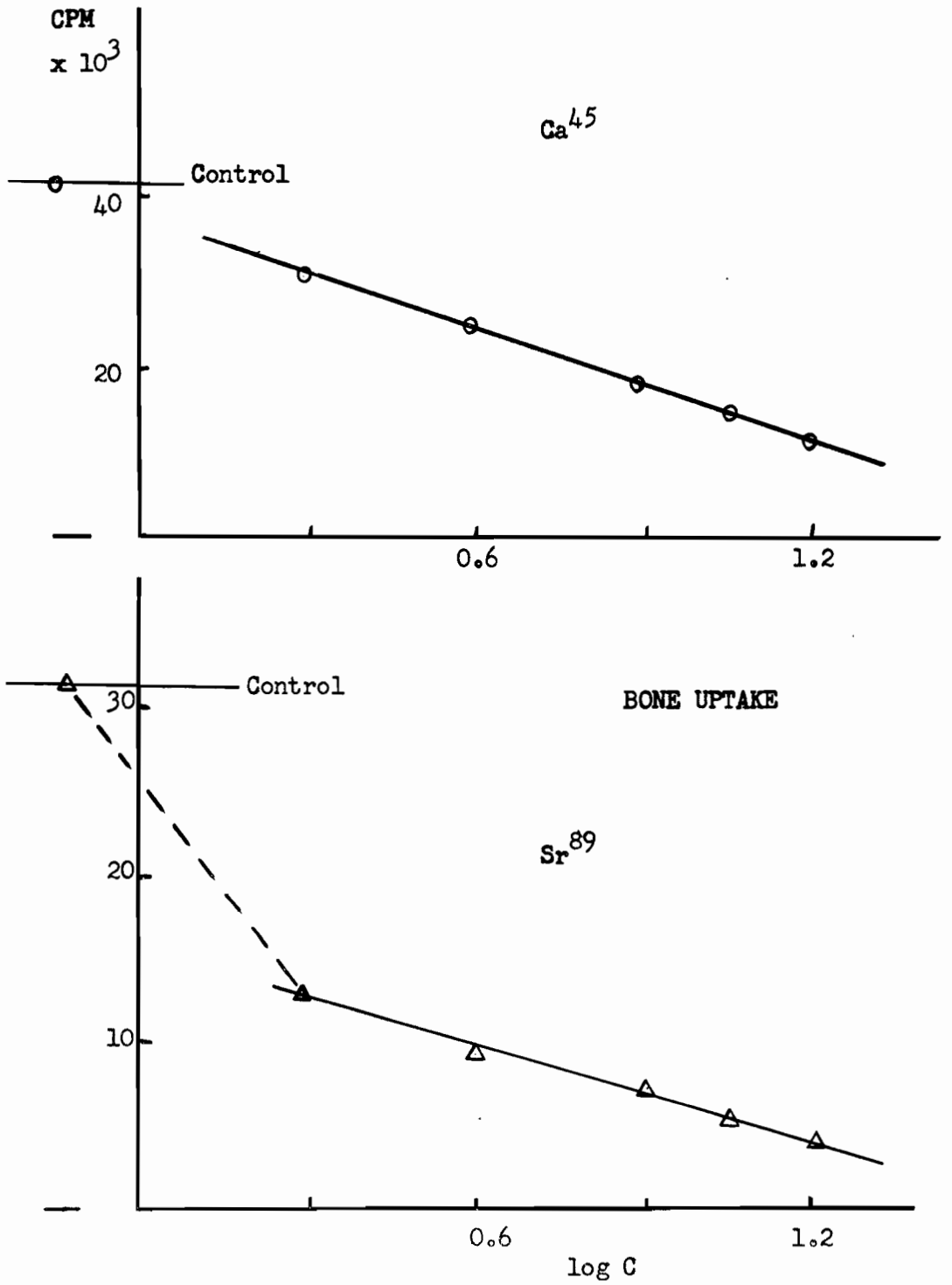


Fig. 27 Effect of increasing concentrations of sodium alginate on Sr^{89} and Ca^{45} bone uptake after injection into ligated duodenal segment.

T A B L E XXXIII

EFFECT OF INCREASING CONCENTRATIONS OF SODIUM ALGINATE ON Sr^{89} AND Ca^{45} ABSORPTION THIRTY MINUTES AFTER INJECTION INTO THE LIGATED DUODENAL SEGMENT

	STRONTIUM^{89}		CALCIUM^{45}	
	5 µc Sr^{89} 0.45 µM inactive $SrCl_2$	Expt. % Control	5 µc Ca^{45} 0.55 µM inactive $CaCl_2$	Expt. % Control
Control	41.9% (±11.5)		65.4% (±6.1)	
2 mg alginate/ml	18.7% (± 6.6)	44.6	60.5% (±6.3)	92.5
4 mg alginate/ml	17.8% (± 5.4)	42.4	64.8% (±8.4)	99.0
8 mg alginate/ml	10.8% (± 4.3)	25.7	49.6% (±9.1)	75.8
12 mg alginate/ml	6.1% (± 2.6)	14.5	40.1% (±8.0)	61.3
16 mg alginate/ml	8.0% (± 4.0)	19.0	31.5% (±10.4)	48.1

Each figure represents mean percentage of absorption and standard deviation for studies in ten rats.

TABLE XXXIV

EFFECT OF INCREASING CONCENTRATIONS OF SODIUM ALGINATE ON Sr^{89} AND Ca^{45} LEVELS IN BLOOD 30 MINUTES AFTER INJECTION INTO THE LIGATED DUODENAL SEGMENT

	STRONTIUM^{89}		CALCIUM^{45}	
	5 μc Sr^{89} + 0.45 μM inactive $SrCl_2$	Expt. % Control	5 μc Ca^{45} + 0.55 μM inactive $CaCl_2$	Expt. % Control
Control	20,926 (±3676)		16,215 (±1438)	
2 mg alginate/ml	11,720 (±2535)	56.0	16,087 (±1968)	99.2
4 mg alginate/ml	6,906 (±1888)	33.0	15,075 (±2764)	92.9
8 mg alginate/ml	3,047 (± 514)	14.5	9,387 (±1172)	57.9
12 mg alginate/ml	2,931 (± 832)	14.0	7,764 (±1787)	47.8
16 mg alginate/ml	1,570 (± 292)	7.5	4,891 (± 677)	30.1

Each figure represents mean counts per minute in one ml. of blood and standard deviation for studies in ten rats.

TABLE XXXV

EFFECT OF INCREASING CONCENTRATIONS OF SODIUM ALGINATE ON Sr^{89} AND Ca^{45} UPTAKE IN BONE 30 MINUTES AFTER INJECTION INTO THE LIGATED DUODENAL SEGMENT

	STRONTIUM^{89}		CALCIUM^{45}		O.R.
	5μc Sr^{89} + 0.45μM inactive $SrCl_2$	Expt. % Control	5μc Ca^{45} + 0.55μM inactive $CaCl_2$	Expt. % Control	
Control	31,023(±10,300)		42,750(±9536)		0.72
2 mg alginate/ml	12,401(±3587)	39.9	30,483(±5883)	71.3	0.40
4 mg alginate/ml	9,111(±2736)	29.3	24,966(±7682)	58.4	0.36
8 mg alginate/ml	7,089(±928)	22.8	17,808(±4485)	41.6	0.39
12 mg alginate/ml	5,298(±2033)	17.0	13,733(±1861)	32.1	0.38
16 mg alginate/ml	3,672(±1472)	11.8	10,461(±1782)	24.4	0.35

Each figure represents the mean counts per minute in one femur and standard deviation for studies in ten rats.

DISCUSSION

In recent years the fear of increased dietary contamination with radioactive Strontium^{90} has stimulated considerable effort towards finding a safe and effective means of decreasing the body burden of radioactivity. It is known that although the physiological processes by which dietary calcium and strontium are accumulated in the body show some notable similarities, there are also some important differences. The possibility of reducing the gastrointestinal absorption, and consequently the body burden of radioactive strontium by binding in the intestine with a non-absorbable, naturally occurring macro - molecular substance, has been explored in this study. Sodium alginate in particular, administered by any of the methods described, effectively reduces the intestinal absorption of radioactive strontium; its effect has been compared concurrently with other naturally occurring and synthetic polymers.

The addition of 20 mgs. (100:1 micromole) sodium alginate to a single dose of radiostrontium given simultaneously by orogastric tube reduced Sr^{89} uptake in bone by 77% as measured at the end of 72 hours. On the other hand, calcium uptake was reduced by less than 45% of the control. The amount of sodium alginate required for effective suppression of Sr^{89} after a single dose to a fasting animal is relatively low. A dose of sodium alginate given thirty minutes before or after the ingestion of radiostrontium was also effective, although

reduction in bone uptake was not quite so large as with simultaneous ingestion. This finding would be of great importance in the first aid management of accidental ingestion of radiostrontium by people employed in an atomic energy plant. In order to obtain the maximum effect with sodium alginate, it would be necessary to treat such cases in the shortest possible time.

Bone uptake of Sr^{89} was also reduced by all other macromolecular reagents tested except carrageenin; the polyuronic acids and sulphonic acid resins being the most effective (Fig. 22). The efficiency of the ion exchange resins in preventing absorption of Sr^{89} confirms the results of Michon and Guilloux (87) using synthetic resins. Polygalacturonic acid, although effectively suppressing Sr^{89} uptake, was relatively less efficient than sodium alginate (Tables XIX and XXIII).

MacDonald et al (86), in a search for agents to inhibit strontium absorption concluded that sodium alginate, among other materials, actually slightly increased the skeletal accumulation of ingested strontium. In their experiments bone uptake of non-radioactive strontium ion was measured by mass spectrometry; in order to detect measurable differences, large doses of strontium chloride were administered. Their dosage of sodium alginate was inadequate, with a molar ratio Sr/alginate of approximately 200:60 (cf 1:10 and 1:80 used in the experiments described in this thesis). These workers found that the following materials in order of decreasing efficiency, decreased Sr^{++} content in the femur: magnesium sulfate, sodium sulfate, the

ammonium salt of amido-polyphosphate, two carboxylic type cation exchange resins, a colloidal phosphorylated glucoside, calcium phytate, pectin, bran cereal, castor oil and a hydrophilic laxative derived from plantago. Many of these materials are laxatives and for obvious reasons cannot be given to human subjects for prolonged periods of time.

Sodium alginate appears to bind Sr^{89} to the same extent in all parts of the small intestine: absorption from each of a series of ligated segments, 5 cms. long, measured from the duodenum to the ileum, was equally inhibited by the binding agent.

The "Observed Ratios" in the experiments described in this chapter were calculated from measurements in a separate series of animals under identical conditions. By simultaneous dosing with sodium alginate, the "O.R." was reduced to less than one half control values, 24 hours after orogastric administration of Sr^{89} and Ca^{45} (Table XIX). A similar reduction in the "O.R." was noticed with polygalacturonic acid, and the two synthetic ion exchange resins, though the actual percentage uptake of both isotopes varied greatly (Tables XXI, XXVII, and XXIX). Thus although sodium alginate reduced Sr^{89} uptake to 25% of control (24 hours), Carboresin reduced it only to 41%, yet the "O.R." in either case was reduced to 0.25 and 0.26 from the control value of 0.53. This demonstrates that factors that inhibit calcium absorption from the gastrointestinal tract are considerably more effective in reducing strontium absorption: this is the converse of the phenomenon observed using "Trisma" buffer, as

described in the previous chapter; in that case the agent promoted strontium absorption to a greater extent than calcium.

Strontium^{89} absorption from ligated duodenal segments decreased proportionately when the concentration of sodium alginate was increased. In the proportion of 1 equivalent of Sr^{++} to 5 equivalents of carboxyl groups (alginate) absorption was sharply decreased by 55%. By a step-wise increment of alginate into the lumen of the duodenum, absorption was further decreased (Tables XXXIII, XXXIV, and XXXV). This decrease showed a linear relationship with the logarithm of the dose (expressed as mg/ml) of sodium alginate (Figures 26 and 27). At the maximum practicable dose, Sr^{++} was introduced with 40 equivalents of alginate, with a resulting inhibition of 81%. Percentage absorption and blood levels of Ca^{45} were not appreciably affected by sodium alginate until more than 20 equivalents of alginate (carboxyl) for each equivalent of calcium were present. Bone uptake of Ca^{45} however, showed a much slower but steadier decline with increasing dosage of alginate. At 40 microequivalents of alginate Ca^{45} absorption was reduced by 52%.

CHAPTER VII

INHIBITION OF STRONTIUM ABSORPTION: PRACTICAL APPLICATION OF THE INHIBITORY AGENTS

Introduction.

Effect of continuous administration of sodium alginate supplied in the drinking water on Sr^{89} and Ca^{45} uptake.

Effect of continuous administration of sodium alginate with foods on absorption of Sr^{89} and Ca^{45}. Alginate supplied in dry form.

Aqueous solutions of alginate mixed with laboratory chow.

Effect of feeding increasing concentrations of sodium alginate on uptake of Sr^{89} and Ca^{45}.

Dose response of sodium alginate to increasing dose of Sr^{89}.

Effect of continuous administration of polygalacturonic acid on Sr^{89} and Ca^{45} uptake in bone.

Metabolic effects of continuous administration of alginate in diet.

Discussion.

CHAPTER VII

INHIBITION OF STRONTIUM ABSORPTION: PRACTICAL APPLICATION OF THE INHIBITORY AGENTS

In the preceding chapter the effects of sodium alginate and polygalacturonic acid on Sr^{89} absorption from the gastrointestinal tract were described. These effects were demonstrated in a strictly limited experimental environment. That is, by injection into ligated intestinal segments and by intubation. It was apparent that these polyuronic acid polymers would have to be tested for competence in preventing radiostrontium absorption from contaminated food and drinking water over prolonged periods of time. The form of administration and also the optimum dosage levels relative to the radiostrontium content of the food materials capable of reducing the radiostrontium uptake effectively, must be known before the findings can be applied to human subjects.

The use of sodium alginate as an inhibitor of radiostrontium absorption has been studied in some detail. The study of polygalacturonic (or pectic) acid has been more limited because it is considerably more expensive, and because the compound is not commercially available in sufficiently large quantities. Furthermore, the preliminary trials with pectic acid did not seem as promising as those with sodium alginate.

Sodium alginate is obtained commercially as a fine powder which was fed, mixed with dry chow in the earlier feeding experiments. This treatment resulted in severe constipation and the rats lost weight at the higher levels of intake. When the alginate was dissolved in the minimum water to form a stiff paste or gel and then mixed with the chow, the rats ate with relish and there were no further digestive troubles. Sodium alginate can also be dissolved in the drinking water; the rats will imbibe a fairly viscous syrup without difficulty.

Experiments were carried out to demonstrate the effect of varying the concentration of sodium alginate in the diet on the inhibition of absorption of a constant intake of radiostrontium. The experiments were extended for a prolonged period until uptake of Sr^{89} had reached a plateau, and the percentage inhibition to be expected on a chronic basis could be calculated.

The dose rate of Sr^{89} was also varied and from widely different levels the inhibiting activity of sodium alginate at three different concentrations in the diet was assessed.

In the preliminary trials discussed in the previous chapter, the inhibition of Ca^{45} was also observed; at low levels of sodium alginate, the effect was found to be negligible, but at higher dosages, inhibition increased to as much as 52% of control values. These facts suggested that on a long term basis, some evidence of calcium deficiency might become apparent. In many of the chronic feeding experiments described in this chapter a parallel series with Ca^{45} instead of Sr^{89} was done.

There have been no reports in the literature concerning the effect on a mammalian metabolism of relatively large doses of sodium alginate over a prolonged period of time. For this reason the weight gains and general condition of rats on a high sodium alginate diet have been observed and recorded. A preliminary check on all bones and organs has been made both macroscopically and microscopically on rats maintained for several months.

Effect of continuous administration of sodium alginate supplied in the drinking water on Sr^{89} and Ca^{45} uptake

Rats were fed on standard Purina chow ad libitum but their drinking water was replaced by a solution containing 0.7 gms. sodium alginate and 1.0 μc Sr^{89} per 100 ml. The rats consumed approximately 20 ml. of drinking water per day. The average daily intake of Sr^{89} therefore was 0.2 μc with 140 mg. sodium alginate. Groups of six rats drinking pure water containing 0.01 μc Sr^{89} per ml. were taken as controls. Six animals were sacrificed at 5 days interval for 55 days and one femur was taken from each under identical conditions.

The results are given in Table XXXVI and Figure 28. Sodium alginate given at the rate of 0.14 gms. per day had an appreciable effect (75% controls) on reducing Sr^{89} uptake after 5 days, and this effect continued during the period over which studies were done.

The uptake of Ca^{45} in the presence of such small quantities of alginate almost coincides with the controls. No interference with calcium

TABLE XXXVI

EFFECT OF CONTINUOUS ADMINISTRATION OF SODIUM ALGINATE IN DRINKING WATER ON Sr^{89} AND Ca^{45} UPTAKE

The drinking water contained 0.01 μc Sr^{89} or Ca^{45} with 7 mgs sodium alginate per ml. 0.2 μc per day.

Number of days	STRONTIUM^{89}			CALCIUM^{45}			OBSERVED RATIO	
	Control	Experiment	$\frac{\text{Expt.}}{\text{Control}}$ %	Control	Experiment	$\frac{\text{Expt.}}{\text{Control}}$ %	Control	Experiment
1	785 (±184)	750 (±212)	95	2541 (±824)	2216 (±172)	87	0.30	0.33
5	2971 (±546)	2191 (±366)	74	7950 (±1812)	8010 (±2903)	100	0.37	0.27
10	5270 (±1073)	4031 (±651)	76	14850 (±2850)	13433 (±1888)	90	0.35	0.30
15	6194 (±3017)	4586 (±1622)	74	26108 (±1644)	27230 (±4800)	104	0.23	0.16
20	8421 (±1567)	4307 (±1293)	51	26660(±4617)	25980 (±6654)	97	0.31	0.16
25	8128 (±1478)	5161 (±1476)	63	30583 (±4759)	32366 (±8180)	104	0.26	0.15
30	8583 (±1366)	7571 (±1493)	88	50516 (±5532)	42150 (±8148)	83	0.16	0.17
35	10002(±2643)	7287 (±1761)	72	46858(±10209)	33016 (±9370)	70	0.21	0.22
40	9909 (±1702)	7155 (±988)	71	46982 (±2699)	46191 (±4712)	98	0.21	0.15
45	8091 (±1815)	4660 (±2318)	57	28950 (±15110)	35133 (±21533)	121	0.27	0.13
50	7133 (±735)	5624 (±931)	78	39416 (±6438)	45150 (±9588)	114	0.18	0.12
55	11424 (±2015)	6885 (±1368)	60	53450(±8726)	43566(±9196)	81	0.21	0.13

Each figure represents mean counts per minute (femur) and standard deviation for studies in six animals.

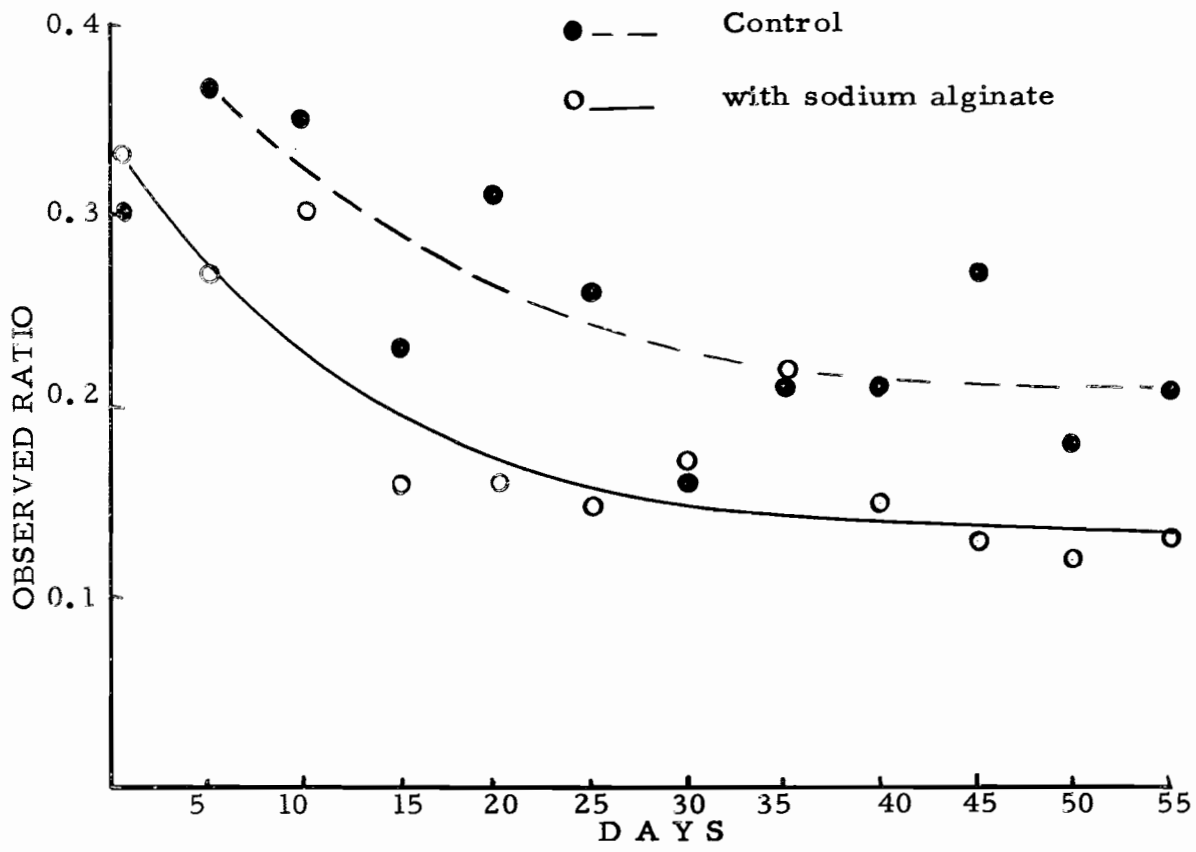


Fig. 28 Effect of continuous administration of sodium alginate on Sr^{89} and Ca^{45} uptake in bone. 0.2 μc per day.

intake is therefore to be expected.

Effects of continuous administration of sodium alginate with foods on absorption of Sr^{89} and Ca^{45}. Alginate supplied in dry form.

Sodium alginate is tasteless and odourless; rats can be induced to eat it when mixed with laboratory chow in proportions as high as 30% of the total diet. The effect of a continuous diet of sodium alginate on the absorption of Sr^{89} and Ca^{45} was investigated.

Rats in groups of six were fed with standard laboratory chow mixed with powdered dry sodium alginate in the proportion 10%, 20%, 30% of food, for five days. On the fifth day Sr^{89} (1 µc/ml) or Ca^{45}, (1 µc/ml) was added to the drinking water of rats. The rats averaged an intake of 20 - 25 ml. drinking water per day and therefore, imbibed 20 - 25 µc radioisotope. At the end of 24 hours the animals were sacrificed and blood and bone samples assayed as before.

Bone uptake of Sr^{89} by the control rats (Figure 29, Table XXXVII), receiving a total of 25 µc over 24 hours, was of the same order as that obtained 24 hours after a single dose of 10 µc administered by intubation (Figure 1, Tables I and II). This is probably due to the fact that absorption, accumulation in bony tissue and elimination from the body of low levels of Sr^{89} proceeds simultaneously and continuously, whereas after a large single dose much higher blood levels are obtained at the peak of the absorptive phase, resulting in a greater transfer of blood Sr^{89} to the bone. The relatively much higher CPM obtained in blood level

TABLE XXXVII

EFFECT OF FEEDING SODIUM ALGINATE MIXED WITH LAB. CHOW ON Sr^{89} AND Ca^{45} LEVELS IN BLOOD AND BONE UPTAKE

Rats were fed with sodium alginate mixed with laboratory chow in different proportions for five days. Twenty four hours prior to sacrifice Sr^{89} and Ca^{45} was given in drinking water (1 μc/ml).

BLOOD

	STRONTIUM^{89}			CALCIUM^{45}		
	Control	20% alginate	30% alginate	Control	20% alginate	30% alginate
*	1114 (±242)	442 (±125)	469 (±191)	4032 (±560)	5197 (±1896)	3093 (±1130)
**		39.6%	42.1%		128.7%	76.7%
	BONE					
*	68130 (±6622)	24485 (±12760)	19390 (±8299)	263429 (±95622)	263296 (±142600)	208122 (±101210)
**		35.9%	28.4%		99.9%	79.0%

* Mean counts per minute and standard deviation for six animals.

** $\frac{\text{Experiment}}{\text{Control}}$ in percentage.

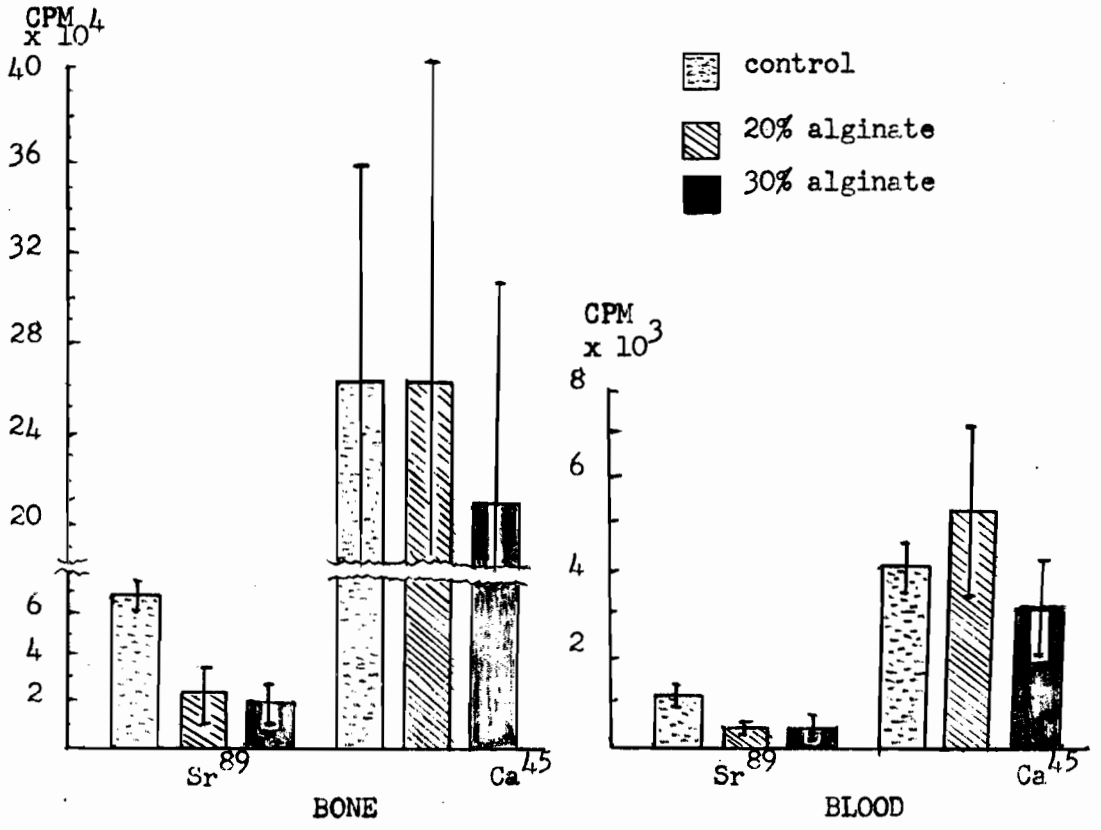


Fig. 29 Distribution of Sr^{89} and Ca^{45} following the administration of isotopes with drinking water. Sodium alginate in dry form was added to the diet in the proportion indicated. C.P.M. - counts per minute.

and bone uptake of Ca^{45} reflect the physiological discrimination against strontium.

The addition of sodium alginate to the food at the 10% level resulted in a decrease in bone uptake of Sr^{89} to 58% control value. Increasing the concentration of binding agent to 20% and 30% resulted in a further decrease in bone uptake to 36% and 28% respectively. Twenty per cent sodium alginate in the diet had no effect on the absorption of Ca^{45}. With 30% alginate in the diet, blood level and bone uptake of Ca^{45} was reduced by 20%.

Total intake of alginate per rat per diem was calculated to be 2.0 gms. at 20%, and 3 gms. at the 30% level. Rats fed continuously for 5 days on sodium alginate at the 10% level remained in good condition and gained in weight. Animals fed with 20% and 30% diet however failed to gain weight and became constipated. At higher concentrations of alginate, the daily consumption of food decreased and the rats showed some loss of weight. Constipation was caused by the dry alginate which swells to a hard mass when mixed with the gastric juices in the stomach.

Aqueous solutions of alginate mixed with laboratory chow.

To overcome the difficulties of constipation noted in the preceding experiment and in order to administer higher concentrations of alginate, powdered sodium alginate was mixed with a small amount of water. The alginate swells considerably on wetting, and at a concentration of 5 gms.

TABLE XXXVIII

EFFECT OF CONTINUOUS FEEDING WITH SODIUM ALGINATE ON Sr^{89} AND Ca^{45} UPTAKE

Rats were fed a diet containing 0.5 μc Sr^{89} or Ca^{45} plus 0.24 grams sodium alginate as a jelly per gram chow. Five microcuries per day.

No. of days	STRONTIUM^{89}			CALCIUM^{45}			OBSERVED RATIO	
	Control	with 24% alginate	Expt. % Control	Control	with 24% alginate	Expt. % Control	Control	with 24% alginate
2 days	21185 (±4250)	6660 (±1505)	31%	65454 (±23396)	42770 (±8946)	65%	0.32	0.15
5 days	44605 (±11621)	10297 (±2730)	23%	145350 (±27238)	84950 (±35587)	58%	0.30	0.12
10 days	72219 (±14166)	15073 (±2013)	21%	308227 (±52389)	131714 (±24905)	43%	0.23	0.11
15 days	102129 (±29492)	20804 (±3880)	20%	462504 (±63043)	226659 (±35690)	49%	0.22	0.09
20 days	117776 (±24735)	20078 (±3081)	17%	451325 (±62568)	239045 (±64683)	53%	0.26	0.08

Each figure represents mean counts per minute (femur) and standard deviation of studies in twelve rats.

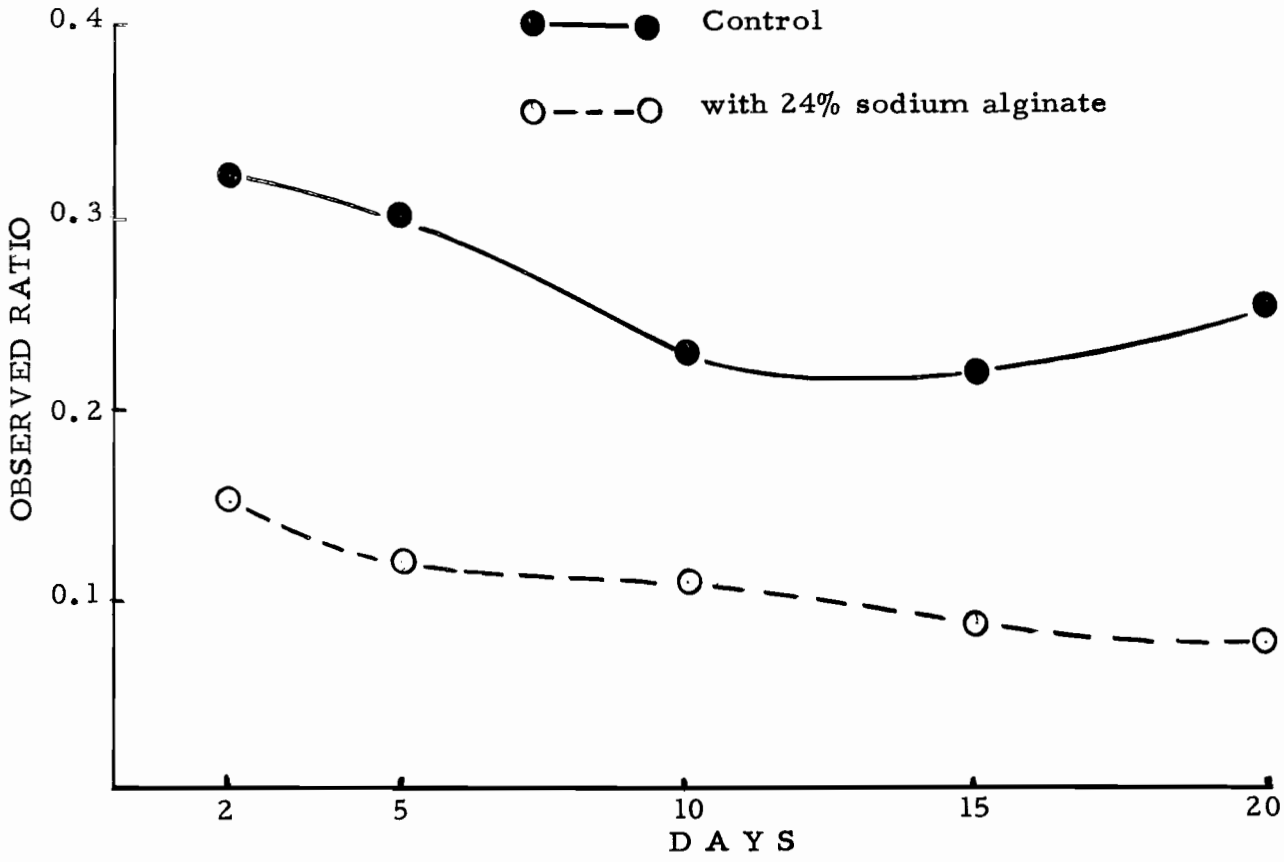


Fig. 30 Effect of continuous feeding of 24% sodium alginate on Sr^{89} and Ca^{45} uptake in bone. Five microcuries per day.

per cent forms a stiff, pasty gel which can be mixed with the laboratory chow. Even at high concentrations the rats eat this mixture with great relish. A total of 200 rats kept continuously on such a diet for several months developed no evidence of constipation. Animals fed on the chow alginate diet ingested enough water in their food to satisfy their thirst and no drinking water was required. In order to ensure that control rats consumed a similar quantity of water, boiled corn starch paste of a similar concentration was mixed with the chow. This procedure also facilitated the incorporation of Sr^{89} into the food.

Rats were fed on a diet containing 0.5 μc Sr^{89} or 0.5 μc Ca^{45} and 0.24 gms. sodium alginate per gram chow, ad libitum for twenty days. Groups of twelve rats were sacrificed at 2, 5, 10, 15, and 20 days. An equal number of rats in the control series fed on starch plus 0.5 μc Sr^{89}/gm. chow diet were treated in the same way.

The results are given in Table XXXVIII. Sr^{89} uptake in the femur increases steadily in the controls, whereas the rate of uptake with alginate is much lower (Figure 30), reaching a steady state plateau after 15 days. As would be expected, the uptake of Ca^{45} in the bone is much more rapid than Sr^{89}. A plateau or equilibrium state is reached after 15 days. Ca^{45} absorption is reduced by this high level of alginate but to a much lower degree than with Sr^{89}.

TABLE XXXIX

EFFECT OF INCREASING CONCENTRATIONS OF SODIUM ALGINATE ON Ca^{45} AND Sr^{89} UPTAKE

Diet contained 0.5 μc Sr^{89} or Ca^{45} per gram standard laboratory chow with varying amounts of alginate in jelly form, for two days.

	Strontium^{89}	Expt. % Control	Calcium^{45}	Expt. % Control	Observed Ratio
Control	21,185 (±4,250)		65,454 (±22,394)		0.32
3% Alginate	13,781 (±2,905)	65%	66,054 (±10,116)	101%	0.22
6% Alginate	13,088 (±1,986)	62%	58,129 (±11,293)	89%	0.22
12% Alginate	9,609 (±1,805)	45%	53,637 (±16,026)	82%	0.17
18% Alginate	8,054 (±1,449)	38%	42,791 (±11,366)	65%	0.18
24% Alginate	6,660 (±1,505)	31%	42,770 (±8,946)	65%	0.15

Each figure represents mean counts per minute (femur) and standard deviation of studies in twelve animals.

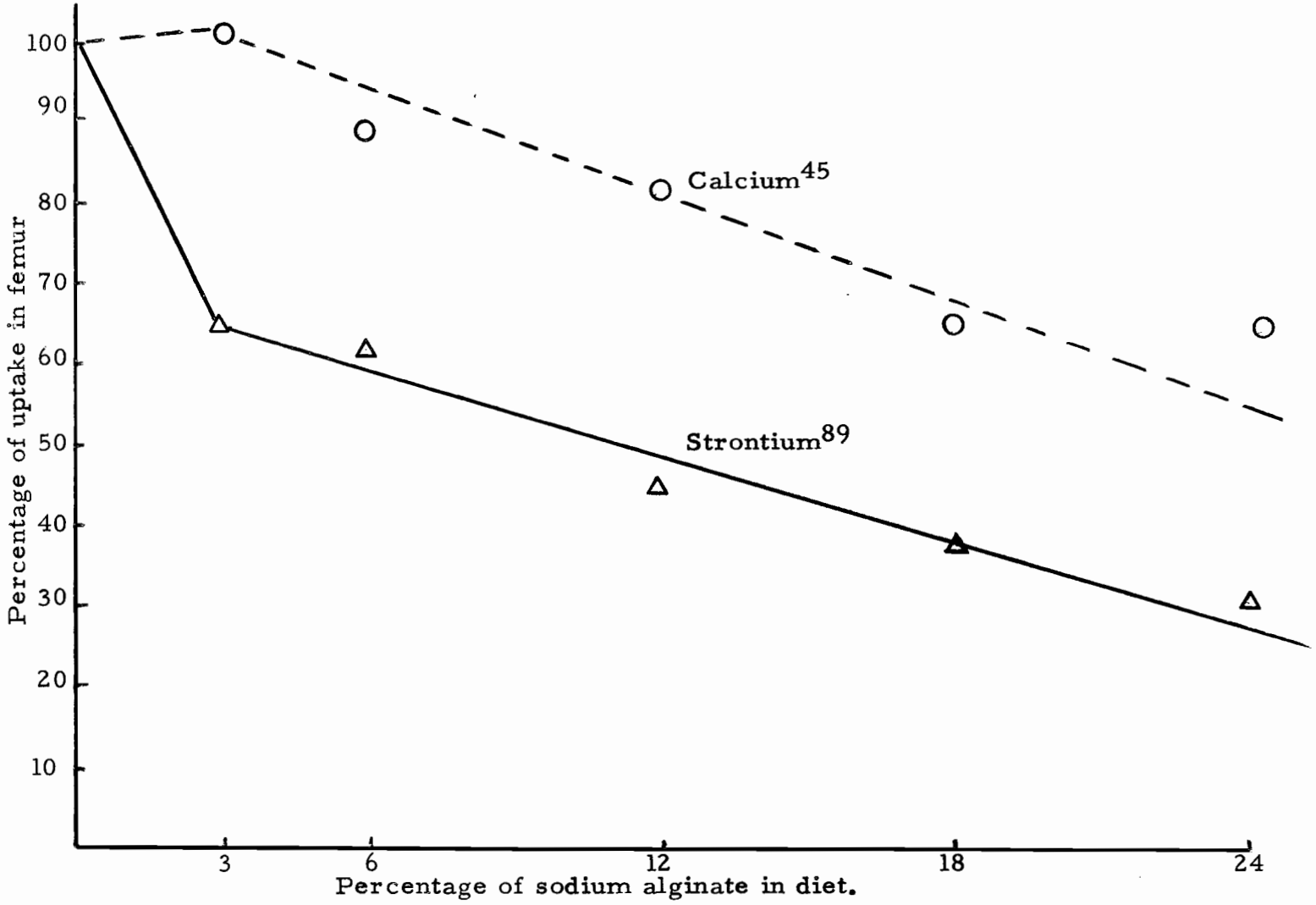


Fig. 31 Effect of adding increasing amounts of sodium alginate on Sr^{89} and Ca^{45}. The alginate was mixed as a jelly with standard laboratory chow. and given for two days.

Effect of feeding increasing concentrations of sodium alginate on uptake of Sr^{89} and Ca^{45}

Sodium alginate dissolved in a little water, in the proportions 3, 6, 12, 18, and 24% was mixed with laboratory chow as described previously and fed to groups of 12 rats in each case. 0.5 μc Sr^{89} or 0.5 μc Ca^{45} per gram laboratory chow was included at the same time. At the end of 24 hours the food mixture had been completely consumed. It was calculated that each rat had ingested, on average, 10 grams of chow, 5 μc Sr^{89} and 0.3 to 2.4 grams alginate respectively. An equal number of rats were fed with a starch plus chow plus Sr^{89} or Ca^{45} mixture as control. These diets were continued for two days by which time they had completely consumed the prescribed dose. Animals were sacrificed and one femur assayed for radioactivity.

By increasing the amount of alginate in the diet, progressively less Sr^{89} was taken up in the femur (Table XXXIX). The mean radioactive count after ingesting 0.3 gms. sodium alginate per day, was two-thirds that in the controls. By increasing the intake to 2.4 gms. per day, uptake of Sr^{89} in the femur was reduced to one-third (31%) on the controls (Figure 31). Larger amounts of alginate were also given, in an attempt to reduce Sr^{89} absorption even further. The volume of chow mixture became so large however, that the rats did not consume their prescribed dose of alginate plus Sr^{89} in the two day period.

In the parallel experiments with 0.5 μc Ca^{45} per gram chow, sodium

TABLE XXXX

EFFECT OF ADMINISTRATION OF DIFFERENT DOSES OF SODIUM ALGINATE ON Sr^{89} UPTAKE BY BONE. 0.2 μc Sr^{89} PER DAY.

Rats weighing 100 - 120 gms. were given 0.01 μc Sr^{89} + 7 mgs. sodium alginate per 1 ml. of drinking water of 0.02 μc Sr^{89} per gram chow with different proportions of alginate in food. At five days interval, 12 rats were sacrificed and one femur assayed for radioactivity.

	IN DRINKING WATER			IN FOOD				
Days	Control	1.4% alginate	$\frac{\text{Expt.}}{\text{Control}}$ %	Control	12% alginate	$\frac{\text{Expt.}}{\text{Control}}$ %	24% alginate	$\frac{\text{Expt.}}{\text{Control}}$ %
5	2,971 (±546)	2,191 (±366)	74	2,270 (±611)	824 (±193)	36	467 (±126)	20
10	5,270 (±1,073)	4,031 (±651)	76	4,374 (±863)	1,885 (±732)	43	901 (±139)	20
15	6,194 (±3,017)	4,586 (±1,622)	74	4,955 (±1,561)	2,320 (±461)	46	1205 (±188)	24
20	8,421 (±1,567)	4,307) (±1,293)	51	4,656 (±1,236)	2,108 (±482)	45	1133 (±231)	24

Each figure represents mean counts per minute and standard deviation for studies in twelve rats.

TABLE XXXXI

EFFECT OF ADMINISTRATION OF DIFFERENT DOSES OF SODIUM ALGINATE ON Sr^{89} UPTAKE. FIVE MICROCURIES Sr^{89} PER DAY.

Rats weighing 100 - 120 grams were given 0.25 microcuries Sr^{89} plus 7 mg alginate per ml. or 0.5 μc Sr^{89} with different percentage of alginate per gm chow. At five day intervals 12 animals were sacrificed and one femur assayed.

	In drinking water			Mixed with Food				
No. of days	Control	1.4% Alginate	Expt./Control %	Control	12% Alginate	Expt./Control %	24% alginate	Expt./Control %
5 days	48548 (±12534)	27598 (±5814)	57%	44605 (±11621)	14544 (±3921)	33%	10297 (±2730)	23%
10 days	88410 (±33050)	54180 (±16966)	61%	72219 (±14166)	20583 (±5192)	28%	15073 (±2013)	21%
15 days	114791 (±41921)	69090 (±19436)	60%	102129 (±29492)	28938 (±6442)	28%	20804 (±3880)	20%
20 days	151930 (±48223)	90007 (±19706)	59%	117776 (±24735)	32977 (±11074)	28%	20078 (±3081)	17%

Each figure represents mean counts per minute and standard deviation for studies in twelve animals.

TABLE XXXXII

EFFECT OF ADMINISTRATION OF DIFFERENT DOSES OF SODIUM ALGINATE ON Sr^{89} UPTAKE. Twenty microcuries of Sr^{89} per day.

Rats weighing 100 - 120 grams were given 1 μc Sr^{89} plus 7 mg sodium alginate per ml, in drinking water or 2 μc Sr^{89}/gm chow with different proportion of sodium alginate for 5 days. Animals were then sacrificed and one femur assayed for radioactivity.

In Drinking Water		Mixed with Food		
Control	1.4% Alginate	Control	12% Alginate	24% Alginate
* 265,410 (±61,102)	128,365 (±40,606)	215,439 (±45,066)	79,604 (±11,625)	35,227 (±8,301)
**	48.3%		36.9%	16.3%

* Mean counts per minute and standard deviation for studies in twelve rats.

** $\frac{\text{Experiment}}{\text{Control}}$ in percentage

TABLE XXXXIII

EFFECT OF ADMINISTRATION OF DIFFERENT DOSES OF SODIUM ALGINATE ON Sr^{89} UPTAKE BY BONE. FIFTY MICROCURIES OF Sr^{89} PER DAY.

Rats weighing 100 - 120 grams were given 2.5 µc Sr^{89} plus 7 mg alginate per ml. of drinking water or 5 µc Sr^{89} per gram chow with different proportions of alginate. After 5 days, animals were sacrificed and one femur assayed for radioactivity.

In Drinking Water		In Food		
Control	Experiment	Control	with 12% alginate	with 24% alginate
* 584,401 (±163,100)	338,908 (±91,631)	370,470 (±103,700)	136,797 (±27,311)	104,411 (±20,797)
**	57.9%		36.9%	28%

* Each figure represents mean counts per minute and standard deviation for studies in twelve rats.

** $\frac{\text{Experiment}}{\text{Control}}$ in percentage.

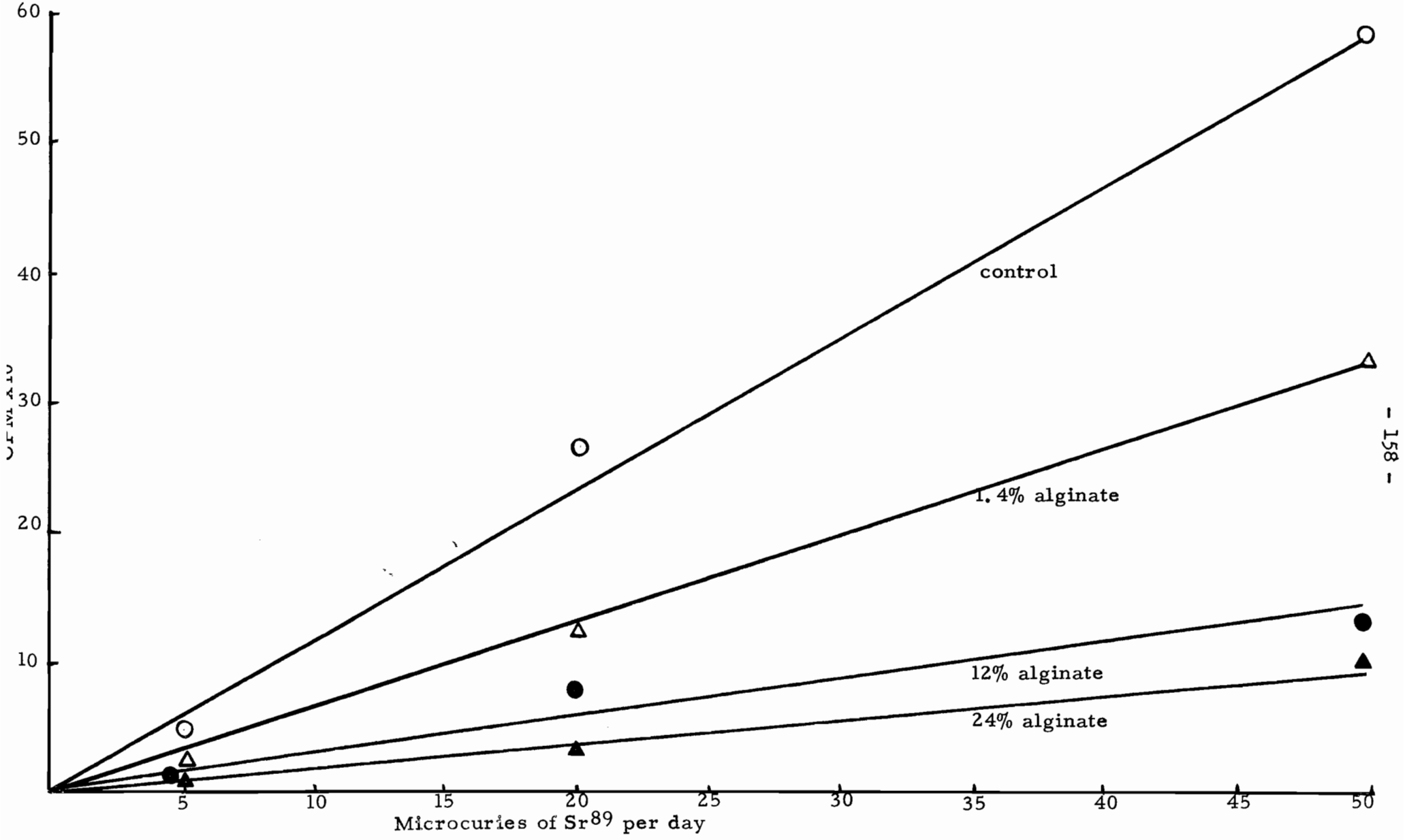


Fig. 32 Sr^{89} and alginate administered in drinking water and food for five day.

alginate at the rate of 0.3 gms. per day had no effect on bone uptake (Table XXXIX and Figure 31). By increasing the dose of alginate Ca^{45} uptake was reduced; at a dosage of 2.4 gms per day, uptake was 65% that in the control.

Dose response of sodium alginate to increasing doses of Sr^{89}.

In the preceding two experiments, by increasing the concentration of sodium alginate with a constant amount of strontium a progressive reduction in the amount of Sr^{89} taken up by bone was demonstrated. In this study doses of radiostrontium were varied at different levels of alginate consumption. Sr^{89} was given at the rate of 0.2 μc, 5 μc, 20 μc and 50 μc per day, while three different concentrations of sodium alginate (1.4% , 12%, and 24% of the diet) were administered. The experiments were continued for 20 days at the lower dose levels of Sr^{89}, ie. 0.2 μc and 5 μc per day; while at the higher levels, it was necessary to restrict that to 5 days. In one experiment sodium alginate was administered in the drinking water at the rate of 1.4% per diem together with Sr^{89}. At higher concentrations, both alginate and radiostrontium were given in the food.

The results are given in Tables XXXX, XXXXI, XXXXII and XXXXIII. The radioactivity of Sr^{89} (CPM x 10^4) in the femur at the end of 5 days was recorded in the Figure 32. At given concentration of alginate intake, a definite percentage reduction of radiostrontium in the bone can be obtained irrespective of the amount of Sr^{89} in the diet. On the

TABLE XXXXIV

EFFECT OF CONTINUOUS ADMINISTRATION OF POLYGALACTURONIC ACID ON Sr^{89} AND Ca^{45} UPTAKE IN BONE

Rats weighing 100 - 120 grams were given 0.25 μc Sr^{89} or Ca^{45} with 25 mg polygalacturonic acid per ml. in drinking water. At five day intervals 12 animals were sacrificed and one femur assayed for radioactivity.

No. of days	STRONTIUM^{89}			CALCIUM ^{45}			OBSERVED RATIO	
	Control	Experiment	$\frac{\text{Expt.}}{\text{Control}}$ %	Control	Experiment	$\frac{\text{Expt.}}{\text{Control}}$ %	Control	Experiment
5	48,548 (-12534)	23,085 (-8836)	47	205,358 (-74,626)	150,528 (-42,140)	73	0.23	0.15
10	88,410 (-33050)	38,091 (-19,062)	43	515,555 (-104,200)	347,259 (-64,296)	67	0.17	0.10
15	114,791 (41,921)	95,698 (-30,112)	83	686,680 (-124,700)	447,845 (-99,840)	65	0.16	0.21
20	151,930 (-48,223)	94,658 (-23,598)	62	664,565 (-208,700)	441,591 (-145,400)	66	0.22	0.21
25	201,791 (-31,668)	98,831 (-29,085)	49	856,080 (-192,500)	622,800 (-119,900)	73	0.23	0.15
30	205,916 (-63,064)	95,321 (-35,292)	46	904,390 (-99,813)	644,485 (-108,700)	71	0.22	0.14

Each figure represents mean counts per minute and standard deviation for studies in 12 animals.

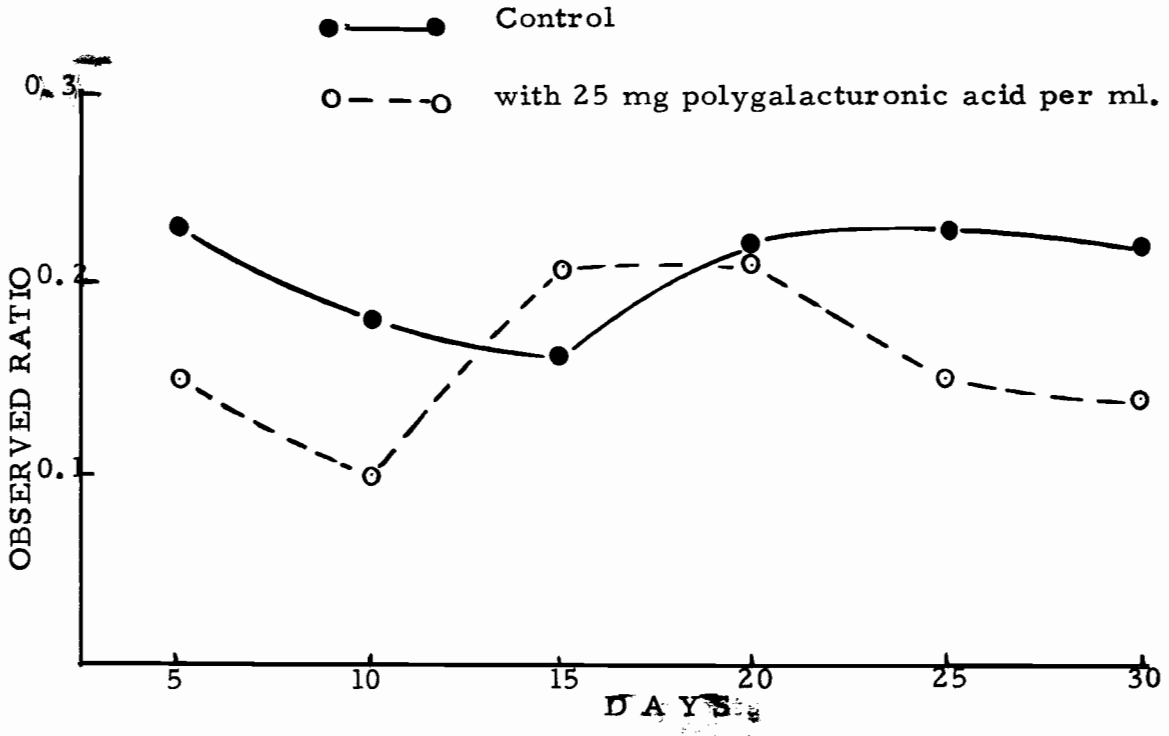


Fig. 33 Effect of continuous administration of polygalacturonic acid in drinking water on Sr^{89} and Ca^{45} uptake in bone. Five microcuries per day.

other hand irrespective of the amount of alginate used a small percentage of radiostrontium appears to be free to be absorbed and thence taken up by the bone.

Effect of continuous administration of polygalacturonic acid on Sr^{89} and Ca^{45} uptake in bone

Polygalacturonic acid was dissolved in sodium bicarbonate solution. 0.25 μc Sr^{89} or 0.25 μc Ca^{45} with 25 mg polygalacturonic acid per ml. in drinking water were administered to rats weighing 100 to 120 grams. At 5 days interval 12 animals were sacrificed and one femur assayed for radioactivity (Tables XXXXIV and Figure 33).

At the dose given, polygalacturonic acid reduced Sr^{89} uptake by more than 50% of the control value. The effect on Ca^{45} absorption was more pronounced than that with sodium alginate. Polygalacturonic acid at the rate of 4% in the diet causes a 30% reduction on bone uptake of Ca^{45} (Table XXXXIV), whereas sodium alginate at the rate of 6% in the diet produced only 10% reduction in Ca^{45} uptake (Table XXXXIX).

Metabolic effects of continuous administration of alginate in diet

Over 500 rats have been used to evaluate the various effects of long term administration of sodium alginate in as high a dose as 24% of diet. Rats were introduced to the alginate diet at an average age of six weeks, that is, approximately three weeks after weaning. Some animals received sodium alginate in jelly form mixed with food in the

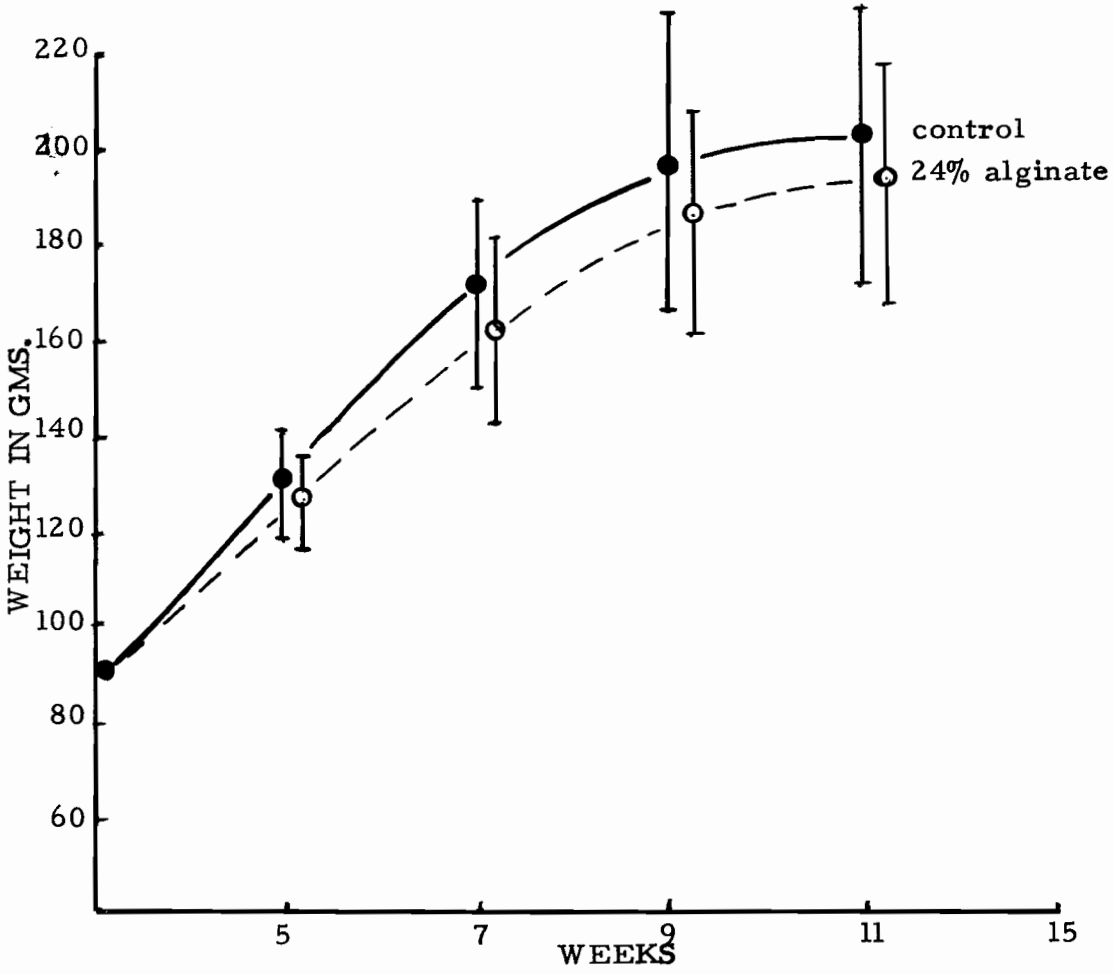


Fig. 34 Effect of continuous feeding of alginate on weight.

proportions 24:100 for over six months. This represents one-fourth life span of rat. So far no clinically detectable untoward symptoms have been noticed. The weight gain and growth was similar to control animals receiving normal lab-chow (Figure 34). A group of rats were sacrificed after three months of continuous feeding with 24% sodium alginate diet. All organs (brain, pituitary, thyroid, heart, lungs, adrenals, liver, spleen, ovaries, testis, bone, etc). were examined for any histological evidence of toxic effects. No abnormal histopathological pattern was detected. A preliminary analysis has detected significant differences in the electrolyte content of the faeces and urine; no firm conclusion however can be reached at this stage.

DISCUSSION

The results of the attempts to control Sr^{89} absorption by feeding sodium alginate in the normal diet were encouraging. The acute problem of constipation developed by mixing powdered alginate with the dry chow was obviated by first dissolving the powder in a minimum of water, forming a stiff jelly and mixing this thoroughly with the chow. Sodium alginate may also be added to drinking water; a heavy syrup is formed and the maximum daily intake by rats through this route is 140 mgs. Even at this low level there is an appreciable reduction in the bone uptake of Sr^{89}.

At low levels of chronic Sr^{89} dosage, uptake in the bone reaches a steady state between 15 and 21 days. Bone levels of Ca^{45} continue to increase for a more prolonged period. When sodium alginate is added to the drinking water or food, Sr^{89} uptake is immediately lowered. At the end of 55 days the cumulative effect of the inhibition of Sr^{89} absorption is reflected in the reduced bone levels. The inhibitory effect is considerably less with Ca^{45} so that the Observed Ratio is gradually reduced to the remarkably low level of 0.08 (control 0.26) after 20 days when rats are treated with 2$\frac{1}{4}$% alginate in their diet.

Sodium alginate given at the rate of 3 grams per 100 grams of chow reduced Sr^{89} uptake by one third. Increasing the dose of alginate to 2$\frac{1}{4}$% caused a further drop in bone uptake of Sr^{89} by approximately two thirds. Interference with Ca^{45} uptake on the other hand, was negligible with three per cent of alginate, but

became appreciable on increasing the percentage of binding agent; at 24%, Ca^{45} uptake is reduced by one third. The Observed Ratio diminishes with increasing dosage of sodium alginate.

A direct linear relationship exists between dose Sr^{89} (μc/day) and bone uptake of Sr^{89} (CPM x 10^4). This relationship holds good when Sr^{89} absorption is partially inhibited by sodium alginate. The percentage reduction of bone uptake for each level of sodium alginate remains constant at 40 for 1.4%, 62 for 12% and 80 for 24%. This remains true for all given dosage of Sr^{89}.

The capacity of sodium alginate to bind radiostrontium in preference to radiocalcium, in the presence of an excess of dietary calcium is remarkable. As in the experiments on the binding capacity of sodium alginate in intestinal loops, described in the previous chapter, calcium does not appear to be bound until a certain, relatively high level of alginate is attained, whereas strontium absorption is appreciably affected even at the lowest levels of alginate.

From a practical point of view therefore in problems concerning long-term exposure to low levels of radiostrontium, in food or milk, small doses of sodium alginate administered with all meals, either in drinking water or with meals may represent an advantage in the human subject. At higher levels of Sr^{90} contamination, it would be desirable to bind as much of the radioisotope as possible by taking the maximum amount of alginate.

Thompson (137) reported that by feeding a diet containing 2.0%

calcium (0.5% calcium providing the normal intake) there was an approximately two fold reduction in Sr^{90} deposition. Other workers have not claimed such an enhanced effect, and none has attained the efficiency of sodium alginate in reducing Sr^{89} deposition. Ion exchange resins were utilised with success by Michon and Guilloux (87). These workers reported results similar to those reported in the previous chapter, using orogastric intubation of suspensions of such resins. However, it is more difficult to persuade rats to consume large quantities of resin; human subjects have found them to be unpalatable when taking them for medication. Moreover, the speed of food transit through the digestive tract is markedly reduced and the efficiency of treatment decreased with time (87).

General effects of chronic ingestion of sodium alginate

Feeding young rats with sodium alginate continuously, at the maximum level, had very little effect on their growth rate and general clinical condition. Their coats and general appearance were excellent except that after two months the rats developed a slight pot belly. This condition was reversed within a few days of removing alginate from the diet. At lower levels of alginate in the diet, for example at 12%, no difference could be detected between treated rats and the controls.

As some interference with calcium metabolism was made apparent by the reduced uptake of Ca^{45} at high levels of alginate intake, symptoms of calcium deficiency were sought. Special attention was

paid to bone development. X-rays of skeleton and histological sections of bone showed no evidence of rickets.

Two factors may be responsible for the maintenance of adequate calcium nutrition in spite of the excess binding agent in the diet. These are firstly, the excess calcium present in the normal laboratory chow, and secondly, the homeostatic control of calcium metabolism.

An average days ration of chow for a rat contained about 150 mgs. of calcium. The apparent absorption of calcium by adult animals is 20% or less of the daily intake of this element (111). Therefore, even if 45% of the calcium ingested is sequestered by a high intake of sodium alginate, there is still sufficient calcium available for nutrition. In the studies with chronically ingested Ca^{45}, the radionuclide served only as a tracer, for the enormous amount of inactive calcium present in the diet. Therefore, even if an adequate amount of calcium is absorbed, the specific activity of Ca^{45} is diluted by the available calcium pool in the body and thus the bone uptake of Ca^{45} reflects only the specific activity of the deposited calcium in bone and not the total radiocalcium in the body. This fact is evident from the experiments with single dose of radioisotope. Tables XVIII and XIX demonstrate that while the Ca^{45} activity in one femur was reduced by 45% at the end of 24 hours, the activity in blood was almost the same as that of the control. Similar anomalies were observed when "Trisma" buffer was administered; Ca^{45} uptake in bones of young rats were equal to control, whereas the blood values were

very considerably higher (XIII). On the other hand, at all times blood levels of Sr^{89} in rats dosed with sodium alginate paralleled the bone uptake. The same is true when increased absorption of Sr^{89} takes place in the presence of "Trisma" buffer (Tables XIII and XIV).

These differences are due to the role of homeostatic in calcium metabolism, an ion which is physiologically essential. Absorption of substances of physiological importance like Ca^{++} takes place against a concentration gradient. To explain this phenomenon, a process of "active transport" or the concept of a "biological pump" must be postulated. Active transport of Ca^{++} across biological membranes is energy dependent and is closely associated with oxidative phosphorylation. Sodium alginate forms an insoluble precipitate with calcium and strontium in vitro. From this study, it can be postulated that the absorptive mechanism or mechanisms involved in the "active transport" of Ca^{++} across the intestinal mucosa successfully competes with alginate molecules for Ca^{++} but not for Sr^{++}. There are substances like Vitamin D, lysine and lactose which can enhance the calcium absorption. On the other hand, citric acid and Versene (140) can bind Ca^{++} and thus hinder the absorption of calcium. Magnesium and phytic acid also adversely affect the absorption of calcium (141). It is likely that depending upon the magnitude of demand made by bony tissue upon the plasma calcium pool, the intestine is capable of determining within wide limits the amounts of calcium entering the pool from the lumen

of the intestine. The capacity of the intestine for avid calcium absorption is well known when, for some reason, a serious threat is posed against calcium homeostasis. Nicolaysen (142) observed that rats which had been maintained on a Vitamin D deficient diet for eight months absorbed calcium with an apparent efficiency of as much as 78%. When the diet was supplemented with Vitamin D, the efficiency of calcium absorption dropped to 25%.

The effect of sodium alginate on other cations such as iron, magnesium, zinc, copper, manganese, etc. has not been investigated. However, from the clinical appearance of the animals fed with 2½% alginate in jelly form for more than six months no deficiency sundrome or imbalance in mineral metabolism has been noted. The preliminary results on studies on the possible toxic effects of long term administration of sodium alginate failed to reveal any lesions detectable by routine histology in the brain, endocrine glands, lungs, heart, liver, spleen, kidneys, etc. It is possible that more detailed histochemical differentiation may show some deviation from the normal, some such studies are planned.

Sodium polygalacturonate

Sodium polygalacturonate was given at the rate of 4% in the diet. This is the maximum which can be given in drinking water which will be consumed by the rats. The order of efficiency of pectate in inhibiting Sr^{89} absorption is in the same range as of sodium alginate. However, as was demonstrated (Table XXXXIV) Ca^{45} is also bound rather more firmly by polygalacturonate than by alginate and thus the "Observed Ratio" did not reach such a low level.

CHAPTER VIII

CONCLUSIONS

CHAPTER VIII

CONCLUSIONS

In the present study Calcium^{45} uptake was found to be considerably higher than that of Strontium^{89}, confirming the observations of a number investigators on the gastrointestinal process of discrimination against strontium and in favor of calcium. The "Observed Ratios" calculated from the experimental evidence described, were in agreement with those previously reported for the rat. The animals attained equilibrium in Sr^{89} uptake during the third week of administration of the isotope. With a constant amount of calcium in the diet, radiostrontium uptake in bone increases linearly with radiostrontium concentration in the food. Further investigations should be carried out to determine the effect of either low or high calcium diet.

Studies with ligated intestinal segments, in vivo, revealed that the duodenum has the greatest rate of absorption for both calcium and strontium in conformity with the earlier work of Wasserman (46) and Lengemann (116). In the intact animal the distal regions of the small intestine are likely to play a much more important role in the absorption of calcium and strontium.

Effect of pH on absorption

Absorption of strontium and calcium was not increased in the duodenal segment with either "Trisma" buffer pH 8.5, ionic strength 0.1, or phos - phate buffer pH 5.75, ionic strenght 0.2. Sr^{89} absorption from the distal

regions of small bowel was significantly increased when injected in the alkaline buffer (Trisma). Phosphate buffer on the other hand reduced the rate of absorption of Sr^{89}. Somewhat similar, but less pronounced effects were noted on Ca^{45}. Orogastric administration of Sr^{89} in "Trisma" buffer both in young and adult rats produced an enormous increase (over 250%) in the Sr^{89} blood level and uptake by bone. Phosphate buffer reduced appreciably bone uptake and blood levels of Sr^{89} on orogastric administration. Ca^{45} absorption was also affected by the buffer but to a lesser extent by the use of either buffering agents. Adult female rats which had had several litters and assumed to be in negative calcium balance had a higher uptake of Ca^{45} when given with "Trisma" buffer. Whether this mechanism of "Trisma" in reducing absorption of Sr^{89} and Ca^{45} is similar to those of lysine and lactose needs further study.

Inhibition of strontium absorption

Of the five macromolecular, non-absorbable materials tested, two naturally occurring polysaccharides, sodium alginate and polygalacturonic acid have shown considerable promise in reducing radiostrontium absorption. Sodium alginate decreased the bone uptake of Sr^{89} by 80% of the control value. Carrageenin was not effective in reducing Sr^{89} absorption, although it produced a slight but consistent enhancing effect on Ca^{45} uptake in bone. Two synthetic ion-exchange resins also decreased strontium absorption, Rexyn 101 (Na^{+}) a strong sulphonic acid resin was more effective than carboresin.

A single dose of sodium alginate, thirty minutes before or after Sr^{89} ingestion was also effective, but the optimal effect was noted in

simultaneous administration of the binding agent and the isotope.

Sodium alginate administered into ligated intestinal segments decreased Sr^{89} absorption from all regions of the small intestine.

Strontium^{89} uptake was decreased linearly with dose rate of sodium alginate. Somewhat similar but less marked effect was noted on Ca^{45} uptake. "Observed Ratio" was also reduced.

Sodium alginate is odourless, palatable and can be easily administered either in drinking water or mixed with food. However, the dry powder mixed with food tends to produce intractable con - stipation and at higher dose rate it is positively harmful. On the other hand, if sodium alginate is dissolved in small quantities of water into jelly like consistency it is harmless. Several hundred rats have been on sodium alginate (in jelly form) diet in as high a proportion as 24% for over six months and so far no evidence of con- stipation or other clinically detectable untoward symptoms have been noticed. They gained weight corresponding to those rats on normal chow. Preliminary histological studies of various organs removed from animals which had been on 24% alginate diet for three months did not reveal any detectable abnormality. Balanced metabolic studies are in progress and further studies are needed before a definite conclusion can be reached.

Finally, it is demonstrated from the experimental data given in this thesis that administration of sodium alginate mixed with food or drinking water is an effective and safe method of blocking gastro - intestinal absorption of radiostrontium and can be given for a prolonged period of time.

BIBLIOGRAPHY

1. Lindell, B.
Present Day Assessment of Radiation Hazard.
Phys. Med. Biol., 6: 173 - 198, 1961.

2. Martell, E.A.
Atmospheric Aspects of Strontium^{90} Fallout.
Science, 129: 1197 - 1206, 1959.

3. Ichikawa, R. et al
Sr^{90} and Cs^{131} absorbed in rice plants in Japan 1962.
Science, 135: 1072, 1962.

4. Olson Jr., T.A.
Sr^{90} in 1959 U.S.A. Wheat Crops.
Science, 135: 1064, 1962.

5. Vase, P.B. and Koontz, H.V.
Uptake of Strontium by Pasture plants and its Possible Significance in Relation to the Fallout of Strontium^{90}.
Nature, 183: 1447, 1959.

6. Comar, C.L.
The Transfer of Calcium and Strontium Across Biological Membranes.
Editor R.H. Wasserman
Academic Press, New York, 405, 1963.

7. Kulp, J.L.
Strontium^{90} in Man.
Gersback & John Verlag, Munchen, 1961.

8. Bair, W.J. and Smith, G.D.
Absorption of $Sr^{90}SO_4$ from the Lung.
Proc. Soc. Expt. Biol. Med., 113: 16 - 17, 1963.

9. Giese, W. and Comar, C.L.
Existence of Non-exchangeable Calcium Compartments in Plasma.
Nature, 202: 31 - 33, 1964.

10. Balabuha, V.C. and Razumovsky, N.O.
Prespective in Excretion of Radioactive Strontium from Organism.
Meditsinskaia Radiologica, 8: 71 - 76, 1963.

11. Irons, L.I. and Perkins, D.J.
Studies of Interaction of Mg: Ca: Sr: with Native and Chemically Modified Human Serum Albumin.
Biochem. J., 84: 152-156, 1962.

12. Bacon, J.A., Patrick, H. and Hansard, S.L.
Some Effects of Parathyroid Extract and Cortisone on Metabolism of Strontium and Calcium.
Proc. Soc. Expt. Biol. Med., 93: 349 - 351, 1956.

13. MacDonald, N.S., Hutchinson, D.L. and Hepler, M.
The Bidirectional Transport of Radiostrontium Across the Primate Placenta.
Radiation Research, 5: 752 - 766, 1962.

14. Kulikova, V.G.
Passage of Strontium, Caesium, Rutheium and Iron Through Placental and Mammary Barriers.
Med. Radiol., 4: 23 - 27, 1959.

15. Pecher, C.
Biological Investigations with Radioactive Calcium and Strontium.
Proc. Soc. Expt. Biol. Med., 46: 86 - 91, 1941.

16. Nilsson, A. and Ullberg, S.
Uptake and Retention of Sr^{90} in Mouse Tissue Studied by Whole Body Autoradiography and Impulse Counting.
Acta Radiologica, 58: 81, 1962.

17. McClellan, R.O.
Calcium - Strontium Discrimination Miniature Pigs as Related to Age.
Nature, 202: 104 - 106, 1964.

18. Kostial, K.,Grunden, N. and Harrison, G.E.
Turnover of Radioactive Strontium in New-born Rats.
Nature, 201: 1240, 1964.

19. Kulp, J.L., Schulert, A.R. and Hodges, E.J.
$Strontium^{90}$ in man IV.
Science 132: 448 - 454, 1960.

20. Schulert, A.R., Hodges, E.J., Lenhoff, E.S. and Kulp, J.L.
$Strontium^{90}$ Distribution in the Human Skeleton.
Health Physics, 2: 62 - 68, 1959.

21. Kahn, D.S., Makhani, J.S. and Skoryna, S.C.
Studies of the Late Effects of Internal Irradiation by Radioactive Strontium in the Rat.
Laval Medical, 34: 169 - 183, 1963.

22. Appelgren, LE., Nilsson, A. and Ullberg, S.
Autoradiographic Localisation of Sr^{89} in Osteosarcomas.
Acta Radiologica, 1: 459 - 464, 1963.

23. Menzel, J., Posner, A.S., Schraer, H., Pakis, G., and Likins, R.R.
Comparative Fixation of Sr^{89} and Ca^{45} by Calcified Tissues as Related to Fluoride Induced Changes in Crystallinity.
Proc. Soc. Expt. Biol. Med., 110: 609 - 613, 1962.

24. Gynning, I. et al
Localisation with Sr^{85} of Spinal Metastases in Mammary Cancer and Changes in Uptake After Hormone and Roentgen Therapy.
Acta Radiologica, 55: 119 - 128, 1961.

25. Owen, M., Sissons, H.A. and Vaughan, J.
The Effect of Single Injection of Sr^{90} in Rabbits.
Brit. J. Cancer, 11: 229, 1957.

26. Glas, J.E. and Lagergren, C.
Biophysical Studies on Strontium Uptake in Osseous Tissue.
Acta Path. Microbiologica Scand. Supp., 144: 51 - 52, 1961.

27. Cohn, S.H., Spencer, H., Samachson, J., and Robertson, J.S.
The Turnover of $Strontium^{85}$ in Man as Determined by Whole Body Counting.
Radiation Res., 17: 173 - 185, 1962.

28. Dubin, P., and Jones, H.D.
Estimation of Turnover Equation of Sr^{90} for Human Bones.
Proc. 2nd Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 23: 140 - 141, 1958.

29. McCance, R.A. and Widdowson, E.M.
The Fate of Strontium After Intravenous Administration to Normal Persons.
Biochem. J., 33: 1822 - 1825, 1939.

30. Laszlo, D. and Spencer, H.
Radiostrontium Metabolism and Decontamination in Man; Chelation in Biology and Medicine.
Proc. 2nd Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 23: 425 - 433, 1958.

31. Spencer - Laszlo, H, Samachson, J., Hardy Jr., E.P. and Rivera, J.
Strontium^{90} Metabolism during Low Strontium^{90} Intake in Man.
Radiation Res., 22: 668 - 676, 1964.

32. Spencer - Laszlo, J, Samachson, J., Hardy Jr., E.P. and Rivera, J.
Strontium^{90} Balances during Starvation in Man.
Radiation Res., 22: 677 - 681, 1964.

33. Miyao, N.
Studies on Strontium Metabolism following Strontium - Calcium Discrimination in Domestic Animals:
II. Retention and Excretion of Strontium in Goat and Effect of Stable Strontium on Sr^{90} Excretion.
Japanese J. Vet. Sci., 22: 341 - 352, 1960.

34. Charles, M.L., Spencer, H., Goldman, A., Korman, S. and Laszlo, D.
Metabolism and Enhancement of Radiostrontium Excretion in Man.
Federation Proc., 15: 407, 1956.

35. Singer, L., Maqsood, M., Medlen, A.B., and Comar, C.L.
Endogenous and Biliary Excretion of Ca^{45} and Sr^{89}.
Arch. Biochem. & Biophys., 66: 404 - 410, 1957.

36. Greenberg, D.M. and Troescher, F.M.
Study with Radioactive Isotopes of Excretion of Calcium and Strontium by Way of Bile.
Proc. Soc. Expt. Biol. Med., 49: 488 - 491, 1942.

37. Tweedy, W.B.
The Effect of Parathyroid Extract upon the Distribution, Retention and Excretion of Labelled Strontium.
J. Biol. Chem., 161: 105 - 113, 1945.

38. Goel, D.O., Skoryna, S.C., Yafee, L. and Webster, D.R.
Factors Affecting the Distribution and Retention of Radioactive Strontium.
Surgical Forum VIII: 129 - 133, 1958.

39. Comar, C.L., Wasserman, R.H. and Nold, M.M.
Strontium - Calcium Discrimination Factors in the Rats.
Proc. Soc. Exp. Biol. Med., 92: 859 - 863, 1956.

40. Wasserman, R.H., Lengeman, F.W. and Comar, C.L.
J. Dairy Sci., 41: 812, 1958.

41. Jarvis, A.A., Brown, J.R. and Tiefenbach, B.
Strontium^{89} and Strontium^{90} Levels in Breast Milk and Mineral - Supplement Preparations.
Canad. Med. Assoc. J., 88: 136 - 139, 1963.

42. Stevens, L.G.
Injurious effect on the skin.
Brit. Med. J., 1: 998, 1896.

43. Walsh
Deep Tissue Traumatism from Roentgen Ray Exposure.
Brit. Med. J., 2: 272 - 273, 1897.

44. Maximum Permissible Amounts of Radioisotopes in the Human Body and Maximum Permissible Concentration in Air and Water.
Natl. Bur. Standards (U.S.) Handbook 61, 1955.

45. Jammet, H. and Vacca, G.
Various Possible Expressions of the Toxicity of Radioactive Substances as Defined by the Practical Problem it Raises.
Proc. 2nd Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 23: 346 - 353, 1958.

46. Björnerstedt, R. and Engström, E.
Maxmimu Permissible Body Burden of Strontium^{90}.
Science 129: 327 - 328, 1959.

47. Healy, J.W.
The Calculation of Maximum Permissible Concentration of Long-Lived Radioisotopes.
Radiation Res., 4: 367 - 371, 1956.

48. Bugher, J.C. and Mead, P.A.
Frequency of Bone Sarcoma in the United States in Relation to Low-Level Radiation Exposion.
Proc. 2nd Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 23: 165 - 170, 1958.

49. Kahn, D.S., Webster, D.R. and Skoryna, S.C.
Effects of Long Term Oestrogen Therapy on Bone Tumours Produced by Radioactive Strontium.
Proc. Canad. Fed. Biol. Sci., 21: 33 - 34, 1959.

50. Kahn, D.S. and Skoryna, S.C.
Effects of Long Term Cortisone Administration on the Skeletal Tissue of the Rat and on Bone Tumour Produced by Radioactive Sr.
Lab. Invest., 8: 763 - 776, 1959.

51. Skoryna, S.C., Goel, D.P., Szekeres, E.A., Webster, D.R. and Kahn, D.S.
Incidence and Distribution of Bone Tumours Produced by Radio - active Strontium in Rats.
Rev. Canad. Biol., 16: 517, 1957.

52. Skoryna, S.C. and Kahn, D.S.
The Late Effects of Radioactive Strontium on Bone: histogenesis of Bone Tumours Produced by High Strontium^{89} Dosage.
Cancer, 12: 306 - 322, 1959.

53. Finkel, M.P. and Scribner, G.M.
Toxicity of Strontium^{90} and of Calcium^{45} in Mice.
Argonne Natl. Lab. Report. A.NL. 5456, 36 - 37, 1955.

54. Lisco, H., Finkel, M.P., and Brues, A.
Carcinogenic Properties of Radioactive Fission Products.
Radiology 49: 361 - 362, 1942.

55. Nilsson, A. and Ullberg, S.
Uptake and Retention of Sr^{90} on Sr^{90} induced Osteosarcomas.
Acta Radiologica 58: 168, 1962.

56. Poluboiarinova, Z.I., and Strel'tsova, U.N.
Appearance of Multiple Neoplasm in Dogs under the Influence of Sr^{90}.
Vop. Onkol., 8: 16-20, 1962.

57. Kowalewski, K.
Strontium^{89} Induced Bone Tumours in the Rat.
Canad. J. Surg., 7: 204 - 215, 1964.

58. Henricson, B., Knudsen, O. and Nilsson, A.
The Effect of Radiostrontium in Mouse Testis.
Acta Radiologica, 58: 52 - 64, 1962.

59. Budko, L.N. and Strel'tsova, V.N.
Effect on the Rat Body of a Single and Chronic Intake of Radioactive Strontium Through the Gastrointestinal Tract.
Med. Radiol., 4: 20 - 29, 1959.

60. Finkel, M.P., Bergstrand, P.J.,and Biskis, B.O.
The Consequence of the Continuous Ingestion of Sr^{90} by Mice.
Radiology, 74: 458 - 467, 1960.

61. Spencer, H., Kabakow, B., Laszlo, D., and Samachson, J.
Factors Modifying Radiostrontium Excretion in Man.
Clin. Sci., 17: 291, 1958.

62. Carlquist, B., and Nelson, A.
Attempts to Influence the Elimination of Radiostrontium.
Acta Radiologica, 54: 305, 1960.

63. Clark, I. and Smith, M.R.
Effect of Magnesium Ions on Removal of Radiostrontium from Rats.
Proc. Soc. Expt. Biol. Med., 109: 135 - 139, 1962.

64. Talmage, R.V. and Elliott, J.R.
Proc. Soc. Expt. Biol. Med., 99: 306, 1958.

65. Zander - Principati, G.E., and Kuzma, J.F.
Reduction of Sr^{90} Bone Cancer by Zirconium Citrate.
Radiation Res., 16: 583, 1962.

66. Schubert, J. and Wallace Jr., H.
The Effect of Zirconium and Sodium Citrate on the Distribution and Excretion of Simultaneously Injected Thorium and Radio - strontium.
J. Biol. Chem., 183: 157, 1950.

67. Lengemann, F.W., Wasserman, R.H. and Comar, C.L.
The Effect of Growth and Thyroid Hormones Upon the Removal by Lavage of Calcium and Strontium from the Skeleton of the Rat.
Endocrinology, 67: 535 - 539, 1960.

68. Samachson, J. and Lederer, H.
Effect of Chelating Agents on Uptake of Ca^{45} and Sr^{85} by Defatted Bone in Vitro.
Proc. Soc. Expt. Biol. Med., 107: 429 - 434, 1961.

69. Cohn, S.H., and Gong, J.K.
Effect of Chemical Agents on Skeletal Content and Excretion of Injected $Strontium^{89}$.
Proc. Soc. Expt. Biol. Med., 83: 550 - 556, 1953.

70. Spencer, H., Samachson, J., and Laszlo, D.
Effect of Ethylenediamine-tetraacetic acid on Radiostrontium Excretion in Man.
Proc. Soc. Expt. Biol. Med., 97: 565 - 567, 1958.

71. Torizuka, K.
Metabolism of Radioactive Strontium Sr^{89-90}. Influence of various Chemical Agents on the Distribution of Sr^{89-90} in Animals.
Naika Hokan 6: 76 - 95, 1959.

72. Spencer, H., Feldstein, A., and Samachson, J.
Effect of Chelating Agent Bis - 2 - Aminoethyl ether Tetraacetate (Baeta) on Radiostrontium Excretion in Man.
J. Lab. and Clin. Med., 59: 445 - 455, 1962.

73. Ito, Y. and Tsurufuji, S.
Detoxication and Removal of Radiostrontium in the Rat.
Proc. 2nd Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 23: 443 - 450, 1958.

74. Vaughan, J.M. and Tutt, M.L.
Use of Ethylenediamine Tetraacetic Acid (Versene) for Removing Fission Products from the Skeleton.
Lancet, 856: October 24, 1953.

75. Catsch, A.
Retention in the Skeleton of Radiostrontium as Influenced by Tetracycline.
Nature: 302, 1963.

76. Richards, V., Lowenstein, J.M., Phillips, J.W., and Armitage, C.
Tetracycline Accelerates Elimination of Strontium from Bone in Mice.
Proc. Soc. Expt. Biol. Med., 107: 550 - 551, 1961.

77. Schubert, J.
Limitation of Chelating Agents for the Treatment of Acute Radiostrontium Poisoning.
Atompraxis, 4: 393 - 397, 1958.

78. Harrison, G.E., Jones, H.G., and Sutton, A.
The effect of Carrier Strontium as the Absorption of Oral Doses of Radioactive Strontium in Rats.
Brit. J. Pharmacol., 12: 336 - 339, 1957.

79. Hegsted, D.M. and Bresnahan, M.
Dietary Strontium and Calcium on Deposition of Sr^{90} and Ca^{45} in the bones of rats.
Proc. Soc. Expt. Biol. Med., 112: 579 - 582, 1963.

80. Miyao, N. and Hayashi, M.
Studies on Strontium Metabolism Following Strontium - Calcium Discrimination in Domestic Animals.
IV. Effect of Calcium and Strontium on Srtontium^{90} and Calcium^{45} Discrimination in Fowl and Goat.
Japanese J. Vet. Sci., 23: 67 - 74, 1961.

81. MacDonald, N.S., Spain, P.C., Ezmirlian, F. and Rounds, D.E.
The Effect of Calcium and Phosphate in Foods on Radio - strontium Accumulation.
J. Nutrition 57: 555 - 564, 1955.

82. Wasserman, R.H. and Comar, C.L.
Effects of dietary Calcium and Phosphorus Levels on Body Burden of Ingested Radiostrontium.
Proc. Soc. Expt. Biol. Med., 103: 124, 1960.

83. Van Putten, L.M.
Treatment of Radiostrontium Intoxication in Mice. I. The Enhancement of Elimination of Radiostrontium.
International J. Radiation Biol., 5: 471 - 476, 1962.

84. Harrison, G.E.
Effect of Doubling the Oral Intake of Calcium on the Uptake of Radioactive Strontium in Rats.
Internatl. J. Rad. Biol., 8: 177 -186, 1964.

85. Wasserman, R.H. and Comar, C.L.
Effectiveness of Dietary Substances in Reducing the Retention of Chronically ingested Radiostrontium: Studies with Tannin.
Nature, 185: 629, 1960.

86. MacDonald, N.S., Nusbaum, R.E., Ezmirlian, F., Richard, C.B., Alexander, G.V., Spain, P., and Rounds, D.E.
Gastrointestinal Absorption of Ions. I. Agents Diminishing Absorption of Strontium.
Pharmacol. Expt. Therap., 10: 348 - 353, 1952.

87. Michon, G. and Guilloux, M.J.
Use of Ion Exchangers and Early Treatment of Radioactive Contamination in the Digestive Tract.
Proc. 2ndInterl. Conf. Peaceful Uses Atomic Energy, Geneva, 23: 439 - 442, 1958.

88. Migicovsky, B.B.
Removal of Strontium and Caesium from Milk.
Nature, 184: 374 - 375, 1959.

89. Aarkrog, A. and Rosenbaum, H.C.
Removal of Radiostrontium from Milk.
Nature, 196: 767, 1962.

90. George, W.H.S.
Separation of Strontium from Milk and Protein Solutions by Gel Filteration.
Nature, 195: 155 - 157, 1962.

91. Edmondson, L.F. et al.
Removing Radiostrontium from Milk, Current Status of a Pilot Plant Process.
J. Dairy Sci., 45: 800 - 803, 1962.

92. Easterly, D.G., Demott, B.J. and Cragle, R.G.
Strontium in Milk. II. Removal by Batch Ion Exchange Method.
J. Dairy Sci., 43: 146 - 150, 1960.

93. Silverman, J., Ghosh, D. and Belcher, R.L.
Removal of Strontium^{90} from Milk.
Nature, 198: 780, 1963.

94. Harrison, G.E., Raymond, W.H.A., and Tretheway, H.C.
Clin. Sci., 14: 681, 1955.

95. Comar, C.L., Wasserman, R.H., Ullberg, S., and Andrews, G.A.
Proc. Soc. Expt. Biol. Med., 95: 386, 1957.

96. Spencer, H., Li, M., Samachson, J., and Laszlo, D.
Metabolism Clin. and Expt., 9: 916, 1960.

97. Barnes, D.W.H., Bishop, M., Harrison, G.E., and Sutton, A.
Intern. J. Radiation Biol., 3: 637, 1961.

98. Comar, C.L.
The Transfer of Calcium and Strontium Across Biological Membranes.
Editor R.H. Wasserman, Academic Press, New York, 409, 1963.

99. Della Rosa, R.J., Goldman, D., Andersen, A.C., Mays, C.W. and Stover, B.J.
Absorption and retention of ingested strontium and calcium in beagles as a function of age.
Nature, 205: 197, 1965.

100. Draper, H.H.
The Transfer of calcium and strontium across biological membranes.
Editor R.H. Wasserman, Academic Press, New York, 97, 1963.

101. Ingerham, R.C. and Visscher, R.B.
Further studies on intestinal absorption with performance of osmotic work.
Am. J. Physiol., 121: 777, 1938.

102. Rabinovitch,
Factors influencing the absorption of water and chlorides from the intestine.
J. Physiol., 82: 279, 1927.

103. Nasset, E.S. and Parr, A.A.
Passage of fluid and certain dissolved substances through the intestinal mucosa as influenced by changes in hydrostatic pressure.
Am. J. Physiol., 109: 615, 1934.

104. Blickenstaff, D.D., Bachman, D.M., Steinberg, M.E. and Youmans, W.B.
Intestinal absorption of sodium chloride solutions as influenced by intraluminal pressure and concentration.
Am. J. Physiol., 168: 303, 1952.

105. Talmage, R.V. and Elliott, J.R.
Fed. Proc., 17: 160, 1958.

106. Rasmussen, H.
Endocrinology, 65: 517, 1959.

107. Gran, F.C.
Acta Physiol. Scand., 49: 211, 1960.

108. Wasserman, R.H. and Comar, C.L.
The parathyroid and the intestinal absorption of Ca^{45}, Sr^{85}, and P^{32} ions in the rat.
Endocrinology 69: 1074 - 1079, 1961.

109. Cramer, C.F.
Participation of parathyroid glands in control of calcium absorption in dogs.
Endocrinology 72: 192 - 196, 1963.

110. Cramer, C.F., Suiker, A.P. and Copp, D.H.
Parathyroid influence on calcium and phosphorous absorption by the gut.
The Parathyoids. Editors Grup, R.O., Talmage, R.V. and Thomas, C.C., Illinois, 158-166, 1961.

111. Harrison, H.E.
Federation Proc., 18: 1085, 1959.

112. Reynafarje, C. and Ramos, J.
J. Lab. Clin. Med., 57: 848, 1961.

113. Balfour, W.M. et al
J. Exp. Med., 76: 15, 1942.

114. Harrison, H.E. and Harrison, H.C.
J. Biol. Chem., 185: 857, 1950.

115. Harrison, H.E. and Harrison H.C.
J. Biol. Chem., 188: 83, 1951.

116. Lengemann, F.W.
The transfer of calcium and strontium across biological membranes.
Editor Wasserman, R.H. Academic Press, New York, 91, 1963.

117. Schacter, D., Kowarski, S. and Finkelstein, J.D.
Vitamin D_3, direct action on the small intestine of the rat.
Science, 143: 143 - 144, 1964.

118. Wasserman, R.H., Comar, C.L. and Nold, M.M.
The influence of aminoacids, and other organic compounds in the gastrointestinal absorption of Ca^{45} and Sr^{89} in the rat.
J. of Nutr., 59: 371 - 381, 1956.

119. Wasserman, R.H. and Lengemann, F.W.
Further observation in lactose stimulation of the gastrointestinal absorption of calcium and strontium in the rat.
J. Nutrition 70: 377, 1960.

120. Brown, E.J. Jr.
In vitro absorption of radioiron by everted pouches of rat intestine.
Am. J. Physiol., 194: 319 - 326, 1958.

121. Scholar, J.F. and Code, C.F.
Rate of absorption of water from stomach and small bowel of human beings.
Gastroenterology 27: 568, 1964.

122. Benson, J.A., Jr., Chandler, G.M., Vansteenhuyse, F.E., and Gagnon, J.O.
Studies concerning the site of fat absorption from the small intestine of rat.
Gastroenterology, 30: 53, 1956.

123. Kremen, A.J., Linner, J.H. and Nelson, C.H.
An experimental evaluation of the nutritional importance of proximal and distal small intestine.
Ann. Surg., 140: 439, 1954.

124. Ohhawara, Y. et al
Gastrointerology, 44: 611, 1963.

125. Shay, H. et al
Am. J. Digest., 7: 456, 1940.

126. Schedl, H.P. and Clifton, J.A.
Gastroenterology, 44: 134, 1963.

127. Cummis, A.J. and Jussila, R.
Gastroenterology, 29: 982, 1955.

128. Lengemann, F.W. and Comar, C.L.
The effect of whole body x-irradiation on the passage and absorption of strontium and calcium.
Radiation Research 14: 662 - 667, 1961.

129. Cramer, C.F. and Copp, D.H.
Progress and rate of absorption of radiostrontium through intestinal tracts of rats.
Proc. Soc. Expt. Biol. Med., 102: 514 - 517, 1959.

130. Dukay, A.C.
Experimental study on calcium absorption.
M.Sc. Thesis submitted to the Graduate Faculty, McGill University, Montreal, 1963.

131. Mraz, F.R.
Intestinal absorption of Ca^{45} and Sr^{85} as affected by the alkaline earths and pH.
Proc. Soc. Expt. Biol. Med., 110: 273 - 275, 1962.

132. Paul, T.M.
Gastrointestinal absorption of radioactive strontium in rats.
M.Sc. Thesis submitted to Graduate Faculty, McGill University, Montreal, 1963.

133. MacDonald, N.S., Ibsen, K.H., and Urist, M.R.
Effect of tetracycline on retention of calcium and strontium in rodents.
Proc. Soc. Expt. Biol Med., 115: 1125 - 1128, 1964.

134. Palmer, R.F. and Thompson, R.C.
Discrimination of intestinal absorption of strontium and calcium.
Proc. Soc. Expt. Biol. Med., 108: 296 - 300, 1961.

135. Palmer, R.F. and Thompson, R.C.
Effect of calcium on the absorption of Sr^{85} and Ca^{45}.
U.S. Atomic Energy Commission Res. and Develop. Report.
H.W. 69500, 8 - 9, 1961.

136. Rosenthal, H.L.
Accumulation of Sr^{90} and Ca^{45} by fresh water fish.
Proc. Soc. Expt. Biol. Med., 104, 55, 1960.

137. Thompson, R.C.
The transfer of calcium and strontium across biological membranes.
Ed. R.H. Wasserman, Academic Press, New York, 399, 1963.

138. Cramer, C.F.
Sites of calcium absorption and the calcium concentration of gut contents in the dog.
Canad. J. Physiol. Pharmacol., 43: 75 - 78, 1965.

139. Steiner, A.B. and McNeely, W.H.
Natural plant hydrocolloids. Edited by the staff of industrial and engineering chemistry.
American Chemical Society, Washington D.C., 70, 1954.

140. Thomas, R.O., Litovitz, T.A. and Geschikter, G.F.
Am. J. Physiol., 176: 381, 1954.

141. Davis, G.K.
The transfer of calcium and strontium across biological membranes.
Ed. R.H. Wasserman, Academic Press, New York, 129, 1963.

142. Nicolaysen, R.
Acta Physiol. Scand., 5: 200, 1943.