GASTROINTESTINAL ABSORPTION OF RADIOACTIVE STRONTIUM IN RATS.

by

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PREFACE

For nearly a decade, extensive investigation has been carried out in the Department of Experimental Surgery on the metabolism and carcinogenic effect of radioactive strontium. The continuing interest to study the various parameters that influence the gastrointestinal absorption of radioactive strontium has led the present project. I was only too happy to undertake these studies, since in my home state in India, a sizable part of the population lives in close proximity to high level of natural radioactivity in soil. This project also coincided with my personal interest in the use of radioactive isotopes in medicine.

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INTRODUCTION

The man produced radiation had been with us for nearly three-quarters of a century. It represents an element which has significant impact on nearly every phase of human life. In the latter part of last century, the experiments with vaccum dischargers have led to the discovery of X-rays by Roentgen in 1895. This was soon followed by Becherel's (1896) discovery of radiation from radiactive materials. Within months it was recognized that each of these radiation could have injurious effect upon persons exposed to it.

The increasing incidence of radiation damage had culminated in the establishment of International Commission of Radiological Protection in 1928. With the introduction of quantity unit for measuring radiation it was possible for the first time to make recommendations for quantitative protection and to introduce the concept of a permissable dose.

The middle of 1940's had witnessed the enormous increase in quantities and varieties of radioactive materials produced by atomic fission. With the explosion of the first atomic bombs at Nagasaki and Hiroshima, a manifest public awareness of radiation as a potential hazard for orderly life on this planet as well as possible benefit to society became evident. Problems resulting from technical advances, with their concurrent social and political implications are not new to man. For example, one can mention such ancient inventions as gunpowder or perhaps the more recent developments of the automobile and the antibiotics with their benefits as well as disadvantages.

Radiation has one particular characteristic that may cause deeper concern. It is known that apart from its injurious or lethal action; the effects can be transmitted to the progeny by means of mutations. Looking into the future, with an ever expanding use of radioactive isotopes for all conceivable purposes and with the prospect of an ever increasing number of people living in

close proximity with thermonuclear reactors, the radionuclides having long half-life like strontium ^{90} and strontium^{89} are posing a tremendous problem in the field of biology and medicine.

With prevention being preferable to treatment, it seems necessary to have a clear understanding of the pathway of entry of such deleterious substances into the biological systems and the factors affecting it, before a meaningful preventive measures can be devised. It is with this view in mind, that the present studies had been undertaken to explore the possible entities involved in the gastrointestinal absorption of radioactive strontium.

CHAPTER II

BIOLOGICAL EFFECTS OF IONISING RADIATION

Section 1. Definitions.

The term radiation indicates a physical phenomenon in which energy travels through space, even though that space be empty of matter. Two types of radiation are generally distinguished (Fano, 1954), namely:

1) Corpuscular radiation consisting of various types of atomic or subatomic particles which are capable of transfer of their kinetic energy to any matter that they encounter.

2) Electromagnetic radiation consists of self-propagating electric and magnetic disturbances, which affect the internal structure of matter and thus dissipates their energy.

Cosmic rays consist of a complex of radiation which flow constantly through the earth's atmosphere in a downward direction and relatively few penetrate to the surface of the earth. Several components of this complex are identical with corpuscular and electromagnetic radiations studied in the laboratory. There are still other components of energy particles which cannot be produced in the laboratory. The biological significance of these rays on the surface of the earth is not known.

Mesons include a variety of subatomic particles heavier than electrons but lighter than protons or neutrons. They arise from collison of very-high-energy radiations with atomic nuclei, but have only a brief existence and presumably are not of much biological importance.

Neutrino is the constituent of a radiation which is presumed to arise from the emission of Beta-rays and other subatomic processes. These are electrically neutral.

Molecular rays are beams of mono or polyatomic molecules which escape from an enclosure into high vacuum, carrying little enery and no electric charge.

A classification of biologically important radiations is given in table I.

Detection of radiation can be carried out in accordance with the effects of their impact on matter. These methods include:

1. Photography
2. Ionisation
3. Study of biological effects
4. Excitation of luminescence
5. Study of chemical effects
6. Atomic transmutation

Several units are used to express the measurement of radiation.

TABLE I

CLASSIFICATION OF BIOLOGICALLY IMPORTANT RADIATIONS

CORPUSCULAR			ELECTROMAGNETIC		
Electrically Charged		Electrically Neutral			
Light	Heavy				
Cathode Rays (electrons) positrons	Protons, deutrons, and other ion beams	Neutrons	Radio-waves, microwaves	Light (infrared, visible & ultra-violet)	X(or Roentgen rays
Special names of radiation from atomic nuclei					
(Beta) rays	(Alfa) rays	-	-	-	(Gamma) rays

(Quoted after U. Fano, Radiation Biology, McGraw-Hill Book Company, Inc., Part I, 1954.)

Curie is an amount of radioactive material which disintegrates at the rate of 3.7×10^{10} per second; a millicurie is 3.7×10^{7} and microcurie is 3.7×10^{4} (Veall and Vetter, 1958).

Roentgen is defined as the quantity of X-ray or gamma radiation associated with corpuscular emission per 0.001293 Gm of air producing ions carrying one electrostatic unit of either sign.

Rutherford (rd) is an unit representing one million disintegrations of radioactive matter per second.

Roentgen Equivalent Physical is an unit of radiation of any type which yields an amount of energy transferred to the tissue equal to that transferred by one roentgen of X - or gamma- radiation. This amount of energy turns out to be 93 ergs per gram of water or soft tissue.

Section 2. General Considerations.

Less than a year after Roentgen (1895) reported "a new form of radiation", Stevens (1896) described the radiation dermatitis and epilation. Walsh in 1897 reported the untoward effects of acute radiation illness. Shortly thereafter other investigators noted similar reactions produced by the new tool in the medical armamentarium. Following those observations and the increasing usage

of X-radiation 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 response reaction.

Although it is generally true that radiosensitivity is related to growth rate, it is clear that relationship is not linear. For example, with the onset of mitosis, germinating wheat seedlings become more resistant to the growth-retarding effect of x-irradiation (Henshaw and Francis, 1935); in eggs of Drosophila, the sensitivity does not parallel the rate of division (Packard, 1930). The rapidly growing squamous cell epithelioma is fairly resistant to radiation while the more slowly growing basal-cell tumour is sensitive. The erythroblast vulnerability to radiation injury is not enhanced by an increase in mitotic activity; in fact, the hyperblastic erythroid tissue is less sensitive than the normal one (Jacobson et al, 1948).

There is equally convincing evidence with regard to the relationship of primitiveness of the cell and radiosensitivity. While the primitive reticular cells are exceedingly resistant to radiation injury, the blast cells which arise from reticular cells are more sensitive (Bloom, 1947; Tullis, 1949).

In general, the haematopoietic and germinal tissues belong to the most sensitive. These are followed by intestinal epithelium, skin and connective tissue. Bone and glandular tissue are relatively radioresistant while muscle and nerve tissue are the least sensitive. There seems to be no relation between the susceptibility of different tissues and their basal oxygen consumption. Brain and kidney have higher rate of respiration than spleen, yet the former two organs are relatively radioresistant, while the latter is radiosensitive.

The events that are immediatly associated with the absorption of radiation are not entirely dependent on temperature (Patt and Brues, 1945). However, the subsequent reactions to injury and recovery are affected by temperature changes and metabolic activity. The influence of hypoxia and protective substances on the changes produced by ionising radiation in the aqueous solution and in living tissues may be considered to be due to the effect of activated-water(free

hydrogen atom and hydroxyl radical) reactions in vivo as well as in vitro. The mode of action of these modifying factors is not clear. There is reason to believe that the early cytological damage following a moderate dosage of radiation is the result of chemical effects of ionization and excitation. This may be an important factor in the production of overall response to radiation injury in the mammalian organism (Patt and Brues, 1954).

In view of the complexity of the nature of the energy absorption, it is not surprising that the effects of radiation are manifested in many ways and several factors have been shown to influence the response to ionising radiation.

Section 3. Radiation Sickness.

Acute radiation syndrome is the symptom complex appearing after irradiation of large areas of the body and its mode of development may be considered pathognomonic of radiation exposure, (Warren and Bower, 1950). It is necessary to distinguish the relatively mild symptoms which sometimes follow clinical application of radiation, from the complex picture of the severe radiation syndrome.

Our knowledge of acute radiation injury in man is based largely on the studies of atomic bomb casualities and those injured by accidental nuclear explosions (Howland et al, 1946; Hempelmann, 1950;

Hemplemann, lisseo and Hoffman, 1952). The study of the effects of atomic bombings were complicated by the effects of heat, blast and lack of precise information regarding conditions of exposure.

The severe irradiation is followed by a shock like reaction and death within a few days. Severe intestinal damage and central nervous system disturbance are prominent. After medium irradiation initially a mild disturbance is evident, consisting of a brief period of apparent respite, a final phase of progressive injury follows with ultimate death. It is apparent that leukopenia, septicaemia, haemorrhage and gastrointestinal damage are the most significant immediate sequlae following a medium dose of irradiation. The chronic effects consist of anaemia, neoplasia, lenticular opacity and premature ageing. These may appear in the survivors of an acute injury or after protracted or repeated irradiation.

The medical management may be resolved into three basic components: a) correction of panhomatopenia, b) prevention or treatment of infection, c) maintenance of adequate nutrition and fluid balance.

Section 4. Cellular Changes.

Changes in Cell Physiology. Metabolic cellular processes such as respiration and protein synthesis are not usually affected by exposure to radiation doses of less than several thousand roentgens, (Howard, 1962). Some processes which have been reported to be exceptionally radiosensitive show measurable changes only after the appearance of morphological signs of cell death. In non-proliferating mammalian cells such as mature lymphocytes of the mouse pycnotic changes occur in two hours following in vivo exposure to 100 r . According to Howard (1962), a delay in the subsequent mitosis is an early and very general consequence of exposure to radiation in proliferating cell populations. If the radiation dose has not been too great, mitotic activity returns and the chromosomes of a proportion of the dividing cells are seen to be structurally abnormal. Daughter cells of these divisions have a high probability of carrying altered chromosome complements. After rather large doses, giant cells are formed through the permanent inhibition of mitosis and continuation of synthesis of cell constituents. The effect of a dose of 450 roentgens on regenerating liver cells cause disappearance of alphacytomembranes and glycogen. This loss is accompanied by complete cytoplasmic

vaculation. The cytoplasm remains vaculated for about 9 to 10 hours after irradiation, but after this new alphacytomembrane and glycogen are formed. (Davies, 1962).

Changes in Tissue Metabolism. Of all the metabolic activities, the synthesis of nucleic acid seems to be the most sensitive to the action of ionising radiation. In animals bearing two tumours, irradiation of one (while the second is shielded) causes inhibition of nucleic acid synthesis in both tumour (Ahlstram, 1945). Furthermore blood transfusion from an irradiated rabbit to a non-irradiated rabbit also produces inhibition of nucleic acid synthesis in the kidneys of the receipient.

Inhibition of respiration, uptake, and the rate of absorption of glucose by small intestine has been also noted. Definite decrease of both the glycolysis and glycolysis-related incorporation were observed(Commarava, 1963). In protein exposed to ionising radiation, the most marked effect is that the secondary structure is disrupted. The denaturation is believed to be due to the disruption of H-bonds as a result of ionization and not the consequence of covalent chemical changes which may also follow exposure of ionising radiation. (Hamilton, 1962).

Changes in Enzyme and Antibody Production. The pre-existing enzymes are in general as resistant as other proteins. The formation of new enzyme molecules can be inhibited by radiation. Ionising radiation may act on either of the two components of which enzymes are made up, that is, the protein and the prosthetic group. When acting on protein, they may oxidise reactive groups in the side chain of the molecule such as sulphydryl groups; or they may act by breaking the hydrogen bonds thus interfering with the structure of the molecule (Barron, 1954). When acting on the prosthetic group, they may produce chemical changes which alter the biological activity of the enzyme.

The radiosensitive nature of the antibody response has received wide attention. Ionising radiation appears to have a specific effect on antibody formation whereby the normal antibody response is supressed. Several studies have demonstrated that the primary antibody response to soluble antigen is more radiosensitive than the secondary response to the specific antigen (Stone, Hale, 1962). This seems to depend upon various factors such as animal species, form of antigen (soluble or particulate) route of antigenic stimulus, time of antigenic stimulus in relation to radiation exposure, total radiation dose and rate and the time interval from exposure when

serum was obtained for antibody determination.

Genetic effects. The most important action of radiation on the cell is the production of changes in the genetic or chromosomal apparatus. Once established, these changes are largely irreversible and may alter the fate of the cell and its progeny (Barran, 1954). The immediate effect of radiation on the transmission of its genetic material is its inhibition of mitosis. If a cell has already progressed in mitosis, when radiation is applied, as in the late prophase, metaphase, anaphase, or telephase stage, the cell will complete division; if it is approaching prophase, it will be inhibited; if the cell is in early or middle prophase it may even appear to regress (Muller, 1954).

In the fertilization period of the egg, radiation interferes with the separation of polar bodies, thus producing polyploid nucleus. Disarrangement of cell division and induction of anenplidy may be noted. Abnormal distribution of chromasomes to daugther nuclei, both at mitotic and meiotic divisions can be seen. (Muller, 1954).

A mutation induced by radiation is essentially indistinguishable from a natural one. Analysis of the relation between mutation

rate and radiation dose has led to the conclusion that mutation may require but a single ionization of the gene; the frequency of mutation characteristically rises in linear proportion to the dose without evidence of a threshold (Upton, 1960).

Section 5. Systemic changes.

Effects on gastrointestinal tract. Because the dividing cells lining the small intestine are extremely radiosensitive, relatively small amounts of radiation applied to the abdomen elicit profound effects on the gastrointestinal system. These vary from slight disturbance of motility and secretion, to ulceration and sloughing of the lining of the bowel. Nausea, vomiting and anorexia were described by Walsh in 1897. Sloughing of the intestinal lining may lead to ulceration, and invasion of the blood stream by bacteria that normally inhabit the lumen of the intestine. This sequence of events is usually fatal and constitutes one of the major causes of death after massive irradiation of the whole body. (Upton, 1960).

Effects on hamopoietic system. The radiosensitivity of blood forming tissues and the consequent hazard of blood damages have attracted considerable attention since the classic work of

Heinke in 1903. Changes in the peripheral blood cells reflect to a considerable extent the alterations in the bone marrow, lymph nodes and spleen (Lawerence et al, 1948, Bloom, W. and Jacobson, 1948). While the site of action is mainly on the blood forming organs, indirect or remote effect on haemopoietic tissues and direct effects on the morphological components of peripheral blood are not known. The alteration of lymphoid tissues in areas distinct from the site of irradiation is an example of indirect action (Barnes 1943, Leblond, 1942).

Blood exposed in vitro to moderate dosage, that is, in the lethal range of mammals, shows only slight changes. Haemolysis has been seen in blood subjected to heavy irradiation. The radiation anaemia may be due to, in part, to a haemolytic reaction as suggested by the rapid decline of red blood cells, which is in excess of that resulting solely from the absence of the erythrogenesis (Davies et al, 1950, Schwartz et al, 1947).

The histological picture is characterized by the disappearance of mitotic figures, cell degeneration, aplasia of marrow and the lymphoid tissue (Bloom, 1949). Edema and hemorrhage with subsequent fatty infiltration of the hypoplastic marrow cavity may occur several days after irradiation. The first of the blood forming

cells to be destroyed are the erythroblasts and hemocytoblasts, next the myelocytes and finally megakaryocytes. In each series the younger cells are more sensitive than the older forms of the same cell types.

A prolonged bleeding time, impaired clot retraction, fragility, thrombocytopenia, and prolonged whole blood clotting time were observed in laboratory animals following single exposure to L. D. 50 range and above radiation dose. (Shouse, Warren, Whipple, 1931). The hematological effects produced by internal radiations by radioelements like Sr^{89}, Sr^{90}, p^{32} etc., depend upon the dosage and the extend of localization within the hematopoietic tissue. With Sr^{89}, a dose of 2 microcurie per gram body weight, produced marked bone marrow depletion (Kahn et al, 1963).

In the presence of anemia, amisocytosis, poikilocytosis, macrocytosis, microcytosis and polychromasia were found (Jacobson). Nucleated erythocytes, degenerative morphological changes of platelets, clumping of nuclear chromatin were noted. Occasional mitotic blast cells, lymphocytes with split nuclei and fragmentation were seen. The granulocytes showed nuclear deformity and

disintegration, nuclear membrane destroyed and bluish non-specific granules of uneven contour filled the entire cell. Atypical aesiniphils characterized by irregularity in granules, size and shape were also demonstrated.

Effect on gonads. Since the developing germ cells are highly radiosensitive, their irradiation may result in immediate sterility; often this sterility is transitory unless too few precursors survive to resume adquate production of germ cells. In man, as in most mammals studied, permanent sterilization requires amounts of radiation that are lethal when absorbed by the entire body. Hence sterility is not, generally, a complication of whole-body irradiation.

It has not been possible to note even a temporary sterility on the basis of comparison of frequency of pregnancies in the exposed and unexposed people of Marshall islands, 4 years after exposure to significant amounts of fallout radiation(Conard et al 1959).

The radiation may produce gene mutation and be passed on, via eggs and sperms to successive generations of progeny. There are very few human data to guide quantitative estimates of the genetic hazards. The studies which have been made of children that are born to parents who were exposed in Hiroshima

and Nagasaki (more than 70,000 births have been investigated) show no significant increase of either major congenital abnormalities, stillbirths or neonatal mortality (Lindell, 1961).

Effects on central nervous system. The response of the nervous system to the ionizing radiation has been studied in terms of biochemical, histological and functional alterations. At the behavioural level, many investigators have reported measurable changes in behaviour after exposure to whole-body or skull irradiation for doses ranging from 0.1 to 700 rads, depending upon the phylogenetic states of the experimental subjects (Zeman, 1961).

Low-level irradiation induced behavioural changes have not been related to specific morphological alterations, since doses in excess of 1000 rads are usually necessary to produce histologically detectable changes in tissue components of the central nervous system (Ordy et al, 1963). However irradiation induced biochemical and electro-physiological changes have been reported with much lower dose levels (100 - 500 rads) than those necessary for producing cell necrosis (Caster et al, 1958; Rosenthal et al, 1961; Gangloff and Haley, 1960).

There is no doubt that the changes arising in the central nervous system modify activity of various organs and thereby may

results in severe consequences for the organism. Livanove (1958), noted that the role of certain nervous formation can be truly evaluated only if it is approached from the evolutionary aspect. Others have demonstrated that the adult mammalian brain can withstand more irradiation without the development of neurological signs than the newborn brain. In rats noticeable and significant radioresistance of the central nervous system developed within first 6 to 8 days of life (Clemente et al 1958).

Changes in the vascular system. Transitory dilatation of blood vessels, causing erythema or reddening of the skin, is one of the earliest known reactions to ionizing radiation. It occurs after only few hundred rads and may be accompanied by increased permeability of blood capillaries. Early circulatory changes such as fall in blood pressure are seen in some species. Hypotension may represent a reflex phenomenon as vagotomy and atropinization can reduce the fall of blood pressure (Painter, 1958). After initial hypotension, arterial pressure rises for a few days to a level slightly below normal and then, decline gradually. The myocardium itself appears to be radioresistant. However, the changes in body fluid equilibrium due to alterations in other systems may influence the circulatory system.

Renal changes. Kidney damage is generally not apparent in irradiated animals. There is some evidence of inhibition of kidney respiration and of oxidation of substrates requiring sulphydryl enzymes (Barron, 1946). Acute necrosis of the developing tubules and glomurulii of baby chicks is an exception to the usual lack of abnormalities with moderate amount of radiation (Patt, Brues, 1954).

Effects on endocrines. It is recognized universally that the adrenal glands constitute a buffer agaisnt a variety of traumatic conditions. The non-specificity of the adrenal response to stress and its role in the general adaptation syndrome are well known. Ionizing stimulii induce changes that are presumed to reflect an increased demand for adrenal hormones. It does not seems to represent to be mediated by the pituitary gland and closely resemble that is seen following a severe traumatic injury (Patt and Brues, 1954).

Although degenerative changes have been seen after heavy local irradiation, dosages of 1000 r. result only in minimal morphological alterations in the adrenal cortex and medulla. Notwithstanding the apparent resistance of the adrenals to structural changes, functional responses may be elicited with relatively low dosage.

Loss of adrenal cortical lipids and adrenal ascorbate occurs soon after irradiation and urinary excretion of 17 ketosteroids may be increased several days later (Dougherty, White, 1946).

Normal thyroid tissue exhibits considerable resistance to ionizing radiation in contrast with the relative sensitivity of the hyperplastic gland. Partial destruction of the thyroid with colloid degeneration has been seen in rats 6 days after injection of approximately 70 mecrocuries of Iodine ^{131} (Findlay et al, 1948).

It is generally agreed that the pituitary is only slightly sensitive to X-rays. The early transient physiological effects of pituitary irradiation are regarded as secondary to increased vascularity or altered permeability rather than primary stimulation of hypophyseal activity.

Dermal changes. Since erythema is the first visible sign of radiation effect on skin and runs parallel to later and more serious effects, much attention has been given to it in clinical practice. Use of large doses results in denudation of epithelium after a few weeks. It seems probable that some of the mechanisms are similar to those following thermal burns, although the changes are much slower in developing.

Temporary epilation is produced by a dosage of the order of 375 to 500 r . Permanent epilation requires a considerably higher dosage. Greying of the hair is seen in mice following relatively low dosage.

Changes in the eyes. Superficial effects of irradiation on the cornea and conjunctiva parallel the effects on the skin. Lenticular opacity are among the most serious non-fatal consequences of irradiation.

Changes in bone and cartilage. Long-continued irradiation of adult bones by radium deposited in the skeleton results in the appearances of areas of rarifaction and aseptic bone necrosis, appearing late and progressing during the course of several years.

Although cartilage is not remarkably radiosensitive, its recovery after irradiation of the exposed area where necrosis had occurred, is very poor. Internal radiation produced by Sr^{90} and Sr^{89} seem to affect the epiphyseal cartilage even at relatively low dosage (Skoryna et al, 1963).

Section 6. Effect on Prenatal Development.

The earliest report of abnormalities following irradiation with a high dose in unborn rabbits on days 7 - 12 after firtilization, was given by Von Hippel and Pagenstecher in 1907, who obtained cataracts, microphthalmia and lid coloboma. Injection of Sr^{89} and platinium^{239} into pregnant females, had produced increased percentage of stillbirths, retardation of growth, fragility, shortening of long bones anaemia and osteogenic sarcoma (Finkel, 1947).

The effects of radiation depend on the stage of development of the embryo at the time of irradiation because different parts of the body are formed sequentally according to a definite order and time schedule. Malformation of almost any organ may be induced by irradiation at the appropriate moment during or preceding its development. How radiation causes malformation is not yet fully known, although interference with organs development through the killing of embryonic cells must play an important role in the progress (Russell, 1954).

The types of human abnormalities enumerated in literature include microcephaly,blindness, microphthalmia, coloboma, cataract, chorioretinitis, ankgloblephron strabismus, nystagnus mental deficiency, hydrocephaly, co-ordination defects, mongolism,

spina bifida, skull malformations, cleft palate, deformed arms and feet, genital deformity and general mental physical subnormality. Although administration of high doses to mother may indirectly affect the viability of the conceptus, there seems little doubt that most of the major embryonic abnormalities are due to the action of radiation directly on the embryo (Russell, 1950).

Section 7. Carcinogenic Effect of Radiation.

It is paradoxical but not unique that ionising radiation which represents such a potent weapon in the treatment of cancer, should also be capable of causing cancer. It is but one of the many agents including viruses and a variety of chemicals that are known to have carcinogenic properties. The earliest known example of radiation-induced tumour was reported in 1902, less than 10 years after the discovery of X-rays. Since then, numerous instances have been observed in man and the process has been studied extensively in experimental animals.

Although susceptibility to cancer induction varies widely among species and organs, virtually all types of cancer have been induced experimentally and it is clear that all types of ionizing radiation share cancer-forming potency. In man, cancer

of the skin, leukemia, cancer of bone and lung have been reported.

The exact mechanism of induction of neoplasms by radiation is still far from clear. It is possible, that the production of carcinogenic substances under the influence of radiation, especially after a chronic exposure to small doses, may have a major role. (Longendorff, 1962). The carcinogenic effect of irradiation appears to be non-linear in character (Brues, 1959).

CHAPTER III

RADIOACTIVE STRONTIUM AND ITS METABOLISM

Section 1. Chemistry of Strontium.

William Cruikshank in 1787, first detected the existence of strontium in the strontianite found at Strontian in Argyllshire; the metal was later isolated by Sir Humphry Davy in 1808, who electrolyzed a mixture of moist hydroxide or chloride with mercuric oxide, using a mercury cathode. Strontium belongs to the alkaline earth family of elements in group two of the periodic table, having an atomic weight 87.63, and atomic number of 38. It composes about 0.02 per cent of the entire crust of earth and its principal sources are strontianite ($SrCO_3$) and celestite ($SrSO_4$). Because calcium and barium, which show close similarity, occur in much greater abundance, strontium is not produced in commercially important quantities.

There are four stable isotopes of the element: their mass numbers, in the order of abundance, are 88, 86, 87 and 84. Several radioactive isotopes are produced by nuclear reactions. Of these, the isotope having a mass number of 90 has received most attention because of its presence in the radioactive fall-out from atomic explosions.

Physical properties. Strontium has a silvery white colour. It is malleable and ductile and is a good conductor of electricity. It has an atomic radius of 2.13 Å and ionic radius of 1.13 Å. Ionisation potential for the first electron is 5.69 V; second electron 10.98 V. Single electrode potential (between metal and molal solution) is 2.89 V. It has a melting point 757 $\pm$ 1°C and boiling point 1366° C. Its density is 2.60 gm. per cc.

Chemical properties of strontium are similar to those of calcium and barium, in accordance with its position in the periodic table, where it is above that of barium and below calcium. Its base forming characteristics are similar to calcium and barium. It has a valence number of 2. As indicated by its atomic radius, ionisation potential and single electrode potential, the atom of Sr easily looses the two electrons in the 5 s level when it reacts with non-metallic elements thus forming Sr^{++} ion.

The metal is an active reducing agent. It reacts readily with water to form hydrogen and the hydroxide $Sr(0H)_2$. It is oxidased rapidly when exposed to air and burns brilliantly when heated in air, oxygen, chlorine, and bromine and sulphur vapour. With oxygen, strontium forms both monoxide and peroxide.

Strontium compounds.

Compounds of strontium are not as extensively used as those of calcium and barium. The solubility of the salts of the element are intermediate between those of corresponding salts of calcium and barium.

The hydride, SrH_2, is a white solid, which readily decomposes water in the cold, and behaves as a strong reducing agent. The monoxide, Sr 0, is a white powder which resembles lime in its general character, it readily slakes with water and the aqueous solution yields a crystalline hydrated hydroxide. It is used in the extraction of sugar from molasses.

Strontium chloride, $SrCl_2$, is obtained by dissolving the carbonate in hydrochloric acid or, commercially, by fusing the carbonate with calcium chloride and extracting the melt with water. Strontium sulfide, SrS, sulfate, $Sr\ S0_4$, nitride, Sr_3N_2, nitrate can also be prepared. Of these, $Sr(N0_3)_2$ is used in pyrotechny for the manufacture of "red fire".

Radioactive Strontium.

The content of non-radioactive strontium in the human body is normally of a low magnitude, with an average concentration of 4.5 per 10,000 parts of calcium. Radioactive strontium shares with radium and other similar isotopes the ability to be deposited in the skeleton. Of several radiostrontium isotopes, two have attracted attention from the field of Medicine and Biology. These are Sr^{90} and Sr^{89}.

Radio-strontium 90 has physical half-life of 25 years. Its biologically effective half-life, that is, the time required for the activity of a single dose ingested by an animal to diminish to half of its original activity has been estimated to be about 7 1/2 years (Hawthorne, 1959). It releases Beta-rays of 0.54 meV. Radio-strontium 89 has a physical half-life of 54 days. It also releases Beta-rays of 1.5 meV. Neither of these isotopes release any Gamma-rays.

One of the products of fission of thermo nuclear explosion is krypton 90, which is an inert gas-like helium or neon. Probably about 5 per cent of all fission events produce Kr^{90}. As it is a gas, it possibly rises to a great height with the mushroom cloud

in the first minute after explosion, by which time, owing to its very short half-life (33 seconds) most of it, has been changed to Rubidium^{90}.

Rubidium^{90} (half-life 2.7 minutes) changes into strontium 90 which in turn slowly produces Yttrium ^{90} having half-life of 64 hours. Yttrium^{90} yields a stable isotope of Zirconium (Heckstall Smith, 1958). In less than an hour after the explosion therefore the only radioactive elements of this chain still existing in effective quantities are 25 years Sr^{90} and its product, 64 hour Yttrium^{90}.

The cloud produced by the explosion then rises out of troposphere into the stratosphere. It drifts east with the prevailing west winds at about 15 - 30 miles per hour, circling the globe every 4 - 7 weeks. It continues to circulate indefinitely. The contents of the cloud reach the earth's 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.

Section 2. Pathway of Radioactive Strontium to Animal System.

During the past few years a great deal of effort has been devoted to discover how much radioactive debris has settled upon the earth and how much more will probably be added as a

result of the continuing thermonuclear weapon testing. Much research has been carried out to determine what proportion of this material will become incorporated in the living organism.

After the radioactive debris has reached the land surface, some of the activity will be carried to the sea in drainage water while the remainder is held in the soil. More than 70 per cent of Sr^{90} in the soil is held in the upper strata of undisturbed soil (Russel, 1958). Radiostrontium reaches plants either from the soil through the plant roots, or by direct deposition on the aerial surfaces of the plant. There is good evidence to show that soil retention is an important factor; however, direct deposition is probably a greater significance at the present moment.

This relative position may change in course of time and varies from country to country. In Japan it was observed that in rice plants 40 per cent of the Sr^{90} contamination was from the aerial parts of the plant and 60 per cent from the soil (Ichkawa et al, 1962). 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 jr., 1962). There

is a species difference in the absorption by plants. Legumes takes up more strontium than grasses by 3 to 6 times (Vase and Koontz, 1959).

It is well known that cattle fed on pasture plants contaminated by radioactive strontium are the chief source of radio strontium to the human being. Inhalation of air-suspended particles and drinking water provide further sources. There is evidence that inhalation of air-borne particles is unlikely to be of major importance and the drinking water does not contribute more than 10 per cent of the total intake. Food represents, therefore, the major source of radiostrontium in human bone (Hawthorne, 1959).

Section 3. Incorporation of Radiostrontium in the Body.

The distribution and relative retention of radiostrontium by the various systems in the experimental animals have been extensively studied. Nilsson et al (1962) using the method of whole body autoradiography and impulse counting had noted that shortly after intraperitoneal injection of Sr^{90}, activity was seen in all tissues, but gradually accumulated in the hard tissues and simultaneously declined in the soft tissues. By 5 minutes Sr^{90} activity was

greatest in the bones and accumulated specifically in growth zones. About 4 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 takes place in the metaphysis. Kahn (1963) and co-workers studying the turnover of radiostrontium in fractured femur had concluded that the period of high uptake at the fracture site corresponds to the period of active osteogenesis, and the amount of the uptake of 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 persistance of this radioactivity depended on the amount of bone remodelling and reconstruction that subsequently occurs.

In the young growing animals tibial ends incorporate more Sr^{89} and Ca^{45} than the incisor, while the opposite was found in older animals (Menezel et al, 1962). Boner and Scoccianti (1961) demonstrated an increased spinal uptake of intravenously

injected Sr^{85} in cases of vertebral fractures, spondylitis and other localized vertebral lesions. Intravenous injection of Sr^{85} also showed localized high concentration in the spinal metastases in uptake after hormone and roentgen therapy (Gynning et al, 1961).

Owen et al (1957) reported that following the injection of one microcurie of Sr^{90} per gram body weight to young rabbits, dose rate of the order of 200 rad per hour where measured in localized region of the epiphyseal tissue of the tibia, representing the dose rate some twenty times greater than the calculated mean skeletal value. This evidence suggests that active osteogenesis is a major determining factor in the focal concentration of radiostrontium in the skeletal system.

Mechanism of Strontium Deposition in Bone.

The strontium ion is associated with bone minerals, the molecular structure of which is very close to that of calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). Owing to the small size of the apatite crystals, bone tissue possesses a large active surface which enables it to serve as an efficient ion exchanger. The exact site of deposition of radioactive strontium is still being debated. Glas (1961) concluded on the basis of his results

obtained by X-ray fluorescence and diffraction studies, that the major portion of strontium is absorbed onto the surface of the apatite crystals. It is possible that a portion is incorporated into the apatite lattice of the bone crystals, or a combination of both the above mechanisms may take place.

Factors Influencing the Deposition of Strontium in Bone.

Certain factors will modify the site and amount of deposition of radioactive strontium. Body discrimination in favour of calcium had been noted by various investigators. Comar et al (1956) by double tracer studies of radiocalcium and radiostrontium concluded that absorption from the intestinal tract and urinary excretion were the major process by means of which the rat discriminated against dietary strontium in favour of calcium. Talmage (1957) from comparisons of Sr^{85} and Ca^{45} in peritoneal lavage solution, estimated that strontium was preferentially released from the bone by a factor of 1.3 over calcium.

Bohr (1960) studied calcium and strontium uptake in normal and rachitic rats. He noted that uptake in the bone during the first hour after injection is the same in normal and rachitic rats, while activity remained practically constant for the

following 24 hours in the normal animals, it was shown that activity in the rachitic rats decreased within 8 hours to about one half of the maximal value. It thus seems that the immediate primary uptake must be considered to be due mainly to a reversible exchange between the blood and the bone tissue and that accretion takes place only through a secondary and slower uptake (Bohr, 1960).

Bacon et al (1956) by giving parathyroid extract was able to increase the urinary excretion of Ca and Sr as well as the deposition of minerals in the kidney tissue. Administration of cortisone has prevented accumulation of Sr and Ca in the kidney tissue but did not influence urinary excretion or diuresis.

Section 4. Elimination of Radiostrontium.

Various agents have been investigated in an attempt to eliminate radiostrontium fron the bone. Spencer et al (1961) studied the use of calcium gluconate and ammonium chloride in the removal of Sr^{85} in man, two weeks after exposure. They noted that combined use of both agents was more effective in removal of radiostrontium than either agent alone. Magnesium ions were effective in increasing the elimination of Sr^{89} in rats which had been given the isotope 30 days before treatment (Clark and Smith, 1962).

The effects of strontium chloride, strontium lactate, calcium chloride, calcium lactate, sodium lactate, sodium lactate EDTA and $CaNa_2$ citrate on the deposition of radiostrontium had been studied by Carlquist and Nelson (1960).

Of these salts only the strontium salts and Ca Na_2 citrate were able to decrease the retention if given within half an hour before or after administration of Sr^{90}.

Barium sulphate was tried as first aid treatment by Volf (1961) after internal radiostrontium contamination in man. Richards et al (1961) noted that parentally administered tetracycline accelerates the elimination of strontium from the bone, while not significantly affecting the amount deposited in bone.

Samachson and Lederer (1961) by using defatted bone powder, demonstrated that strong chelating agents like ethylene-diamine-tetraacetic acid and cyclohexane-diaminetetraacetic acid decreased the ratio of calcium 45/strontium 85 uptake considerably in the presence of calcium, calcium plus strontium or strontium as carrier. They also noted that citrate and adenosine triphosphate had similar but less pronounced effects.

Section 5. The Late Effects of Radioactive Strontium in Bone.

The late effects of radioactive strontium had been studied in experimental animals at various centres in the world. In this laboratory, Skoryna and Kahn (1959) have made extensive investigation in this field. They noted that there was a minimal latent period of 188 days before the first gross tumour was observed. Neoplastic changes were found on microscopic examination in all animals surviving the minimal latent period. The majority of these animals had pulmonary metastases.

In addition to tumours, the basic changes observed were: disturbace of osteogenesis; fibrosis of marrow; and cellular proliferation. The changes were maximal in the metaphysis in relation to the epiphyseal cartilage growth, corresponding to the area of active osteogenesis. Periosteal and endosteal new bone formation of marked degree was also observed.

The extent of fibrosis of bone marrow varied considerably and the most extensive changes occurred in corresponding relationship to the area of highest uptake of the isotope. The presence of foci of cells with large hyperchromatic nuclei were noted. Cellular proliferation without some degree of fibrosis in the area was never found, whereas fibrosis without cellular proliferation was often noted.

CHAPTER IV

STRONTIUM ABSORPTION

Section 1. Introduction.

Absorption is a complex phenomenon, the exact nature of which is still far from being understood. The ultimate aim of digestion is absorption. Only after the products of digestion have passed through the epithelial lining of the digestive tract and entered the blood stream, are they able to serve their purpose. Absorption of most materials takes place almost entirely from the small intestine; although the stomach is capable of absorbing small amounts of water and certain drugs.

The mucous lining of the small intestine is admirably adapted to its absorptive functions. It is one of the most remarkable organs of the body. It produces a variety of hormones which help to regulate gastric secretion and motility, the secretion of the pancreas and of the intestine itself. During absorption the mucosal cells perform a variety of hydrolytic and synthetic processes upon the food substances passing through them (Thomas, 1961).

Epithelial cells of the intestinal mucosa transfer water and other dissolved substances from the lumen of the intestine into the interstitial fluid to be taken up by the blood. This absorption activity is highly selective and frequently involves the expenditure of considerable energy. Probably nowhere, except possibly in the liver are such a great variety of functions performed by a single tissue. It has been estimated that the presence of the villi result in an increase of seven or eight fold in the surface area of the mucous membrane of the intestine.

Section 2. FactorsAffecting Absorption.

The simple process of diffusion is inadequate to explain absorption. Some substances are absorbed against a concentration gradient; absorption goes on until the concentration falls far below that in the interstitial fluid and in the blood (Ingerham and Visseher, 1938). Several agencies are now known which, either directly or indirectly influence the transport of food materials from the intestinal lumen to the circulation.

Osmotic effect of solutions influences the absorption of water and salt. Rabinovitch (1927) noted that absolute amount of salt absorbed increased with increasing concentration of

salt up to 0.8 per cent.

Hydrostatic pressure has some influence on absorption. Nasset and Parry (1934) showed that negative hydrostatic pressure tended to accelerate the net flow of fluid into the intestine whereas increasing the pressure produce net absorption. Blickenstaff (1952) and others by using Omi-type fistula, found that there were statistically significant differences in the mean rate of absorption of chloride and water at different levels of hydrostatic pressure.

The nervous system probably influences absorption, by its control on the gastrointestinal secretions and motility.

Hormones are said to have considerable influence in the absorption of sugars, possibly exerted indirectly through sodium chloride deficiency. In adrenal cortical insufficiency, the absorption of sugar is diminished in the presence of sodium chloride deficiency (Thomas, 1961). Absorption of sugar is accelerated in hyperthyroidism and depressed in hypothyroidism, and in the latter case, administration of thyroxin improves absorption.

The nutritional status of the body may exert some effect on the rate of absorption. Calcium is said to be absorbed more readily by individuals suffering from calcium deficiency (Thomas, 1961). For the normal absorption of sugars pantothemic acid, thyamine and pyridoxine, members of the vitamin B group are essential. In patients on salt restricted diet, sodium practically disappears from the contents of terminal ileum, thus suggesting a sodium conserving action by the upper intestine.

Active participation of mesenterie lymphatic system in the absorption of water has been noted by Lee (1963). In vitro, a 40 per cent decrease in absorption of water was observed in jejunal preparations when lacteal ducts were sectioned near the wall; no decrease occurred when blood vessels were sectioned. During absorption the lymphatics showed a rhythmical contraction with an average frequency of 10 per minute.

Temperature is also said to influence absorption (Smyth and Taylor, 1957).

Stereochemically selective absorption has been also reported. Wiseman(1951) has shown that the levoratory, or naturally occurring forms of alanine, phenylalanine, isoleucine, methionine and histidine are absorbed more rapidly than the dextrorotatory isomers

and that levo-forms could be transferred through the intestinal wall against a concentration gradient. Eldsen et al (1950) have found that if a racemic mixture of aminoacids is introduced into the intestine, the levoratatory isomers will be absorbed as much as six times more rapidly than the dextro-isomers.

The presence of certain substances such as an excess of magnesium or phytic acid is said to interfere with calcium absorption. On the other hand vitamin D accelerates the absorption of calcium from the lower ileum, but not from other parts. Aminoacid absorption is inhibited by absence of oxygen and by several metabolic inhibitors such as dinitrophenol and desoxypyridoxine (Friedlander and Quastel, 1955).

The different anatomical levels of gastrointestinal tract are said to have selective regional maximal absorption. The absorption of iron from the stomach is well known. Scholar and Code (1954) (using heavy water as tracer) have noted that water is ten times more rapidly absorbed from the small intestine than from the stomach. Benson and co-workers (1956) 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 (1954) demonstrated that in dogs the proximal 50 to 70 per cent of the small intestine could be removed with no apparent ill effects on food absorption, particularly fat absorption. However, if the distal 50 per cent were removed, there was a profound interference with fat absorption with loss of weight and from 80 to 90 per cent of the fat intake was lost in the fæces.

H ion, concentration in the intestinal tract may influence the absorption of food material. Perhaps this factor is least understood.

Section 3. Techniques of Studying Radioactive Strontium Absorption.

It is well known that absorption studies can be carried out by either in vitro or in vivo preparations of intestine.

In vivo methods include administration of a known quantity of the isotope by a gastric tube and consecutive assay of the amount of radioisotope deposited in the femur, as well as a total body count. Another technique is to introduce a known quantity of isotope in the isolated segment of intestine and to determine at intervals the amount removed. This may be combined with determination of the amount deposited in the bone.

In vitro techniques include the use of an excised segment of intestine or an everted sac. A number of techniques for this purpose has been described by various authors. All depend upon maintaining the vitality of a portion of the intestine that has been removed from the body by keeping it in contact with oxygenated fluid of appropriate composition and temperature. The preparation can be so arranged that the fluid inside the intestine which is in contact with the absorbing surface can be kept separate from that on the outside in contact with the serosal surface. The changes in the composition of the internal and the external fluid can be measured and this gives the degree of absorption by the mucosa.

Cramer (1959) studied radiostrontium absorption in vivo after the administration of the isotope by a gastric tube and using the tail of the rat for estimation of the amount absorbed. The animals were sacrificed at known intervals and carcass and intestine ashed separately and counted. He noted that strontium absorption has a latent period of 20 - 30 minutes; the absorption is slower and continued for a longer time. When comparing the rate of maximal absorption of phosphorus and strontium, he concluded that there is a difference in the site and transfer rate

of these ions in different parts of the intestinal tract.

Wasserman (1960) studied the absorption of calcium^{45} and strontium ^{85} by means of isolated everted intestinal segments. He noted that in the duodenal segment, calcium^{45} but not strontium^{85} was transferred against a concentration gradient from mucosa to serosa. In the ileal segment Sr^{85} but not Ca^{45} was transported against a concentration gradient from serosa to mucosa. Wasserman and Comar(1961) also investigated the parathyroid influence on calcium^{45} and strontium^{85} absorption. In vitro studies with isolated everted gut segments indicated that transfer of Ca^{45} and Sr^{85} across the duodenum and ileum was not influenced by the parathyroid status of the donor rat.

Carr et al (1961) made a comparative study of the absorption and retention in rabbits following the addition to the diet of Sr^{90} incorporated into plant material and $Sr^{85}Cl_2$. They observed no difference in absorption rate in these two forms of strontium radionuclides after simultaneous administration.

Mraz (1962) extensively investigated the influence of alkaline earth elements and pH on the intestinal absorption

of Sr^{85} and Ca^{45} by means of a perfusion technique. He demostrated that Sr^{85} was absorbed to a lesser extent than Ca^{45}; calcium was more effective in reducing the absorption of Ca^{45}. The effect on strontium was less pronounced. An increase in the inert carrier strontium slightly reduced Sr^{85} and Ca^{45} absorption. Strontium was least effective of the alkaline earths in reducing the absorption of Sr^{85}, except beryllium. Magnesium was more effective in reducing Sr^{85} absorption. There was remarkably little effect of pH in the range of pH from 4 to 11. In the range of pH 2 to 4 there was marked increased absorption of Sr^{85} but not Ca^{45} suggesting that absorption of the two elements occurs by different mechanisms.

Rosenthal (1960) using fresh water fish was not able to observe any discrimination between Sr^{90} and Ca^{45} uptake. Addition of non-radiactive strontium or calcium depressed the accumulation of Sr^{90} and Ca^{45} in the fish. At low concentrations of sodium ions, the fish discriminated against Sr^{90} as compared with Ca^{45}; however, as Na ion concentration increases, this discrimination becomes less obvious. Similar results were obtained using different concentration of alkaline

earth elements.

Palmar et al (1961) studied the absorption of Sr^{85} and Ca^{45} in the rat by the technique of in vivo intestinal perfusion. With no inert calcium added to the perfusion solution, the percentage of Sr^{85} absorbed was about 0.3 that of Ca^{45}. With increasing amount of calcium in the perfusion medium the percentabe of absorbed strontium^{85} and Calcium^{45} decreased; however, the ratio of percentage of absorption of Sr^{85} and Ca^{45} increased, reaching the value of about 0.6 in 25 mM calcium solution. There was no evidence of discrimination of Sr^{85} and Ca^{45} in the reverse direction from blood to intestine.

Lengemann and Comar (1961) studied the distribution of absorbed Sr^{85} and Ca^{45} as influenced by lactose. A greater proportion of absorbed Sr^{85} than Ca^{45} was taken up by bone within the first four hours after administering the dose. Only in the ileum did the presence of lactose, lysin and glucose cause the difference to disappear. On the other hand, Stover et al (1961) studied the integrated effect of an exposure of a beagle to an essentially constant

dietary Sr^{90}/Ca level which began in utero and continued to young adulthood. The relative Sr^{90}/Ca content of the bone was the same as that of the dietary environment.

The discrimination which occurs in the absorption process would appear to be the dominant factor in determining the over all strontium calcium relationship. It is necessary to have more precise knowledge about the mechanism and factors affecting the absorption of radiostrontium. The present study had been undertaken with this objective in mind.

CHAPTER V

EXPERIMENTAL METHODS AND MATERIALS

Aim of the Experiments.

The aim of this investigation was twofold: firstly, to evaluate the hydrogen ion concentration in the gastrointestinal tract of normal rats and its influence on the absorption of radiostrontium; secondly, to find out whether there is a region of maximum absorption of strontium in the gastrointestinal tract.

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. It would be preferable to reproduce physiological conditions by giving the experimental animal radiostrontium in food or drinking water; an assay may then be made of strontium retained in the body tissues, in the intestinal tract and in the excreta. It may be possible to adopt this method for the study of segmental absorption by means of multilumened tubes having multiple cuffs or balloons. This technique, however, is hazardous for use in small animals such as rats; in addition

the process of intubation is very time consuming, thus limiting the number of animals that can be studied.

In this study an in vivo technique has been utilised involving surgery under anaesthesia. These conditions are more close to the normal physiological state than the in vitro technique previously mentioned. Cramer (1959) found essentially no difference in gastrointestinal absorption of strontium between anaesthetised and unanasthetised rats. How far operative trauma influences and modifies the absorptive process is not known.

The effect of pH on the rate of intestinal absorption of calcium has been investigated by Dukay (1962) in this laboratory. He noted that Ca^{45} had a maximum rate of absorption from the duodenal region when it was administered in a buffered solution of neutral pH. The absorption was appreciably reduced at pH values of 4, 5, 6 and 8. This is in contrast to the results obtained in perfusion experiments by Mraz (1962) who did not find any remarkable effect on Ca^{45} or Sr^{85} intestinal absorption between the pH ranges 4 to 11. At the extreme pHs of 1 and 12, there was a sharp decline in the absorption of calcium.

These results necessitated a further study of the relationship between pH and the intestinal absorption of radiostrontium. A buffering solution having a pH value similar to the average physiological value in the small intestine was considered more suitable for this study, because it is likely that an extreme pH either acid or alkaline may disrupt the normal mechanism of absorption. Therefore, the first part of the study was to evaluate the normal pH value of the intestinal tracts of the rat. Subsequently, a buffering agent "Trisma" (a neutralized crystalline hydrochloride of Tris((hydroxymethyl) aminomethane) with a pH 7.85 at 25°C was used. Since "Trisma" has a significant temperature coefficient, the pH will decrease on an average of 0.025 pH units per degree centigrade; the solution at the body temperature of 37°C will give a pH value of 7.55.

A number of investigators have reported on the influence of inert carrier strontium in the absorption of radiostrontium (Samachson et al 1961, Rosenthal 1960, Mraz(1962) Hegstead 1963). Two groups of absorption studies, with and without inert carrier $SrCl_2$, was carried out in order to evaluate this factor in the segmental absorption from rat's gastro-intestinal tract. One hundred twenty micrograms of inert strontium chloride

(hydrated) per 2 microcurie radioactive Sr^{89} was used in this study. This amount is equivalent to the quantity of inert calcium present in samples of Ca^{45} used in an earlier study on Ca^{45} absorption (Dukay, 1962). In one set of experiments, the radioactive strontium^{89} dose was increased by 5 in order to detect any change in the absorption pattern at higher levels of radioactivity.

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 remain unknown, but it is believed that these rats are the offspring of Wistar rats and Norwegian wild rats. The colony has been thoroughly inbred by continuous brother-sister matings since 1933. Animals weighing 100 to 200 gms were used throughout the experiments and a total of about 1200 rats were studied.

Radioactive strontium^{89} used in this study was supplied by the Atomic Energy of Canada Limited, Ottawa, in monthly shipments. The Sr^{89} was in the form of $SrCl_2$ in aqueous (0.98 N) HCl, with Sr^{90} approximately 0.25 millicuries/ml.

Heavy metals were less than 10 p.p.m, total solid content was 8.5 mg/ml.

Methods.

Operative Technique. The animals were fasted for 24 hours and then anaesthetised with nembutal. The anterior abdominal wall was shaved and a laparotomy performed. The stomach was then ligated with silk at the aesophageal and pyloric ends and was designated as segment No. 1. The total length of small intestine was divided into 9 segments of approximately 5 cms each. The duodenal segment was numbered II and the rest in serial sequence, the terminal ileal segment being number 10. Each segment was ligated at both ends without disturbing the vascular supply. Only one segment was used in each rat. A diagramatic representation of a typical segment is given in Figure 1. The test solution was introduced into the lumen of the intestinal segment by means of a tuberculin syringe with a 27 G 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 of the rat. Any increase in volume gives an undue distension of the segment which may alter the absorption pattern.

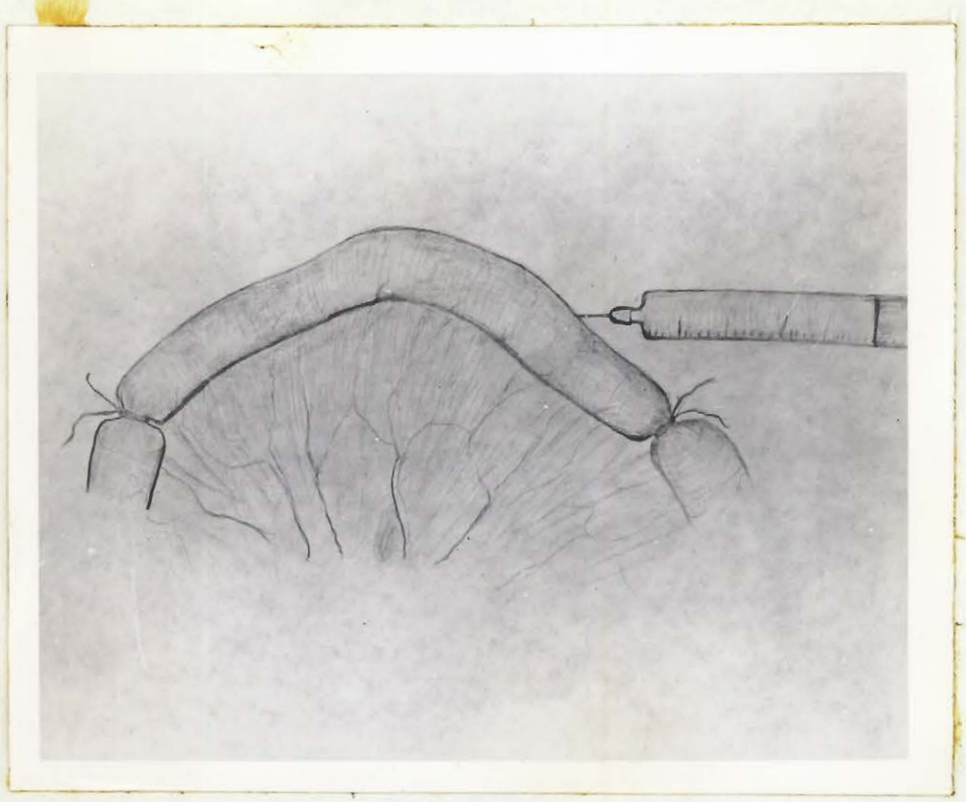

Fig.1 A typical ligated intestine segment.

A very small leakage into the peritoneal cavity of the test solution through the needle puncture was observed in a group of 10 animals tested by injecting a methylene blue solution. It was noted that only a tiny drop of fluid escaped at the moment when the needle was withdrawn from the intestinal wall. 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 laboratory conditions. After the desired length of time animals were sacrificed. The ligated segment was then stripped of its mesentry, resected with ligatures intact and analysed.

Isotope Technique.

The radioactive Sr^{89} after making suitable correction for the decay factor was diluted either in normal saline or in a buffering agent and the volume made up to give 2 microcurie activity per 0.5 ml. In one set of experiments, the volume was made to give 10 microcurie activity per 0.5 ml.

This isotope solution was injected into the lumen of intestine with a disposable tuberculin syringe and 27 G needle; 0.5 ml of the solution was used for each segment and the syringe was used only once. After a period of 30 minutes, the segment of intestine was removed as described earlier and placed in a graduated test-tube containing 3 ml of 38% hydrochloric acid for 24 hours, by which time the intestinal walls had been dissolved by the acid. This procedure ensures that absorption in these experiments means the removal of radioactive ions not only from the lumen of the gastrointestinal tract but also from the walls of the intestine. This acid solution was neutralised with 30% sodium hydroxide, and diluted with water in such a way that the original 12 micro-curie solution was diluted 375 times. This dilution was used after experimenting with several different dilutions. At this dilution one ml of solution after drying in a planchet gives a very thin layer which is scarcely visible. Such a thin layer reduces the error of self absorption of beta-particles by the crystals to a negligible minimum.

The solution was transferred into the planchet using 1 ml capacity pipettes with a safety pipettor. The planchets were dried for 3 hours at 75°C.

Counting was carried out in a Shielded Manuel Changing Chamber, using an 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. Before counting samples, care was taken to check the plateau using a standard sample and making the necessary adjustment in the EHT input. A constant geometrical set up was used in all measurements. Background radiation was counted before and after each set of samples. The background was less than 60 counts per minute.

Each sample was counted for over 3 minutes to give a total count of 10,000 and thus reducing the coefficient of variation to a minimum of 1 per cent. This was calculated using the formula:

$$\text{coefficient of variation} = \frac{100}{\sqrt{NT}} \%$$ (Veall and Vetter, 1958)

With each group of samples a standard sample of the same dose of Sr^{89} was counted. As the standard value of the isotope introduced into the intestinal segment is known, the percentage

of Sr^{89} retained in each segment was calculated. From this value, the amount absrobed was derived.

All glassware and other instruments used in this study were decontaminated by washing with "radiowash" (Atomic Products Corporation, Center Moriches, New York) followed by repeated washings with cold water. All radioactive waste products including the rat's carcass were stored in special containers and periodically shipped to Ottawa. A routine check up of the radioactive room, glassware and instruments was regularly carried out to detect any contamination.

Experiment No. 1. pH of Intestinal Segments Washed with Normal Saline.

The rats were prepared as previously described: 0.5 ml N. saline was injected into the ligated segments. At the end of 30 minutes the segment was resected and the contents of the intestinal lumen collected in a small vessel. The pH was determined by using a glass electrode and a Beckman pH meter. Thirty rats were studied for each segment.

Experiment No. II. pH of Intestinal Segments Washed with Buffer Solution.

In this experiment an attempt was made to modify the pH of

the intestinal segments by using a buffering solution. The buffer "Trisma", hydroxymethylaminomethane, from Sigma Chemicals was used. A series of varying concentrations of the buffer was tried. One with 0.4 molarity, ionic strength of 0.5 and pH of 7.85 at 25°C, was selected for this experiment. Higher concentrations of buffer produced distension of the segments and lower concentrations produced no significant effect on the pH of lumen contents. 0.5 ml of the buffering solution was introduced into the intestinal segments and the pH determined at the end of 30 minutes as described in the previous experiment. Five animals were used for each segment.

Experiment No. III. Absorption of Sr^{89} in Isotonic Saline (a).

In this group, the radioactive Sr^{89} was injected into the lumen of the gastrointestinal segments at a dose of 2 microcuries, (7.2×10^{-5} microgram), dissolved in 0.5 ml of 0.9% sodium chloride solution. At the end of 30 minutes, the animals were sacrificed and the segments removed and radioactivity measured as described earlier. Ten rats were studied for each segment.

Experiment No. IV. Absorption of Sr^{89} in Isotonic Saline (b)

This study was essentially same as the experiment No. III, except that instead of 2 microcurie, 10 microcurie dose was used. Ten animals were used for each segment.

Experiment No. V. Absorption of Sr^{89} at "Controlled" pH.

This study was also similar to the experiment No. III, except that instead of normal saline, "Trisma" (hydroxymethylaminomethane) buffer, 0.4 molarity, 7.85 pH at 25°C and an ionic strength of 0.5 was used. Two microcuries Sr^{89} in 0.5 ml buffer were injected as before. Animals were sacrificed at the end of 30 minutes activity counted as described earlier. Fifteen rats were studied for each segment.

Experiment No. VI. Absorption of Sr^{89} in the Presence of Excess Carrier $SrCl_2$ (isotonic saline).

In this experiment, in addition to 2 microcuries of Sr^{89}, 120 micrograms of inert strontium chloride was added to each of 0.5 ml solution in isotonic saline. This represents an increase of Sr ion in concentration. The study was similar in all other aspects to that of experiment No. III. Twelve rats were used for each segment.

Experiment No. VII. Absorption of Sr^{89} in the Presence of Carrier $SrCl_2$ (controlled pH).

This was the same as experiment No. V, except that 120 micrograms of inert $SrCl_2$ H_20 as carrier was added to each 0.5 ml of "Trisma" buffer solution. Twelve rats were studied for each segment.

Experiment No VIII.

This was a repetition of the experiment No VI to determine the correlation and reproducibility of the technique used in this investigation. Twelve rats were studied for each segment.

CHAPTER VI

RESULTS:

The results of this investigation are in Tables II to XIV. The standard deviation of the mean value of each segment was calculated using the formule:

$$S = \sqrt{\frac{nX^2 - \frac{(n\,X)^2}{N}}{(N-1)}}$$

tsm was determined using student "t". (Gossett W.S. : Biometrika 6, 1908). The significance of difference in the mean values between segments was statistically analysed using "Ducan's Multiple Range Test" for multiple comparisons. (Freund, Livermore and Miller, 1960). The details of calculations and statistical analysis are given in the Appendix.

Experiment No. 1. pH of Intestinal Segments Washed with Isotonic Saline.

The results of this study is given in Table II. From the table it can be noted that the mean pH value in the stomach was 2.35 ($\pm$ 0.89). The duodenal segment was alkaline, 7.75 ($\pm$ 0.37),

TABLE II

pH STUDIES IN RATS STOMACH AND SMALL INTESTINE

I (Stomach)	II (Duodenum)	III	IV	V	VI	VII	VIII	IX	X
1.5	7.8	6.95	7.0	7.05	7.25	7.75	8.2	8.55	8.67
1.6	8.0	6.75	7.65	7.45	7.6	8.0	8.65	8.7	8.35
1.88	7.45	6.65	7.75	7.3	8.15	7.2	8.6	8.81	8.69
1.55	7.85	6.75	7.15	6.75	8.0	8.4	8.3	8.95	8.39
1.45	8.6	6.75	7.85	7.25	7.95	7.95	8.52	8.7	8.75
1.95	7.8	6.65	7.55	7.55	8.1	7.3	8.1	8.42	8.59
1.6	7.75	6.55	6.65	8.05	8.3	7.35	8.15	8.6	8.5
1.5	7.95	6.5	7.7	7.4	7.95	7.65	8.5	8.4	8.8
1.55	7.6	6.65	7.55	7.5	7.9	7.5	7.95	8.43	8.72
1.55	7.6	6.62	7.85	7.75	7.8	8.15	8.8	8.5	8.66
3.7	7.3	7.9	6.95	7.35	6.55	7.8	7.55	8.58	8.72
3.8	7.72	7.01	7.42	7.7	7.0	7.15	8.35	8.4	8.8
3.52	7.87	6.9	7.23	6.85	7.42	7.56	7.23	8.52	8.65
1.72	7.68	7.2	6.86	6.95	7.05	8.27	7.65	8.19	8.9
3.4	7.12	6.78	7.32	6.5	7.55	8.2	7.49	8.6	8.65

(Continued)

TABLE II (continuation)

I (Stomach)	II (Duodenum)	III	IV	V	VI	VII	VIII	IX	X
3.58	8.4	6.8	6.9	7.45	7.08	8.5	8.18	8.9	8.71
4.1	7.8	6.72	6.5	6.7	7.83	7.75	7.2	8.57	8.9
1.49	7.82	6.7	6.95	7.0	7.44	7.65	8.02	8.7	8.82
1.57	7.13	7.05	6.49	7.6	7.45	7.8	8.3	7.9	9.03
2.3	7.69	6.75	6.75	6.4	7.91	8.21	7.65	8.38	9.1
2.0	7.8	7.32	6.9	6.3	8.12	8.21	8.32	8.4	8.9
2.4	2.45	7.0	7.1	7.65	7.70	8.2	8.39	8.62	9.08
3,3	7.83	7.52	6.9	6.65	7.52	8.25	7.7	8.7	9.1
1.8	7.08	6.9	7.39	7.1	7.92	8.7	8.0	8.52	8.8
1.5	8.52	6.52	7.0	6.63	8.35	8.9	7.9	8.61	8.85
1.7	7.5	7.03	7.18	6.82	7.08	7.52	8.27	8.52	8.84
2.45	7.44	7.0	6.83	7.32	7.25	8.23	8.13	8.85	9.02
3.70	7.5	6.9	7.25	6.7	7.81	7.95	8.6	8.79	9.0
3.1	7.79	6.55	7.19	7.21	7.85	7.7	8.5	8.6	8.85
3.22	7.75	6.4	6.7	7.55	7.1	7.12	8.05	8.4	
*2.35	7.75	6.85	7.18	7.15	7.63	7.89	8.1	8.56	8.79
**(±0.89)	(±0.37)	(±0.31)	(±0.38)	(±0.44)	(±0.44)	(±0.56)	(±0.41)	(±0.21)	(±0.56)
***0.113	0.046	0.039	0.048	0.055	0.055	0.071	0.052	0.027	0.071

* Mean pH value
** Standard deviation
*** tsm

in the IIIrd segment it was just on the acid side, 6.85 (± 0.31) and thereafter in the subsequent segments the pH was progressively more alkaline with the terminal segment having a pH value of 8.79 (± 0.56).

The differences in mean pH between the segments are statistically significant.

Experiment No. II. pH of Intestinal Segments Washed with Buffer Solution.

The results of this study on the influence of buffering agent "Trisma" on the pH of rat's gastrointestinal tract is given in Table III. The pH of the stomach was greatly increased but the mean pH value reached was slightly acid. The pH of all others segments varied slightly within the range 7.45 to 8.0, indicating that the lumen was fairly adequately buffered. However, a slight but distinctly rising gradient in mean pH value can be seen progressing towards the distal segment of intestine.

Experiment No. III. Absorption of Sr^{89} in Isotonic Saline (a)

The results of this study are given in Table IV. Maximum absorption took place from the proximal one-third of the small intestine. There was an appreciable amount of absorption in

the stomach. In the distal 3rd of the small bowel, the absorption was about one half of that in the proximal part. The middle third of the small intestine varied between these two extremes.

There is no statistically significant differences between segments II, III and IV; segments V, VII, VIII and IX. The segment VI is not similar to either of the two former groups. Segments I, IX and X do not have significant difference in the absorption.

Table V shows the percentage of frequency distribution of Sr^{89} absorption from different segments in classes of 5%. It can be noted that a higher percentage absorption occurs with greater frequency in the proximal one-third of the small bowel.

Experiment No. IV. Absorption of Sr^{89} in Isotonic Saline (b).

The results of administering 10 microcuries of Sr^{89} is given in Table VI. The features are similar to the previous study using 2 microcuries. Statistical analysis has revealed no significant difference among the segments IV to X in their absorption of Sr^{89}. The segment II and III are significantly different from the other segments as well as between themselves. Segments I, VII and IX are similar in their absorption capacity. The Table VII also demonstrates that in segments II and III there

TABLE III

pH READINGS AT THE END OF 30 MINUTES WITH "TRISMA" BUFFER 0.5 ML; 0.4 MOLARITY, 0.5 IONIC STRENGTH, pH 7.85 AT 25°C.

I (Stomach)	II (Duodenum)	III	IV	V	VI	VII	VIII	IX	X
7.4	7.35	7.2	7.4	7.3	7.5	7.65	7.7	8.0	7.9
6.6	7.3	7.2	7.3	7.4	7.45	7.7	7.8	8.0	8.1
4.3	7.6	7.4	7.4	7.05	7.45	7.65	7.75	7.9	8.0
6.5	7.45	7.2	7.25	7.25	7.65	7.55	7.9	8.0	7.9
4.95	7.3	7.15	7.3	7.2	7.55	7.8	7.85	8.1	8.05
* 5.95	7.45	7.23	7.33	7.24	7.52	7.67	7.8	8.0	7.99
** (± 1.44)	(± 0.3)	(± 0.1)	(±0.07)	(± 0.13)	(±0.109)	(± 0.087)	(±0.077)	(±0.07)	(±0.089)

*Standard deviation

** Mean of pH value

TABLE IV

RADIOACTIVE STRONTIUM 89. ABSORPTION FROM LIGATED SEGMENTS OF RATS' GASTRO-INTESTINAL TRACT

(2 microcurie of Sr^{89} in 0.5 ml of 0.9% normal saline injected. Activity measured after 30 min.)

I (Stomach)	II (Duodenum)	III	IV	V	VI	VII	VIII	IX	X
8.6	26.6	24.4	16.3	15.2	33.0	19.0	13.9	15.5	19.2
4.4	38.1	35.3	37.3	19.3	20.7	23.9	12.0	16.9	18.4
21.5	30.6	16.5	24.9	10.5	12.6	36.3	24.3	13.5	7.5
5.1	35.6	17.8	35.4	19.1	19.1	39.9	12.6	9.1	14.9
9.1	41.3	53.8	30.8	30.4	24.3	17.1	32.8	14.3	28.1
4.9	27.4	47.4	38.8	21.7	32.2	35.2	14.0	13.1	8.6
16.8	22.4	32.4	28.0	28.3	34.1	21.8	12.7	13.1	25.7
11.7	14.4	36.3	34.5	31.0	38.4	18.4	27.5	22.2	12.7
9.3	31.9	40.7	48.3	13.7	33.0	13.7	-	27.3	16.5
-	41.8	-	26.5	-	-	-	-	-	-
* 10.1%	31.01%	33.84%	3208%	21.02%	27.49%	25.03%	18.71%	16.0%	16.8%
** (± 4.47)	(± 8.71)	(±12.64)	(±8.83)	(± 7.41)	(±8.60)	(±9.53)	(±8.18)	(± 5.47)	(±6.92)

* Mean per cent of absorption

** Standard deviation

TABLE V

FREQUENCY DISTRIBUTION OF Sr^{89} ABSORPTION FROM RATS' GASTRO INTESTINAL TRACT (2 Microcurie in 0.5 ml Normal Saline for 30 minutes)

ABSORPTION IN CLASSES OF 5%	I	II	III	IV	V	VI	VII	VIII	IX	X
57.5										
52.5			11.11							
47.5			11.11	10.00						
42.5		20.00	11.11							
37.5		20.00	22.22	30.00		11.11	33.33			
32.5		20.00	11.11	20.00	22.22	44.44		12.5		
27.5		20.00		20.00	11.11			12.5	11.11	22.22
22.5	11.11	10.00	11.11	10.00	11.11	22.22	22.22	12.5	11.11	
17.5	11.11		22.22	10.00	33.33	11.11	33.33		22.22	33.33
12.5	11.11	10.00			22.22	11.11	11.11	62.5	44.44	22.22
7.5	44.44								11.11	22.22
2.5	22.22									

NUMBER OF SEGMENTS

TABLE VI

RADIOACTIVE STRONTIUM 89. 10 MICROCURIE IN 0.5 ML of 0.9% SALINE INJECTED INTO LIGATED SEGMENTS OF RATS' GASTRO-INTESTINAL TRACT.

(Radioactivity measured after 30 minutes)

I (Stomach)	II (Duodenum)	III	IV	V	VI	VII	VIII	IX	X
10.00	20.6	31.1	21.0	27.2	41.4	22.2	19.4	22.1	22.6
20.0	36.6	36.0	16.4	31.0	20.4	16.2	20.1	12.2	32.8
7.8	19.5	22.8	17.4	30.0	24.5	23.2	20.4	19.3	10.1
17.7	35.7	29.7	20.1	20.0	23.0	24.0	20.1	21.3	17.2
7.1	34.3	39.8	25.1	27.1	27.9	20.4	17.3	24.0	29.1
27.8	37.0	26.0	21.9	32.9	33.2	19.3	11.4	22.5	20.1
5.5	37.5	40.4	27.8	25.5	12.9	14.6	19.4	28.7	28.9
15.3	26.3	54.3	16.4	14.5	28.9	11.4	30.9	16.0	17.7
15.3	21.9	46.0	21.4	10.1	24.4	21.6	38.9	17.1	21.6
-	-	-	21.9	-	-	16.5	-	-	-
* 14.05%	29.93%	36.23%	20.94%	24.24%	26.28%	18.94%	21.98%	20.35%	22.2%
**(±7.21)	(± 7.68)	(±10.00)	(±3.60)	(±7.74)	(± 8.00)	(± 4.00)	(± 8.062)	(± 4.79)	(± 7.071)

* Mean per cent of absorption

** Standard deviation

TABLE VII

FREQUENCY DISTRIBUTED OF Sr^{89} ABSORPTION FROM RATS GASTRO INTESTINAL TRACT

(10 microcurie in 0.5 ml of normal saline for 30 minutes)

ABSORPTION IN CLASSES OF 5%	I	II	III	IV	V	VI	VII	VIII	IX	X
57.5										
52.5			11.11							
47.5			11.11							
42.5			11.11			11.11				
37.5		44.44	22.22					11.11		
32.5		11.11	11.11		33.33	11.11		11.11		11.11
27.5	11.11	11.11	22.22	20.00	33.33	22.22			11.11	22.22
22.5	11.11	22.22	11.11	50.00	11.11	44.44	50.00	33.33	44.44	33.33
17.5	33.33	11.11		30.00			30.00	33.33	33.33	22.22
12.5	11.11				22.22	11.11	20.00	11.11	11.11	11.11
7.5	33.33									
2.5										

NUMBERS OF SEGMENTS

is a greater frequency of higher percentage of radiostrontium absorption.

Experiment No. V. Absorption of Sr^{89} at "Controlled" pH.

These results are given in Table VIII. A decrease in the absorption of radiostrontium from the 2nd segment is the marked feature. Maximum absorption occurs in the third segment.(31.9%). Statistical analysis shows no significant difference between segments II, IV, VI, VII, VIII, IX and X. The percentage of absorption in segments I, IX and X are also similar. Segment III is significantly different from the other segments. These result indicate as they stand, that by controlling the pH to steady level, in fact absorption is brought to a more "steady" level. The frequency distibution is given in Table IX. Segment III in particular and the proximal part of small intestine in general continue to show maximum absorption.

Experiment No. VI. Absorption of Sr^{89} in the Presence of Excess Carrier $SrCl_2$ in Isotonic Saline (Table X).

Addition of 120 micrograms of inactive strontium chloride has appreciably changed the mean percentage of absorption. But the pattern of distribution between segments is the same as in the previous three experiments. The second segment has the maximum

TABLE VIII

RADIOACTIVE STRONTIUM 89. ABSORPTION FROM RATS' GASTRO-INTESTINAL TRACT. Sr^{89} MICROCURIE IN 0.5 ML. "TRISMA" BUFFER, 0.4 MOLARITY 0.5 IONIC STRENGTH pH 7.85 AT 25°

(Radioactivity measured after 30 minutes)

I (Stomach)	II (Duodenum)	III	IV	V	VI	VII	VIII	IX	X
20.3	10.8	58.0	13.9	13.8	16.0	34.9	19.5	15.0	27.0
9.2	18.6	28.9	14.0	14.4	18.0	23.3	21.7	29.0	7.1
9.1	14.9	32.2	38.5	27.1	15.3	29.2	23.3	15.7	16.7
9.1	22.4	49.2	19.9	28.0	25.8	21.7	14.2	10.1	17.5
11.4	10.8	43.4	12.0	30.3	26.5	29.1	16.4	24.6	14.7
23.1	20.3	17.4	51.2	26.0	31.9	25.3	14.4	27.2	15.9
10.9	16.9	19.8	18.5	41.8	13.8	39.1	26.8	12.3	16.7
7.5	26.4	22.0	17.8	10.3	19.4	14.0	30.8	21.3	28.1
7.5	31.9	37.2	46.6	30.4	23.1	10.4	30.6	5.5	15.6
-	31.8	22.0	19.1	23.9	11.8	24.7	20.2	9.3	15.3
-	19.8	25.4	14.7	36.8	12.0	16.9	19.4	16.1	20.7
-	20.1	27.4	16.2	33.8	26.4	14.3	22.0	27.9	17.2
-	33.1	-	17.9	28.2	20.2	-	26.5	15.8	-
-	43.1	-	-	34.5	-	-	27.4	29.5	-
-	-	-	-	32.2	-	-	31.0	-	-
* 12.01%	22.9%	31.9%	23.1%	27.4%	20.11%	23.5%	22.9%	18.5%	17.7%
**(±5.65)	(± 9.32)	(± 12.64)	(±13.15)	(±8.77)	(±6.32)	(±8.66)	(±5.65)	(±8.00)	(± 5.47)

* Mean per cent of absorption

** Standard deviation

TABLE IX

FREQUENCY DISTRIBUTION OF Sr^{89} ABSORPTION FROM RATS' GASTRO INTESTINAL TRACT

(2 microcurie in 0.5 ml of "Trisma" buffer for 30 minutes)

ABSORPTION IN CLASSES OF 5%	I (Stomach)	II (Duodenum)	III	IV	V	VI	VII	VIII	IX	X
67.5										
62.5										
57.5			8.33							
52.5				7.53						
47.5			8.33	7.53						
42.5		7.14	8.33		6.66					
37.5			8.33	7.53	6.66		8.33			
32.5		21.42	8.33		26.66	7.53	8.33	20.00		
27.5		7.14	25.00		33.33	23.76	25.00	20.00	28.57	16.67
22.5	22.22	21.42	16.67		6.66	13.38	25.00	26.66	14.28	8.33
17.5		21.42	16.67	46.15		30.76	8.33	20.00	21.42	58.33
12.5	22.22	21.42		30.76	20.00	23.76	25.00	13.33	21.42	8.33
7.5	55.55								14.28	8.33
2.5										

NUMBER OF SEGMENTS

TABLE X

RADIOACTIVE STRONTIUM. ABSORPTION FROM LIGATED SEGMENTS OF RATS' INTESTINE. STRONTIUM 89, 2 MICROCURIE IN 0.5 ML NORMAL SALINE WITH 120 MICROGRAMS OF $SrCl_2$ AS CARRIER INJECTED

(Activity measured at the end of 30 minutes)

I (Stomach)	II (Duodenum)	III	IV	V	VI	VII	VIII	IX	X
16.9	38.8	25.1	26.5	19.0	9.2	8.4	10.5	12.6	11.2
15.6	42.2	19.6	13.8	22.2	12.4	10.8	6.3	12.0	22.1
10.4	53.3	21.2	20.9	20.6	16.5	8.0	6.4	8.4	17.1
6.3	39.4	20.8	18.6	9.1	11.6	5.8	12.0	11.4	12.0
8.5	48.4	19.6	23.8	7.5	9.5	4.8	18.4	15.6	18.0
8.9	49.5	13.6	10.9	12.6	12.7	8.4	12.9	4.5	8.4
4.8	39.3	24.6	11.4	9.3	14.3	11.9	15.7	10.6	14.0
9.0	15.3	16.4	18.2	14.9	15.0	16.3	18.9	7.8	7.3
9.8	44.3	20.8	12.9	8.9	12.2	4.6	12.3	15.5	10.1
15.3	50.2	15.3	12.3	15.1	14.1	18.8	16.1	20.9	5.3
7.5	18.6	20.8	13.7	14.4	22.2	17.2	4.3	13.9	10.2
11.0	41.5	11.6	11.7	12.9	10.4	12.8	5.4	11.6	9.3
*10.4%	40.0%	19.1%	16.2%	13.8%	13.3%	10.6%	11.6%	11.2%	13.1%
**(±3.79)	(±11.78)	(±4.12)	(±5.26)	(±4.81)	(±3.54)	(±4.84)	(±5.09)	(±7.74)	(±4.87)
***0.489	1.52	0.531	0.678	0.621	0.457	0.624	0.657	0.998	0.628

*Mean per cent of absorption

**Standard deviation

***tsm

TABLE XI

FREQUENCY DISTRIBUTION OF Sr^{89} ABSORPTION FROM THE GASTRO INTESTINAL TRACT OF RATS

(2 microcurie with $SrCl_2$ 120 micrograms as carrier for 30 min.)

ABSORPTION IN CLASSES OF 5%	I (Stomach)	II (Duodenum)	III	IV	V	VI	VII	VIII	IX	X
67.5										
62.5										
57.5										
52.5		16.67								
47.5		16.67								
42.5		25.00								
37.5		25.00								
32.5										
27.5			8.33	8.33						
22.5			41.66	16.67	16.67	8.33			8.33	8.33
17.5	25.00	16.67	33.33	16.67	16.67	8.33	25.00	33.33	16.67	16.67
12.5	16.67		16.67	58.33	33.33	66.67	25.00	33.33	50.00	41.66
7.5	50.00				33.33	16.67	33.33	25.00	16.67	33.33
2.5	8.33						16.67	8.33	8.33	
	NUMBER OF SEGMENTS									

rate of absorption, 40.0% (± 11.78); while the third segment follows with 19.1% (±4.12). The difference in absorption between segments IV, V, VI, VII, VIII, IX and X are not statistically significant. So also segments I. V to X are similar in their rate of absorption. Segments II and III are different from each other as well as from the rest.

The frequency distribution of absorption (percentage) is given in Table XI. In this, the wide scattering noted in the previous experiments had been considerably narrowed down. The 2nd segment shows the greater incidence of higher percentage of absorption.

Experiment No. VII. Absorption of Sr^{89} in the Presence of Excess Carrier SrCl2 in "Controlled" pH. (Table XII.)

The maximum rate of absorption is again noted in the duodenal segment; however, this is considerably less than the rate in the experiment No. VI. Statistical analysis showed that difference between segments II, III and IV are not significant. Segments V, VII, VIII, IX and X are also similar. Segments VII, VIII, IX and I showed no significant difference. Table XIII demonstrates the frequency distribution, which is similar to the previous experiment.

Experiment No. VIII.

The result of this study is given in Table XIV. The correlation coefficient with the results of experiment No. VI was calculated using the formula:

$$r = \frac{S(xy)}{\sqrt{S(x^2).\ S(y^2)}} \qquad \text{(Fisher, 1958)}$$

The two experiment correlate very well; in almost all the segments the value is above 0.9 except in segment V and X where the value is 0.873 and 0.846 respectively.

TABLE XII

STRONTIUM 89, 2 MICROCURIE WITH 120.05 MICROGRAMS OF $SrCl_2$ AS CARRIER IN 0.5 ML. "TRISMA BUFFER, 0.4 MOLARITY, 0.5 IONIC STRENGTH, pH 7.85 AT 25°C.

(Activity after 30 minutes)

I (Stomach)	II (Duodenum)	III	IV	V	VI	VII	VIII	IX	X
7.1	34.5	25.5	14.6	18.3	20.3	5.6	14.6	10.1	16.2
8.0	21.3	14.2	14.3	21.0	25.0	7.7	6.1	13.6	23.0
1.7	22.2	38.0	20.8	22.7	22.6	19.9	10.0	15.6	20.7
10.9	29.4	18.8	35.0	16.8	20.8	18.0	15.8	18.5	8.3
2.4	21.6	30.8	13.1	13.0	11.6	15.6	20.0	9.8	20.2
1.7	16.1	15.1	13.5	11.0	24.1	5.1	13.1	10.2	12.4
22.4	25.2	14.1	31.3	18.2	21.5	16.0	15.7	7.3	7.5
8.4	31.7	26.0	24.2	16.3	16.9	12.8	10.9	10.2	15.4
2.4	26.6	13.8	29.7	16.1	21.7	16.6	8.9	18.0	9.5
10.4	25.9	14.4	17.2	15.4	14.6	8.3	18.2	16.8	12.7
9.6	21.1	17.1	30.2	11.7	26.3	8.4	14.5	13.7	10.5
*8.36%	25.54%	21.08%	22.12%	16.58%	20.46%	12.00%	12.90%	12.87%	14.21%
**(±6.164)	(±5.291)	(±7.874)	(±7.810)	(±3.492)	(±4.266)	(±5.099)	(±4.458)	(±3.674)	(±5.069)

*Standard deviation

**Mean per cent of absorption

TABLE XIII

FREQUENCY DISTRIBUTION OF Sr^{89} ABSORPTION FROM RASTS' GASTRO INTESTINAL TRACT

(2 microcurie Sr^{89} with 120 micrograms of $SrCl_2$ as a carrier in 0.5 ml of "Trisma" buffer for 30 minutes)

ABSORPTION IN CLASSES 5%	I (Stomach)	II (Duodenum)	III	IV	V	VI	VII	VIII	IX	X
52.5										
47.5										
42.5										
37.5			9.09							
32.5		18.18	9.09	27.27						
27.5		36.36	18.18	9.09		9.09				
22.5	9.09	36.36		18.18	18.18	63.63				27.27
17.5		9.09	27.27	9.09	54.54	9.09	45.45	36.36	36.36	18.18
12.5	18.18		36.36	36.36	27.27	18.18	9.09	45.45	45.45	27.27
7.5	36.36						45.45	18.18	18.18	27.27
2.5	36.36									

NUMBER OF SEGMENTS

TABLE XIV

RADIOACTIVE STRONTIUM 89 ABSORPTION FROM LIGATED SEGMENTS OF RATS' GASTRO-INTESTINAL TRACT

(Sr^{89}, 2 microcurie in 0.5 ml of normal saline with 120 micrograms of $SrCl_2$ as carrier for 30 minutes)

I (Stomach)	II (Duodenum)	III	IV	V	VI	VII	VIII	IX	X
10.8	44.9	19.6	30.1	11.3	19.2	13.1	12.1	7.9	15.6
8.0	54.5	18.9	23.9	9.2	18.0	7.9	13.1	13.8	6.3
11.4	40.5	28.2	20.1	18.0	23.9	11.1	17.7	15.6	13.7
8.5	48.8	12.9	18.3	14.6	22.0	8.9	8.5	9.1	11.9
13.3	52.2	15.8	16.3	14.6	17.7	13.1	18.0	12.6	10.5
5.8	29.4	20.2	17.0	11.3	13.9	4.6	16.5	17.4	14.6
11.2	58.2	27.7	14.7	21.2	11.8	11.0	8.6	6.2	8.6
10.4	41.0	21.9	20.4	8.0	10.5	10.2	15.0	13.4	9.3
15.0	22.6	17.5	15.6	17.8	12.5	7.3	14.1	15.1	20.4
11.2	22.0	25.2	14.2	12.6	11.4	14.9	12.2	13.6	6.4
7.5	20.0	20.1	17.5	11.6	11.5	11.0	15.2	20.2	16.8
8.4	49.0	15.9	10.3	15.9	9.2	13.6	11.7	11.0	18.9
* 10.1%	40.4%	20.3%	18.2%	13.8%	14.9%	10.5%	13.5%	12.1%	12.7%
**(±2.58)	(±13.67)	(±4.74)	(±5.09)	(±3.87)	(±4.78)	(±2.96)	(±3.13)	(±3.33)	(±4.69)
***0.332	1.76	0.611	0.656	0.499	0.616	0.381	0.403	0.430	0.605
****0.931	0.939	0.99	0.978	0.873	0.913	0.933	0.902	0.907	0.846

* Mean per cent of absorption

** Standard deviation

*** tsm

**** correlation coefficient with Table X

CHAPTER VII

DISCUSSION

Methods.

Ligated segments of rat's intestine have been used previously for in vivo study of absorption in this laboratory by Dukay and Makhani (1961-1962). This in vivo technique has several advantages over the methods used by other investigators. It is also admirably suited to study the regional absorption pattern.

The present study is basically different from those of in vitro experiments. In vitro study may give valuable information about the transport of solutes and solvents across the viable, semipermeable membrane formed by the intestinal mucosa. The blood and lymphatic supply to the intestinal walls, mucosal secretions and neuronal and hormonal control of intestinal mucosa and its secretions do not participate.

There is evidence that strontium is partly excreted into the intestinal lumen (Wasserman 1960). Hence when the total length of the small intestine is used as in perfusion experiments, the result obtained is the difference between the amount absorbed and that excreted into the intestinal lumen.

By utilizing 5 cms segments and only one such segment in each animal, this excretion factor is localized. However, no previous attempt has been made to study it.

The rate of absorption is related to the rapidity of propulsion of food particles and flow rate of soluble material through the intestinal lumen. The use of ligated segments of about 5 cms. eliminates this factor. In perfusion experiments a variable factor of rate of flow through the lumen must be considered. Further, the perfusate mixed with intestinal secretions being a non-Newtonian fluid, particulate constituents are not uniformly distributed in the stream of the fluid. If solutions are used throughout the experiment this may not apply.

The period of study being limited to 30 minutes only, any physio-pathological changes that could have occured due to intestinal obstruction are probably reduced. Throughout the experiment the animals are under the influence of anaesthesia; any variation which may occur in absorption at the time of recovery from anaesthetics is eliminated.

The high degree of correlation noted between the experiments VI and VIII substantiate the relability of this in vivo technique.

Effect of pH on Sr^{89} Absorption.

Dukay (1962) using ligated intestinal segments demonstrated that the maximum absorption of Ca^{45} (about 65%) from the duodenal segment occured when it was administered in a buffer solution of neutral pH. At pHs 4, 5, 6 and 8 absorption was considerably decreased. Towards the distal segment where pH was alkaline, the Ca^{45} absorption (from unbuffered solution) was less than 10% in 30 minutes. Maximum absorption of calcium coincided with a near neutral pH in the intestinal tract. It seemed possible that by controlling the pH of intestinal lumen with a suitable buffering agent, the absorption of alkaline earth elements could also be "controlled".

As a preliminary step, the normal pH values of the gastrointestinal tract of rat under the experimental conditions was determined using isotonic saline. The stomach was highly acid. The duodenal segment had an alkaline pH; however the immediately distal segment was slightly acid. This remarkable difference in the two adjoining segments may be due to the fact, that when the pyloric end of the duodenum was ligated the normal flow of acid secretions of the

stomach was interrupted and consequently the bile, pancreatic secretions and secretions of Brunner's glands accumulated producing an alkaline pH. In the third segment, as there is no accumulation of alkaline secretions other than those of intestinal mucosal glands the pH continued to be slightly acid. It could be presumed that normally the duodenal contents have a neutral or slightly acid pH, but under these experimental conditions it has an alkaline pH. In the rest of the segments the alkalinity increases directly proportional to the distance from the stomach; thus the terminal ileal segment has the highest pH. These values are similar to those of the human beings where the pH varies from 6.3 to 9 (Thomas, 1961).

As a buffering agent "Trisma" was chosen because it has an optimum range of buffering action closely resembling the physiological values in the intestine, 7.0 to 9.0. Secondly, unlike many other buffers "Trisma" does not form an insoluble salt with alkaline earth elements. 0.4 M "Trisma" was used after experimenting with both weaker and stronger solutions (ie., of greater buffer capacity). A sample result using 0.25, 0.35 and 0.5 molarity is given in table XV.

pH READINGS AFTER USE OF DIFFERENT MOLARITIES OF "TRISMA" BUFFER, 7.85 pH at 25°C.

TABLE XV

0.25 Molarity.

Segment	II	III	IV
	7.4	6.85	6.75
	7.85	6.65	7.15
	7.55	6.55	7.25
	7.75	7.25	6.9
	7.85	7.0	7.05
	7.68	6.85	7.02

0.35 Molarity.

Segment	II	III	IX
	7.5	7.25	7.9
	7.65	7.2	8.25
	7.85	6.9	8.3
	7.7	7.15	8.05
	7.55	7.0	8.0
	7.65	7.1	8.1

Usage of 0.5 Molarity gave undue distension of the segments.

Segment	II	III
	7.9	7.4
	7.6	7.45
	7.75	7.4
	7.65	7.6
	7.6	7.55
	7.7	7.48

pH STUDIES IN RAT'S GASTROINTESTINAL TRACT

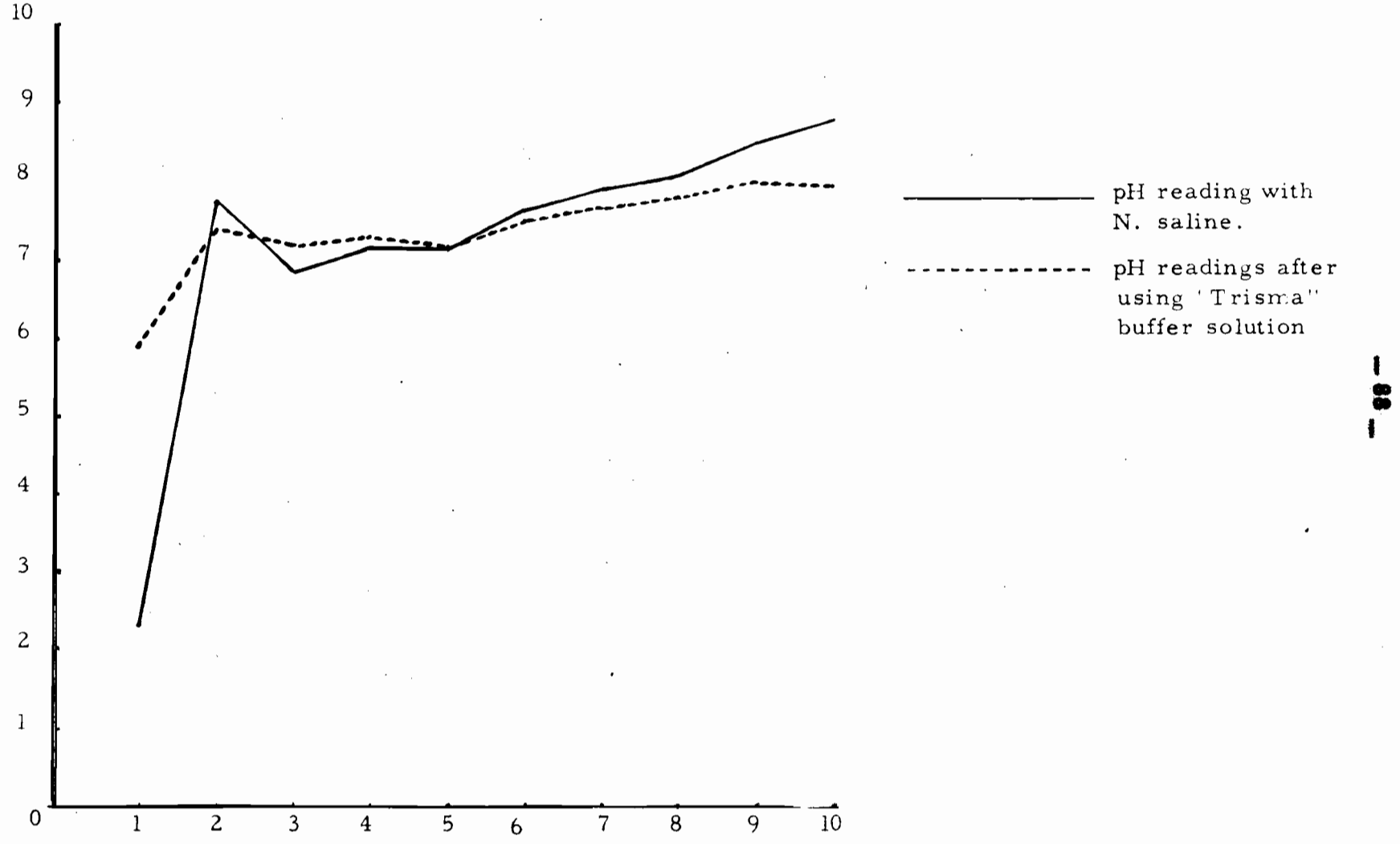

Fig. 2.

Sr^{89} ABSORPTION FROM LIGATED SEGMENTS OF RAT'S GI TRACT USING DIFFERENT AMOUNT OF Sr^{89}, WITH AND WITHOUT BUFFERING SOLUTION

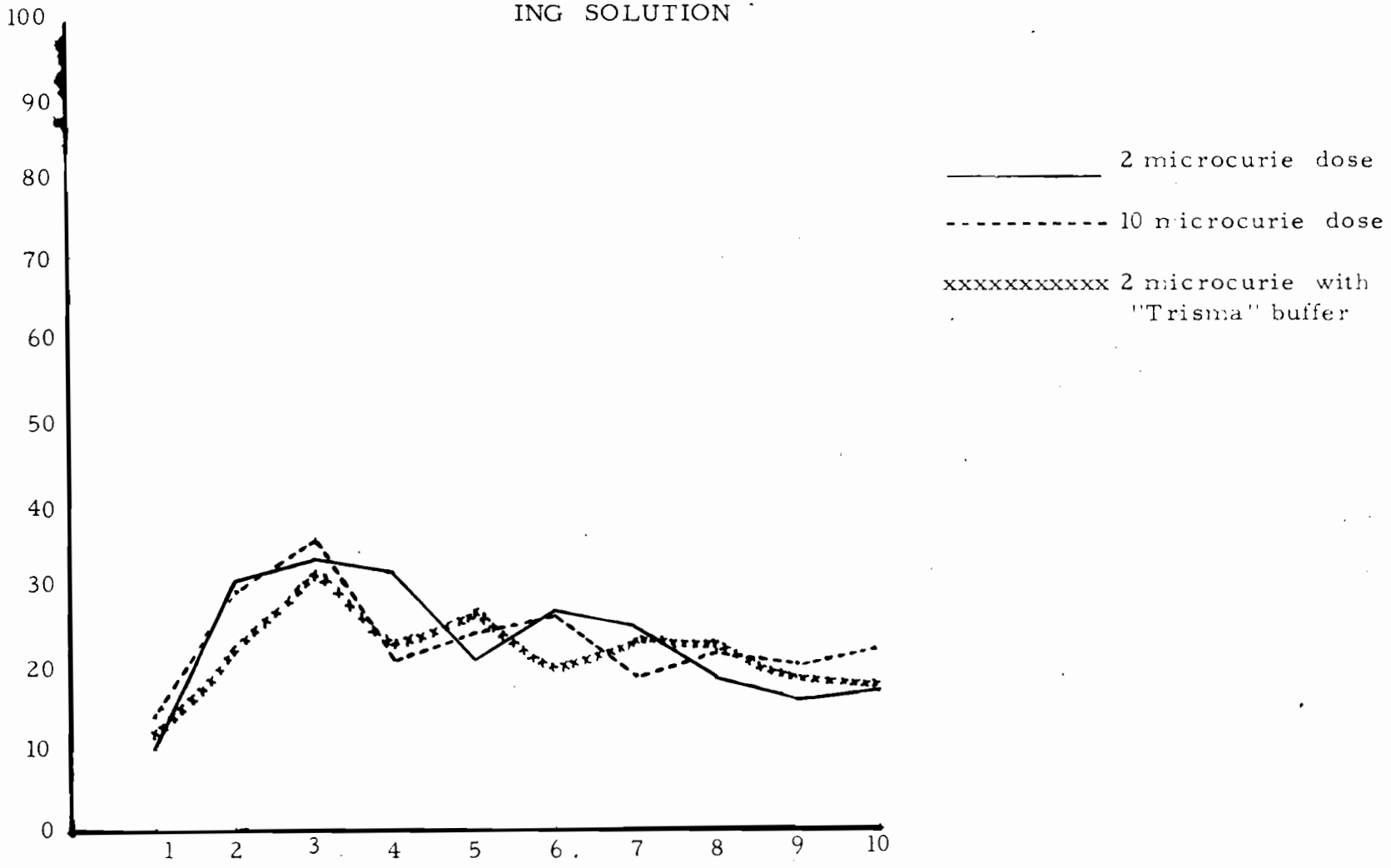

Fig. 3.

Sr^{89} ABSORPTION FROM LIGATED SEGMENTS OF RAT'S GI TRACT

2 microcurie dose with 120.05 microgram of inert $SrCl_2$, with and without "Trisma" buffer for 30 minutes.

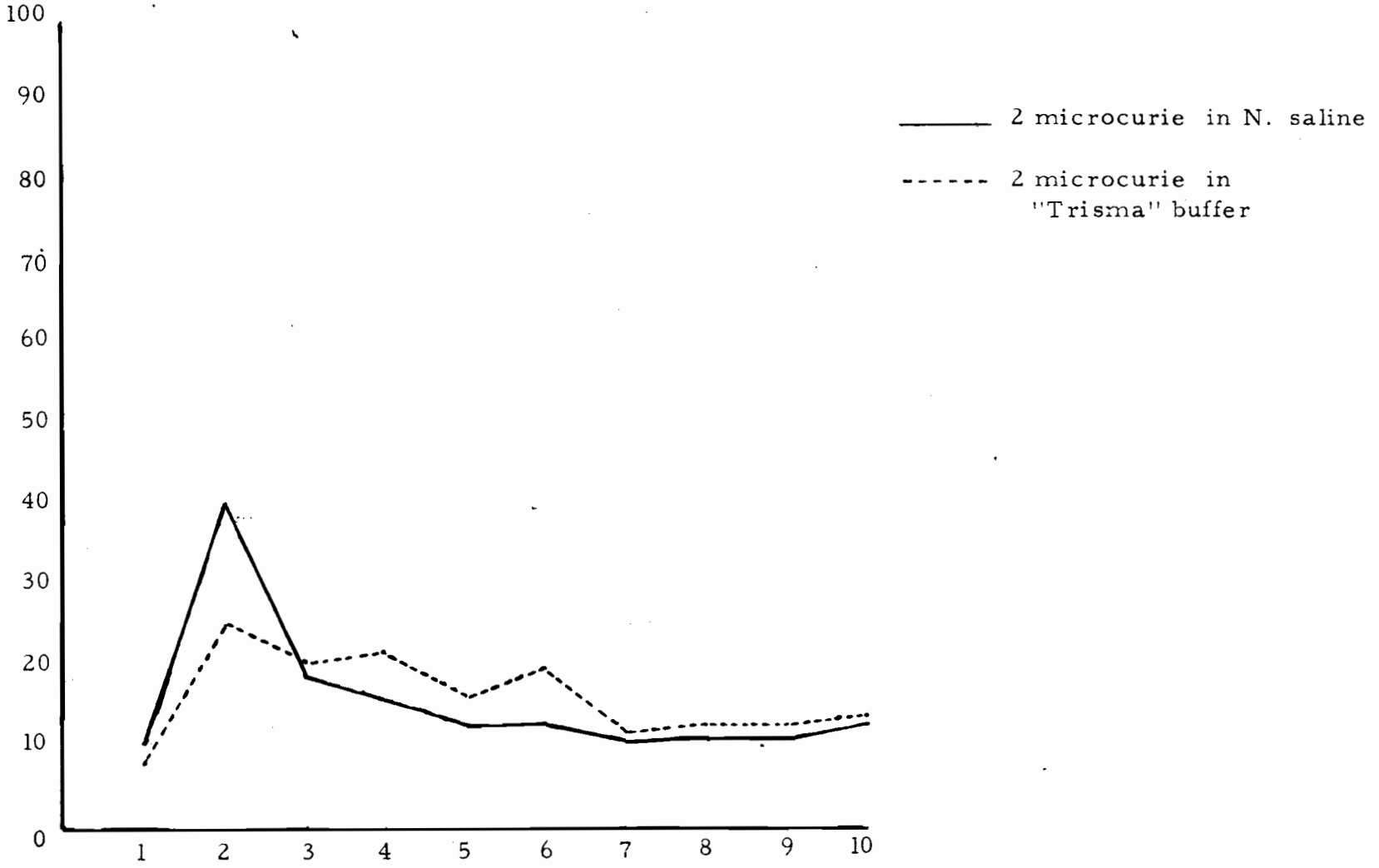

Fig. 4.

DISTRIBUTION OF Sr^{89} ABSORPTION WITH "CONTROLLED" pH

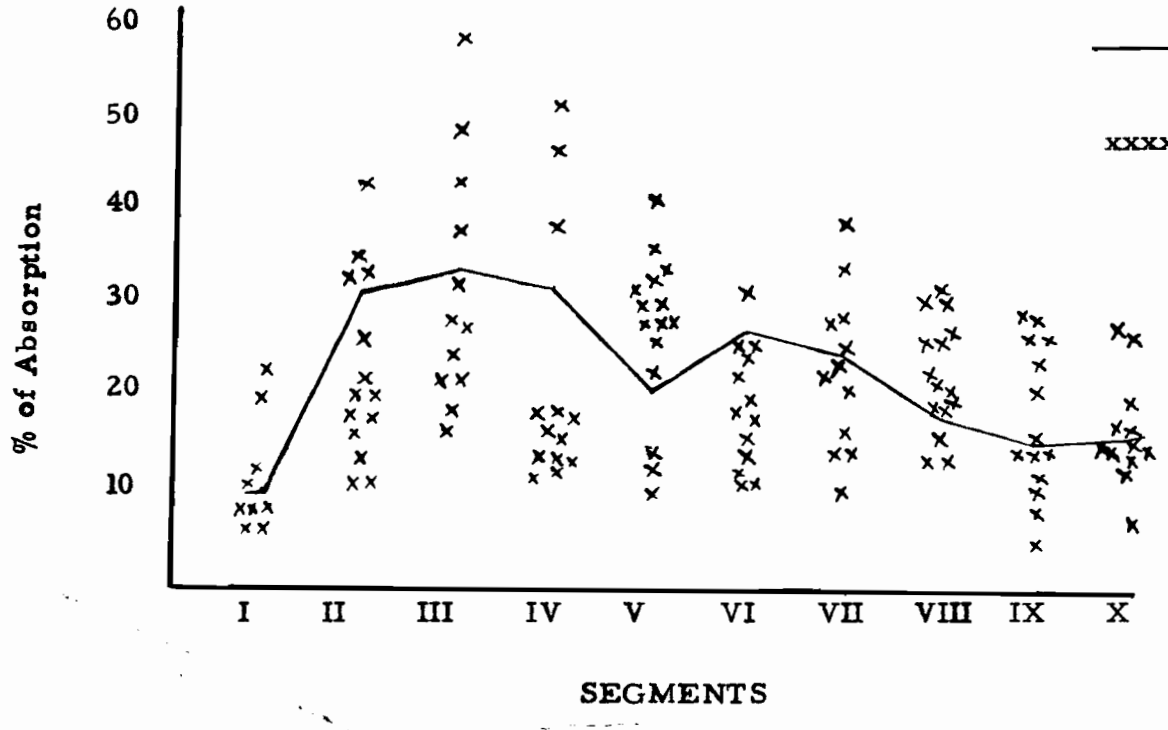

Fig. 5.

DISTRIBUTION OF Sr^{89} ABSORPTION IN ISOTONIC SALINE

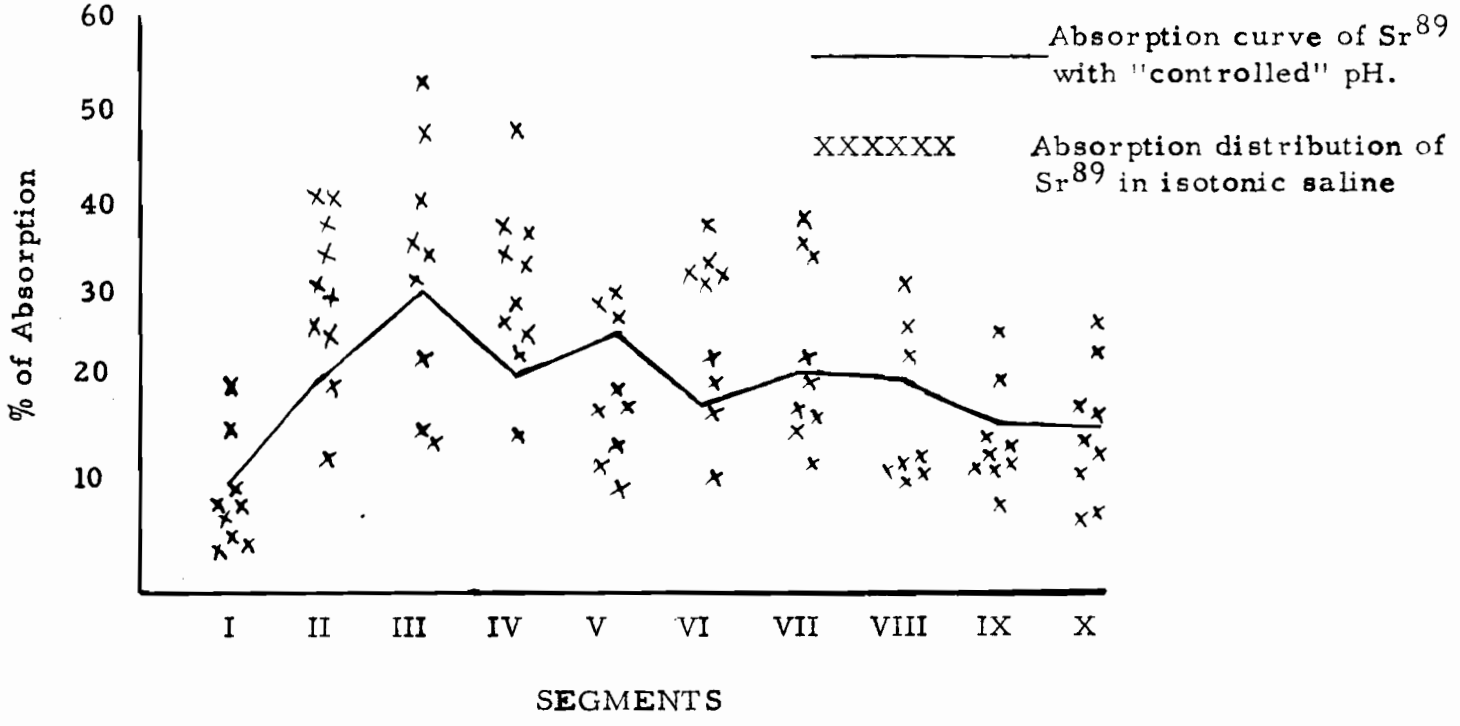

Fig. 6.

0.4M(400mM) "Trisma" is hypertonic compared with the saline (154mM); however no macroscopic damage to the intestinal mucosa was observed. A stronger solution produced distension of the intestinal segment. A weaker solution did not alter the pH to a sufficient degree.

By using "Trisma" the pH of the stomach was raised. In the duodenum the pH was altered to near neutral and in the third segment it was changed to slightly alkaline. Figure 2 shows the amount of control in the pH of intestine produced by "Trisma", in comparison with the value obtained from isotonic saline. Even though the buffering action of the "Trisma" was fairly satisfactory, the pH was not uniform throughout the length of intestine; nor in the same segment. It also was not able to give the same pH as that of the buffer. It is possible that some of the buffering solution might have been rapidly absorbed by the intestinal mucosa, or that the secretions of the mucosa have a high neutralising capacity. A combination of both these processes may occur. If compared in vitro, volume for volume "Trisma" has an excess of buffering capacity for the intestinal secretions. But the continuous process of secretion and absorption by the mucosa has

reduced the capacity of "Trisma" to produce a uniform pH throughout the intestinal tract.

Various investigators have reported on the absorption of radioactive calcium and radioactive strontium after using solutions with pHs ranging from 1 to 12. How far the intestinal mucosa is capable of altering these extremens of pH at the end of 30 minutes is not known. It is conceivable that such a drastic change in pH is likely to produce histopathological reaction in the intestinal mucosa. Further study in this direction is necessary before a definite conclusion could be reached.

In this study a buffering agent was used and the pH maintained at a "controlled" level throughout the period of absorption study. In perfusion experiments by Mraz (1962) and segmental absorption study of Ca^{45} by Dukay (1962), solutions used were not buffered and the pHs of the intestinal contents at the end of the period of absorption study were not determined. Therefore, the relationship of pH on the absorption of Sr^{89} and Ca^{45} cannot be concluded from those studies.

In six series of Sr^{89} absorption studies, two were under controlled pH with "Trisma" buffer. By administering Sr^{89} in buffer at approximately pH 7.55, it would be expected that absorption would decrease in the proximal segments and increase in the distal segments. Figures 3 and 4 show the comparison of mean percenteage of absorption with controlled pH and those of isotonic saline, with and without inactive strontium chloride as carrier. Except in the duodenal segment no appreciable difference can be noted from these graphs. Statistical analysis also did not show any significant difference except in the duodenal segment.

However the influence of the control of absorption by the buffer becomes apparent by a study of the distribution pattern. Figure 5 demonstrates the distribution in accordance with percentage of absorption in controlled pH plotted in comparison with the mean absorption curve of isotonic saline. Here in the proximal segments of small intestine a majority of rats with controlled pH absorb less than the mean absorption with isotonic saline. In segment V this difference is striking. The mean value in isotonic saline

is lower; more animals with controlled pH had a higher rate of absorption. In the distal segments, higher values of absorption have been observed in more animals with controlled pH than those in which isotonic saline preparation has been used.

Figure 6 shows the distribution of Sr^{89} absorption plotted against the mean absorption curve of controlled pH. The phenomena observed represent a reverse picture to that in figure 5. Figures 7 and 8 also show similar distribution to that of figures 5 and 6 respectively, where 120 micrograms of inactive $SrCl_2(6H_20)$ was added to controlled pH as well as in isotonic saline.

These show that "Trisma" buffer by controlling pH was,to a certain extent, able to produce a controlled level of Sr^{89} absorption. Further studies with different pH ranges are necessary before it can be concluded that absorption depends on pH and, as a corollary, that absorption can be controlled at different levels by altering the pH of the intestinal environment.

DISTRIBUTION OF Sr^{89} ABSORPTION WITH "CONTROLLED" pH AND ADDED 120 MICROGRAMS OF INERT $SrCl_2$ AS CARRIER

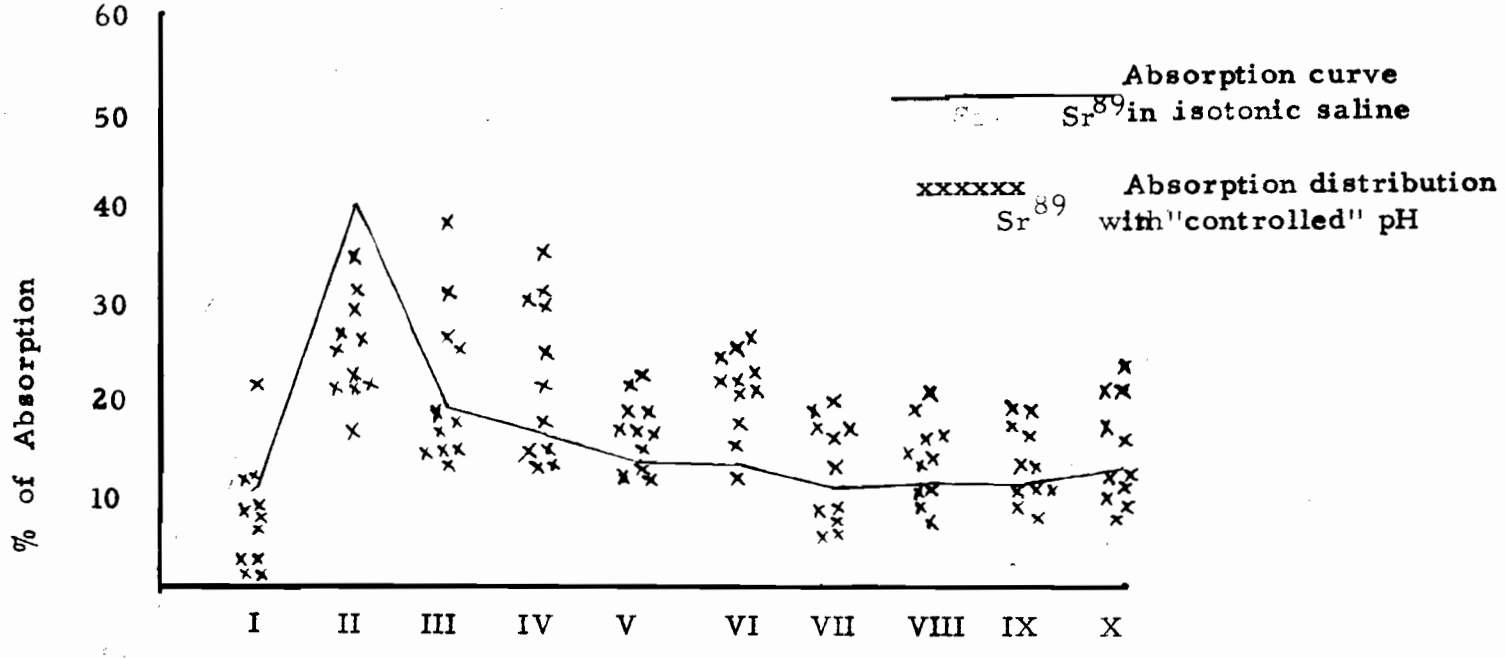

Fig. 7.

DISTRIBUTION OF Sr^{89} ABSORPTION IN ISOTONIC SALINE AND ADDED 120 MICROGRAMS OF INERT $SrCl_2$ AS CARRIER

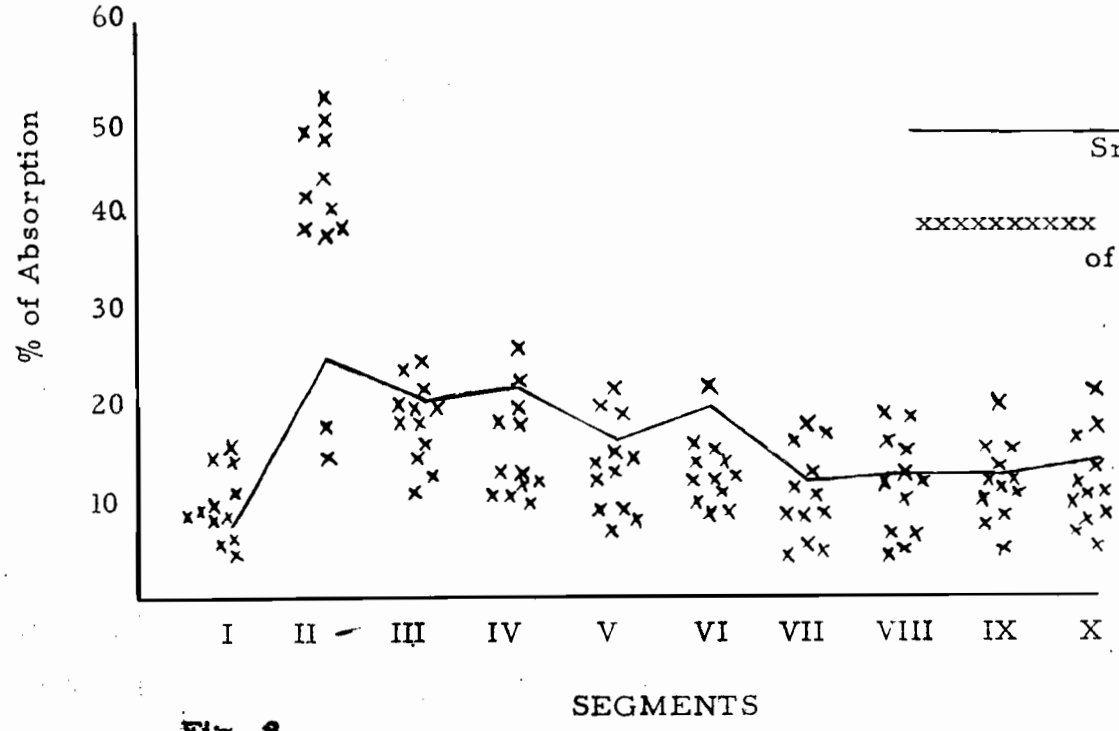

Fig. 8.

Effect of Addition of Inert Strontium Chloride and the Use of a 5 Fold Increase in the Radioactive Sr^{89}

Two microcuries of radioactive strontium represented about 7.2×10^{-5} micrograms of strontium by weight. To this was added 120 micrograms of inactive strontium chloride ($SrCl_2 6H_2O$) which gives about 43 micrograms of Sr^{++}. The addition of the inactive element produced a definite depressing action on the absorption of radioactive strontium.

Figures 9 and 10 show the difference in the mean percentage absorption with and without inactive carrier strontium. The duodenal segment shows a marked increase in the absorption of radioactive strontium after the addition of inactive $SrCl_2 6H_2O$. But all the other segments had considerable reduction in the mean percentage of absorption. Statistical analysis also shows significant reduction in the absorption of radiostrontium from all segments except the IInd. segment. There is an overall reduction in the absorption of Sr^{89} on addition of inert strontium chloride. These results are similar to those obtained by Rosenthal (1960), Smachson (1961) and Mraz (1962).

ABSORPTION OF Sr^{89} IN "TRISMA" BUFFER SOLUTION WITH AND WITHOUT ADDED INERT $SrCl_2$ AS CARRIER

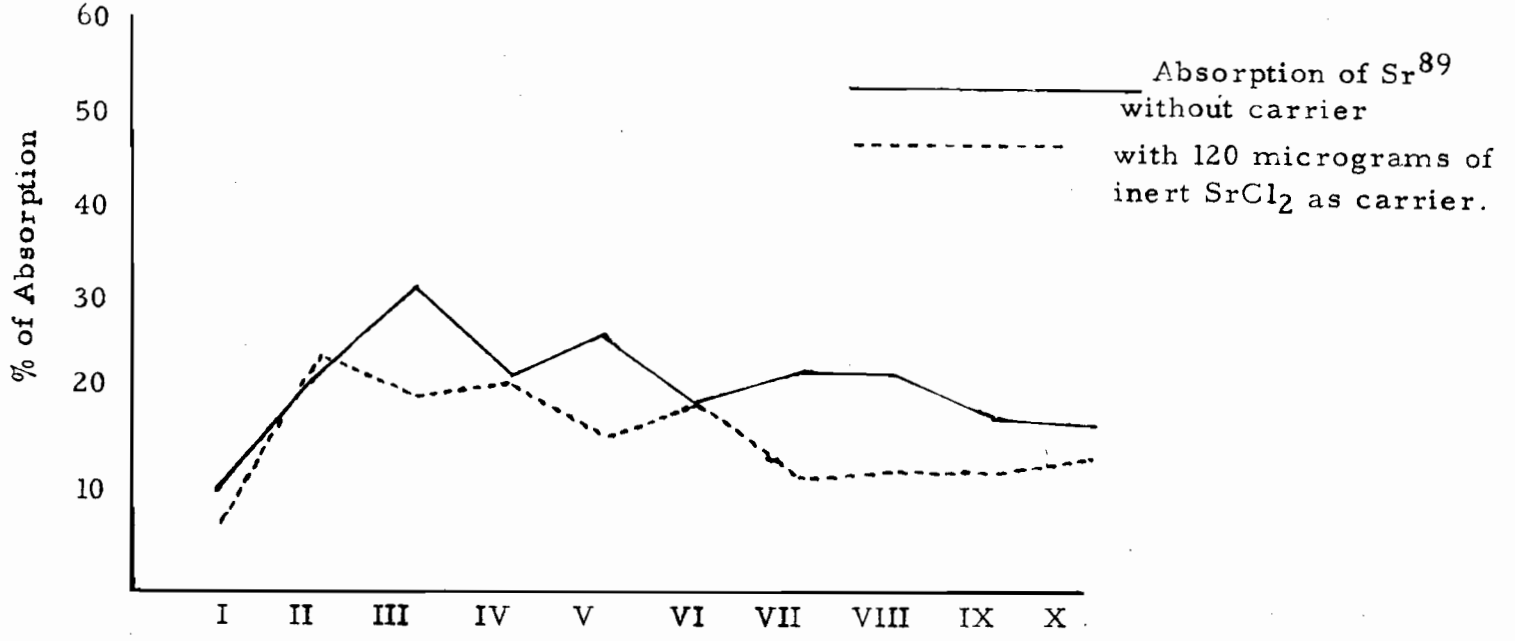

Fig. 9.

ABSORPTION OF Sr^{89} IN ISOTONIC SALINE WITH AND WITHOUT ADDED INERT $SrCl_2$ AS CARRIER

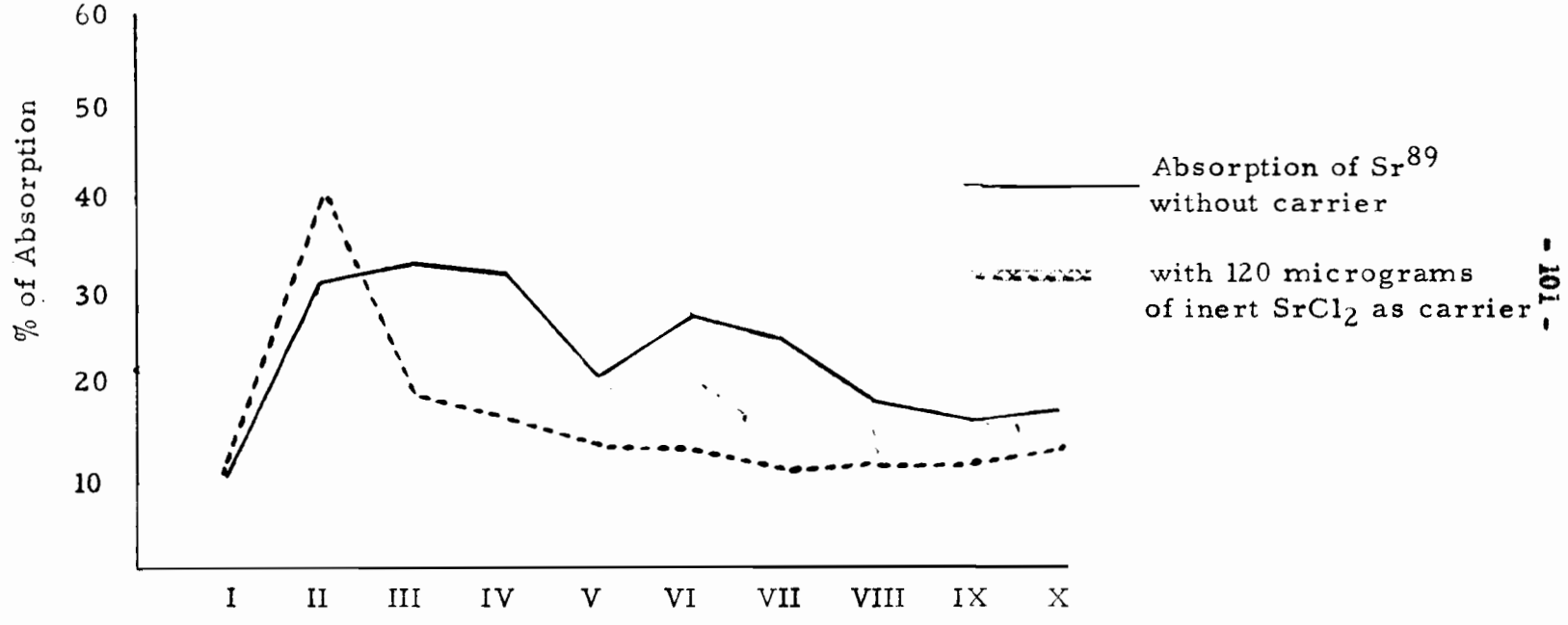

Fig. 10.

Figure 3 shows that an increase of radiostrontium by 5 fold did not produce any appreciable difference in the rate of absorption. The pattern of absorption continued to be the same.

Regional Distribution of Sr^{89} Absorption.

In all the six series of absorption studies which were conducted, the maximum rate of absorption was from the proximal one-third of small bowel. Figures 2 to 10 consistently demonstrate this phenomenon. There was an appreciable amount of absorption from the stomach. The distal third of small intestine absorbed about one-half of that in the proximal part and the middle third was in the middle range between these two values .

This pattern appears to be similar to those of calcium and iron absorption. Dukay (1962) noted that Ca^{45} had a maximum rate of absorption from the proximal part of small intestine. In 30 minutes as much as 65% was absorbed from the duodenal segment, whereas in the distal half of small intestine it was less than 10%. Wasserman (1960) demonstrated that Ca^{45} was actively transported against a concentration

gradient in the duodenal segment.

In the case of radioiron, Brown (1958) noted that iron was taken up equally well by all the segments in vitro; however in vivo studies a distinct gradient was present, with the highest rate in the duodenal region and progressively decreasing towards the distal part of the small intestine. Ohawara et al (1963) reported on the basis of studies of iron absorption in human subjects that absorption of orally administered iron was lower in the distal regions of the intestinal tract.

There is ample evidence to suggest that certain substances have a region of maximum absorption in the small intestine. In the case of fat, several studies in experimental animals as well as in human subjects suffering from regional ilietis and following massive resection of small bowel, have demonstrated that fat is readily absorbed from the distal ileal segments. Removal of the distal 50% of the small bowel produced profound interference with fat absorption in dogs (Kremen et al, 1954). Levy (1958) noted that in all patients with regional ilietis increased amounts of oleic

acid Iodine ^{131} could be recovered from the faeces in both operated and unoperated cases.

It is possible that this regional difference in the absorption of inorganic salts like calcium and strontium from that of fat may be due to the difference in size of the pores of the cell membrane and the permeability in different parts of the small intestine. The present day concept of the cell membrane is that of a semipermeable membrane composed of a lipid layer with adsorbed protein, the lipid layer containing minute pores which permit the extraordinarily rapid passage of very small molecules of low lipid-solubility (Fenton, 1961). The transfer across such a membrane could occur in part by simple diffusion due to difference in concentration of the specific substance on the two sides of the membrane, or by active transport necessitating the expenditure of energy. It may also be influenced by the lipid solubility and the molecular volume of the solute.

CHAPTER VIII

CONCLUSIONS

From the previous studies on Ca^{45} absorption and pH by Dukay (1962), it seemed possible that by controlling the pH in the intestinal tract with a suitable buffering agent the absorption of alkaline earth elements could also be "controlled". As a preliminary step, the normal pH values of the gastrointestinal tract of rat under experimental conditions were determined using isotonic saline. Ten sections were isolated by ligation in vivo. Saline was injected into each loop ; the pH of the contents of each loop was assayed at the end of 30 minutes. The pH of the stomach was 2.35 ($\pm$ 0.89) and in the small intestine it varied from 6.85($\pm$ 0.31) in the proximal jejunum to 8.79($\pm$ 0.56) in the terminal ileal segment.

The influence of a buffering agent "Trisma" at pH 7.55 on stabilising the gastrointestinal pH was studied. The pH of the stomach increased to 5.95($\pm$1.44). In the small intestine a pH ranging from 7.23($\pm$ 0.1) to 8.0($\pm$ 0.07) was

obtained indicating that a certain degree of "control" is possible.

Six series of radioactive strontium^{89} absorption studies were conducted using the same technique of in vivo ligated segments of rat's gastrointestinal tract as in the pH studies. In all the studies carried out on Sr^{89} absorption, the proximal third of amall bowel in general, the duodenum and first part of jejunum in particular had the highest rate of absorption (40.0% in 30 minutes). Absorption decreased towards the distal segments to a minimum of 12 to 16%. This pattern of absorption is similar to those of Calcium and Iron. The stomach absorbed an appreciable amount of Sr^{89} (10.4%).

Two of these series were under "controlled"pH with "Trisma" buffer, with and without inactive carrier strontium chloride. By "controlling" pH the absorption of Sr^{89} was brought to a relatively standard level throughout the small intestine. This was evident by analysing the distribution of the percentage of absorption. With "controlled" pH, decreased absorption from the proximal region of small bowel

was observed in the majority of animals. On the other-hand a higher rate from the distal end was noted in more animals treated with buffer. The evidence is strongly suggestive that by "controlling" the pH of the intestine, absorption of strontium can also be controlled. High level of pH in the distal segments coincided with low level of Sr^{89} absorption. Further studies are necessary therefore to see if absorption can be further reduced by increasing the pH.

The addition of 120 micrograms (43 micrograms of Sr^{++}) of inactive strontium chloride to 2 microcuries (7.2×10^{-5} micrograms) of Sr^{89} produced an overall decrease in the absorption of radioactive strontium both in saline and buffered solution with "Trisma". The percentage of decrease varied from about 10% in the proximal to 5% in the distal parts of small intestine.

A five fold increase in radioactive strontium produced no remarkable difference in the rate of absorption of the radionuclide.

CHAPTER IX

Appendix.

Multiple comparisons (Duncan's multiple range test). (Freund, Livermore, Miller, 1960).

If in an analysis of variance there is a significant difference among several means, it is often desirable to know what mean, or set of means, differ significantly what other mean or set of means.

X_1 , X_2, ... X_k are the means of K random samples of size n and MSE is an estimate of the common variance of the population from which the samples were obtained.

Calculations:

(a) Standard deviation of the sample means

$$Sx = \sqrt{MSE/n}$$

(b) Least significant ranges

$$Rp = sx \,.\, rp \qquad p = 1, 2 \ldots\ldots\ldots k\text{-}1.$$

where rp is obtained from Table VI (Freund, Livermore, Miller, 1960), p is the number of means involved in a given comparison, and the number of degrees of freedom is that of MSE.

Procedure:

(1) Means are arranged according to size.

(2) The difference between all pairs of adjacent means is compared with R_2. If such differences are less than R_2, a line is drawn under the corresponding pair of means.

(3) All sets of three successive means are considered. If the difference between the two extreme means of such a set is less than R_3, a line is drawn under the three means.

All sets of k-1 successive means are considered. If the difference between the two extreme means of such a set is less than R k-1, a line is drawn under the k-1 means.

Note: A line drawn under a set of means indicates that the difference among them is not significant. Also, if a line under a set of means does not extend beyond another line, it is to be disregarded.

Example - Analysis of results of experiment No. VI,

Table X.

Segments and percentage of absorption arranged in order of size.

I	VII	IX	VIII	X	VI	V	IV	III	II
10.4,	10.6,	11.2,	11.6,	13.1,	13.3,	13.8,	16.2,	19.1,	40.0

$$MSE = \frac{368.4 \times 11}{120-10} = \underline{\underline{36.84}}$$

$$Sx = \sqrt{36.84/12} = \underline{\underline{1.74}}$$

rP	P = 2	P = 3	P = 4	P = 5	P = 6	P = 7	P = 8	P = 9	P = 10
	2.8	2.95	3.04	3.12	3.17	3.22	3.25	3.29	3.31
Rp	4.87	5.13	5.29	5.43	5.52	5.60	5.66	5.72	5.76

Successive Means.

(2) $10.6 - 10.4 = 0.2 < 4.87$

$11.2 - 10.6 = 0.6 < 4.87$

$11.6 - 11.2 = 0.4 < 4.87$

$13.1 - 11.6 = 1.5 < 4.87$

$13.3 - 13.1 = 0.2 < 4.87$

$13.8 - 13.3 = 0.5 < 4.87$

$16.2 - 13.8 = 2.0 < 4.87$

$19.1 - 16.2 = 2.9 < 4.87$

$40.0 - 19.1 = 20.9 > 4.87$

(3) 11.2 - 10.4 = 1.8 < 5.13

11.6 - 10.6 = 1.0 < 5.13

13.1 - 11.2 = 1.9 < 5.13

13.3 - 11.6 = 1.7 < 5.13

13.8 - 13.1 = 0.7 < 5.13

16.2 - 13.3 = 2.9 < 5.13

19.1 - 13.8 = 5.3 > 5.13

40.0 - 16.2 = 23.8 > 5.13

---

(4) 11.6 - 10.4 = 1.2 < 5.29

13.1 - 10.6 = 2.5 < 5.29

13.3 - 11.2 = 2.1 < 5.29

13.8 - 11.6 = 2.2 < 5.29

16.2 - 13.1 = 3.1 < 5.29

19.1 - 13.3 = 5.8 > 5.29

40.0 - 13.8 = 26.2 > 5.29

---

(5) 13.1 - 10.4 = 2.7 < 5.43

13.3 - 10.6 = 2.7 < 5.43

13.8 - 11.2 = 2.6 < 5.43

(5) Cont'd

16.2 - 11.6	=	4.6 < 5.43
19.1 - 13.1	=	6.0 > 5.43
40.0 - 13.3	=	26.7 > 5.43

---

(6)

13.3 - 10.4	=	2.9 < 5.52
13.8 - 10.6	=	3.2 < 5.52
16.2 - 11.2	=	5.0 < 5.52
19.1 - 11.6	=	7.5 > 5.52
40.0 - 13.1	=	26.9 > 5.52

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(7)

13.8 - 10.4	=	3.4 < 5.60
16.2 - 10.6	=	5.60 < 5.60
19.1 - 11.2	=	7.9 > 5.60
40.0 - 11.6	=	28.4 > 5.60

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(8)

16.2 - 10.4	=	5.8 > 5.66
19.1 10.6	=	8.5 > 5.66
40.0 - 11.2	=	28.8 > 5.66

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(9) 19.1 - 10.4 = 8.7 > 5.72

40.0 - 10.6 = 29.4 > 5.72

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(10) 40.0 - 10.4 = 29.6 > 5.75

Segments IV, V, VI, VII, VIII, IX and X have no significant difference.

Segments I, II, and III have significant difference from each other as well as from the rest.

Confidence value was calculated using student t (Grossett, W.S., Biometrika 6, 1908).

The 90% confidence limit is therefore $\bar{X}$ -tsm $\leq \overline{\overline{X}} \leq \bar{X}$ + tsm. There is only one chance in ten that the sample mean $\bar{X}$ differs from the true value $\overline{\overline{X}}$ by more $\pm$ tsm.

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