AN EXPERIMENTAL STUDY OF OSTEOGENESIS USING RADIOACTIVE STRONTIUM.

by

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PREFACE.

During previous studies carried out in this Department malignant bone tumours have been produced in one hundred percent of experimental animals by means of administration of repeated high doses of radioactive strontium. The attention of Dr. S.C. Skoryna, Research Director and Dr. D.S. Kahn, Consulting Pathologist was drawn to the relationship of active osteogenesis and tumour formation. They suggested that it might be of importance to study the relationship of bone tumours and fracture healing. This project coincided with my personal interest in bone physiology, particularly in relation to fractures. I was happy to undertake these studies. The physiology of fracture healing and associated metabolic changes are not perfectly understood. The role of various factors, e. g. immobilization, periosteum resection, muscle section and endosteal reaming, has to be evaluated in order to obtain a clearer view of the nature of changes involved.

The use of radioactive strontium has given me the opportunity not only to use this material as a tracer substance in following the changes related to fracture healing, but also to study the long term irradiation effect of the isotope, which appears to be one of the problems of our time. The material in this thesis represents only a fraction of the work which will be needed to elucidate the problem outlined above. However, thanks to an ample supply of experimental

animals and good organizational facilities for work with radioactive isotopes, I was able to obtain significant data on several important points concerning both fracture healing and bone tumour formation.

During my year of study, I have obtained help and encouragement from many members of the Department of Experimental Surgery as well as other Departments of the University.

I wish to acknowledge, with gratefulness, the Fellowship from the Medical Research Council of Canada, under whose tenure this work has been carried out and who gave me an opportunity to work in a great Canadian University.

My sincere thanks to Dr. D. R. Webster, Professor of Surgery and Surgeon-in-Chief of the Royal Victoria Hospital, for providing me with facilities in the Department of Experimental Surgery to carry out this work. To Dr. S. C. Skoryna, Research Director of the Gastro-Intestinal Research Laboratory, I extend my deepest appreciation for supervising this project, for extensive help in planning, discussions and for his keen interest in my problems. The guidance of Dr. D. S. Kahn, Consulting Pathologist and Assistant Professor of Pathology at McGill University and Pathologist-in-Chief, St. Mary's Memorial Hospital was greatly appreciated. His suggestions for various aspects of this project and his supervision have been invaluable.
I hope their interest in the study of the basic problems of radio-active strontium continues so that further investigative work may be planned and carried out during the coming year.

I appreciate the inspiration and help from staff members of other Departments and teaching Hospitals: Dr. H. R. Robertson, Chairman of the Department of Surgery, Dr. C. P. Leblond, Chairman of the Department of Anatomy, Dr. L. Yaffe, Professor of Chemistry, McGill University and Dr. J. R. Robichon, Research Unit, University of Ottawa, for their advice and guidance.

To Miss Unni Mürer I owe greatest regards for her pleasant and charming co-operative help throughout the year; especially for the tiresome job of typing this manuscript. Miss Anne Watkins, biochemistry technician, Department of Experimental Surgery, has always shown keen interest in my problems, has helped in more than one way with endless efforts. Mr. James Byers, technician, has shown great interest in attending the radioactive room and in taking care of a large number of experimental animals. A supply of over 1,500 rats for this project by Mr. Michael Farrell is appreciated. Mrs. Trifonow, histology technician, has very carefully prepared the histological material which has been excellently photographed by Mr. Coletta.

It has been a sincere pleasure to work in the Department of Experimental Surgery, McGill University, with full co-operation from the other Research Assistants and Fellows; Drs. Beaudry, McSweeney, Dukay, Wekselman, Chari, Stellos, David, Wright, Elias, Prohaska, Becerra, and the technician staff.

This has been an excellent opportunity to meet many distinguished personalities, such as Drs. Illingsworth, Humes, Patterson, MacLean, Ian Aird, Irvine, MacKenzie, Kovach, Ellison and many others who visited this Department during the year 1960-61.

To Dr. Gertrude Prohaska, I owe special thanks for translating some of the German and Russian literature.

There is more than one who has helped in his own way, in the completion of this work. It is impossible to mention each one of them but I thank them all in my silent way.

Joginder S. Makhani,
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TABLE OF CONTENTS.

LIST OF ILLUSTRATIONS.

List of Figures

List of Figures. Page

List of Figures

List of Figures

List of Figures — Page

CHAPTER I.

INTRODUCTION.

Sufficient evidence is available to consider radio-activity as a public health hazard. Constantly increasing use of atomic energy products in industry, as well as in the laboratory, presents a real problem. The biological effects of irradiation are presently studied from various points of view. Much remains to be learned about the long range effects of radiation. Only a beginning has been made by the studies of Copeland, Vaughan, Aub, Looney and Gartland Hoffmann, Martland. The carcinogenic activity of radio-active isotopes is well established. Lung carcinoma among the miners in Central Europe as well as bone tumours among radium dial painters is well known. Malignant tumours have also been produced by administration of several radio-active isotopes in experimental animals.

Radioactive strontium is one of the isotopes with a long half life. The inadvertent release of this product in industry raises the question of long term effects of the isotope incorporated in bones. The ingestion of strontium-contaminated material would appear to be the principle route of entry so far as the health hazard of this isotope is concerned.

Studies on the long term effects of administration of high doses of radioactive strontium have been carried out in our laboratories by Skoryna and Kahn for several years. It has been found possible to

produce 100% incidence of tumours. The effect of small doses of radioactivity used in industry and laboratories as compared to large tumour-producing doses given to experimental animals is still not clearly understood. The study of the effects of strontium in relationship with the production of active osteogenesis is of particular interest. This represents the subject of the current investigation. It was thought such an experimental model would be of interest from a public health point of view, because of possibility of dealing with fracture cases during the release period of radioactive strontium. Certain aspects of this project, such as use of low calcium diet are also applicable to the nutritional status of the population in underdeveloped countries.

In the current investigation strontium 89 has been used as a tracer isotope to study the healing pattern of fractures under various experimental conditions. Retention study was carried out in groups of animals injected with strontium 89 and sacrificed at various intervals. The loss of radioactivity in relation to osteogenic activity as well as the cumulative effect of repeated doses of strontium 89 have been investigated. It is hoped that this work will contribute to furthering the knowledge in the new and rapidly expanding field of radiation-research which seems to constitute one of the basic problems of our present life.

CHAPTER II.

BONE AS A LIVING TISSUE.

Bone is a highly specialized form of connective tissue (Stein, Stein and Beller, 1955). It is composed of a combination of organic and inorganic material. It has three structural forms in bones, cortical, cancellous and medullary. Bone may exist as immature (woven) bone or as adult (lamellated), arranged in thin layers of a three dimensional system.

Bone is covered on the outside by periosteum and inside by endosteum (Maximow and Bloom, 1947). The periosteum consists of an outer fibrous, relatively acellular layer and an inner, cellular cambium layer. The endosteum is a single layer of connective tissue, similar in form and function to the cambium layer of the periosteum. These layers of connective tissues have cellular elements in varying forms of differentiation. In their resting state, the periosteal and endosteal cells resemble resting fibrocytes (Stein et al).

The human organism, in the course of his development has 3 types of skeleton (Aegerter and Kirkpatrick, 1958). The skeleton during fetal life is composed in the main of flexible cartilage. The

moderate flexibility lessens the danger of injury to the mother during delivery. The second skeleton is composed of primitive woven bone which predominates throughout the first 18 months of life. It is more rigid than cartilage and more resilient than adult bone. It is adequate for the infant. The third skeleton is the adult-type bone which because of its internal structure is more adapted to withstand the stresses of weight-bearing and movement. Bell, Cuthbertson and Orr (1941) have shown that bone is nearly as resistant as cast iron to bending and twisting stresses.

Cellular Components of Bone.

The cells arise from primitive connective tissue (Ham, 1932). They include osteocyte, osteoblast and osteoclasts. The osteocyte is the final adult bone cell with a life-span of many months. It is located in lacunae in the bone, interconnected by their processes which pass through canaliculi. They are so highly differentiated that they have no reproductive ability. The osteoblast is a form of a connective tissue cell which, it has been suggested, may differentiate into either an osteocyte or osteoclast (Stein et al). It has the capacity to produce osteoid tissue. They are found in the periphery of new and old bone and are universally present at sites where bone is being

formed rapidly. They have a life-span of several months. The osteoclasts have a life-span of 96 hours and have a capacity to resorb bone (osteoclasis) and play a vital role in the remodeling of the bone.

An osteoblast takes 10 days to produce the amount of bone that can be destroyed by osteoclasts in 8 hours. Although it is possible that the osteoblasts and osteoclasts can both form and destroy bone, it is still a reasonable working hypothesis to consider that the osteoblasts are primarily bone-forming and the osteoclasts are for bone absorption (Stein et al).

Development of Bone.

In 1727 Stephen Hales noted that the long bones grew in length only at their extermities. He, and later Duhalmel (1742) and Haller (1757-66) demonstrated the lack of any substantial interestial lengthening in the normal process of growth. This was again confirmed by John Hunter (1837). Endochondral ossification in growing bones was apparently not mentioned until 1836 when Miescher made the first substantial reference to this basic mechanism of the growth process.

Bones are living tissue, not inert structure. New bone is constantly being laid down and old bone being resolved . McLean has

coined the term "osteogenic potency" for the inherent capacity within a cell to form bone.

An excellent study of bone growth by autoradiography after injection of P32 was done at McGill University by Leblond, Wilkinson, Belanger and Robichon (1950). New bone formation according to these authors may be periosteal, endosteal or endochondral in character. Periosteal bone formation does not differ from the endosteal bone formation but the two are not found in the same parts of the diaphysis. They divide a long bone into a central cylinder and the two sub-epiphyseal funnels, the proximal one long while the distal one is a short funnel. New bone formation was studied by autographs and the evidence suggests that in the cylinder the increase in the diameter took place by formation of periosteal bone: At the level of the two funnels, mechanism of bone formation is different. Their studies showed that this portion of the diaphysis grew by addition of endosteal bone. Bone was formed at a fairly even rate along most of the length of the funnel, while at the narrow end of the funnel, the rate was slower.

This study shows that in both the cylinder and the two funnels, active bone formation on one surface is compensated by an equivalent bone resorbtion on the other. Addition of endosteal bone to the lowermost part of the funnel has transformed this region into the central

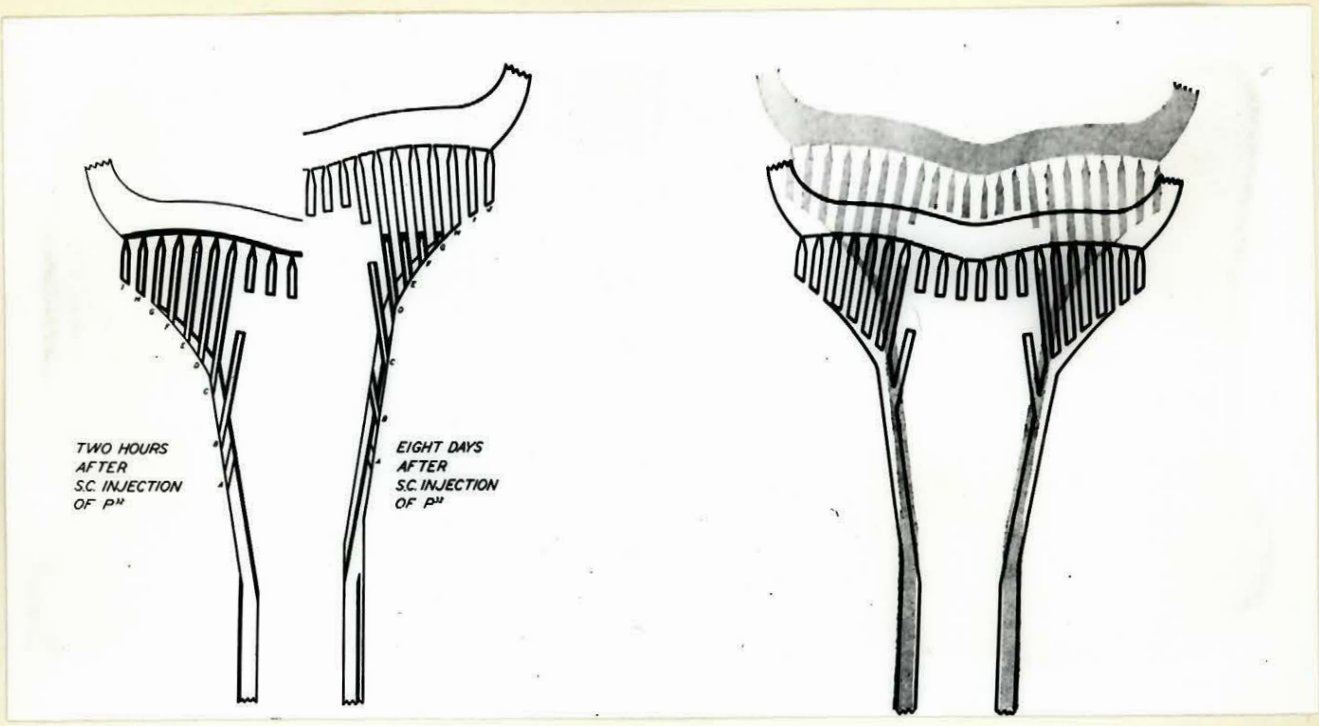


Fig. 1 a.

Fig. 1 b.

Fig. 1 a.

On the left, a diagram represents the head of the tibia of a 50-gm rat at two hours after a subcutaneous injection of radio-phosphorus. The heavy lines indicate the surfaces where a marked deposition of radio-phosphorus occurred immediately after injection.

On the right, a diagram represents the head of the tibia 8 days later. The reactive areas have been drawn as heavy lines in a position symmetrical to that in the left diagram.

Fig. 1 b.

Increase in size of the tibia of the 50-gm rat over an 8-day period.

The bone parts of the grey diagram that are not covered by the white diagram correspond to new bone formed during the 8 days, while the bone parts of the white diagram that do not cover the grey diagram correspond to the bone resorbed during the 8 days.

The rise of the epiphyseal plate, the fate of the individual endochondral spicules, and the lengthening of funnel and cylinder may be analyzed in this diagram. (From: Leblond, Wilkinson, Belanger and Robichon; Radio-autographic visualization of bone formation in the rat. Am. J. Anat., 86: 1950).

cylinder. The net result is that the junction between funnel and cylinder rises and therefore the cylinder increases in length.

Endochondral bone formation is observed mainly on the diaphyseal side of the epiphyseal plate. To start with there is an increase in the width of the epiphyseal plate by addition of cartilage. (Fig. 1). As the plate widens, new spicules are added (spicule J) and progressively the spicules become 'supporting' ones (spicule H). In the meantime, the inner supporting spicules are gradually resorbed. When such a spicule is resorbed, the endosteal bridge on its outside (which was formerly horizontal) is reorganized by resorption from the outside and deposition from the inside until it becomes vertical and fills in more of the space between adjacent spicules. These changes result in an increase in the length of the funnel. Bone resorption inside the funnel smooths down the protruding piece (B) to the level of the inner funnel wall. Later the spicules, forming part of the funnel wall (A), are covered by endosteal bone on the inside like the rest of the funnel, and are gradually resorbed from the outside. Thus near the narrow end of the funnel, the wall is exclusively composed of endosteal bone.

The epiphysis proper also shows endochondral bone formation on the joint side of the epiphysis which constitutes the growing part

of the epiphysis.

It is through the co-operative efforts of periosteal, endosteal and endochondral bone formation processes, along with the process of bone reabsorption, that harmonious growth of a long bone is achieved.

Blood Supply of Long Bone.

Humphry (1885) described a dual blood supply to any long bone, the periosteal and nutrient arteries. Weinmann and Sicher (1947) pointed out that the nutrient artery supplied the marrow and the central part of the metaphysis. The more peripheral parts of the metaphysis are supplied by the metaphysial arteries derived from the periosteum. Trueta and Harrison (1953) in their studies of the blood supply to the adult human femur did not find the nutrient artery supplying the metaphysis which was wholly supplied by metaphysial arteries. Lewis (1956) studied the blood supply of the developing long bones with special reference to the metaphysis. He found that the periosteal plexus of blood vessels supply the compact periosteal bone while the endochondral bone was supplied by the nutrient artery. The metaphysis is at first supplied by the nutrient artery only, but as the bone grows, metaphysial arteries derived from the periosteum

take over the blood supply of the peripheral parts of the metaphysis to an ever-increasing extent. This confirms Trueta's finding that in the adult bone marrow of the medullary cavity is supplied by the nutrient artery.

Bone and its Differentiation.

Sevastikoglon (1958) carried out a morphological and biochemical study of the early stages of osteogenesis in tissue cultures, utilizing isolated embryonic epiphyseal cartilaginous cones of 12 day chickens. In tissue cultures in homologous medium with fresh embryo extract there was morphological evidence of differentiation and of formation of preosseous tissue with the appearance of uncalcified, immature bone. However, in the culture growth in either heterologous or in homologous medium treated with filtered embryo extract, there was no formation of the preosseous tissue. They inferred that differentiation depends on the presence of flattened chondrocytes and some properties of the homologous medium. In the further stages of culture, a perichondral capsule is formed which gradually encloses the culture. From the cells of the inner layer of the capsule, osteoblasts differentiate which in turn form preosseous tissue which must therefore be regarded as periosteal in type.

Morphological study showed that no mature bone was formed in the culture. There is simultaneously a steep rise in the alkaline phosphatase activity, the maximum probably coinciding with the increase in the differentiating activity of the osteoblasts. At the end of the period of high alkaline phosphatase activity and before any decline is evident, there is an increase in the collagen content, with an almost simultaneous rise in acid phosphatase activity. During this phase there appears to be an elaboration of the ground substance as the formation of the preosseous tissue is completed.

MINERAL PHASE OF BONE.

Bone is composed of inorganic and organic components. Structural strength and rigidity are provided by the skeleton from its composition and architecture. About one third of its mass is in the form of mineral crystals. The principal constituents of the mineral are calcium, phosphate, carbonate and hydroxyl ions (Carlström and Engström, 1955-56, 1959; Trautz, 1955).

Agna, Knowles, Alverson in 1958 made a comparative analysis of skull, ribs and ilium for water, calcium, potassium, phosphorus,

carbonate, nitrogen, chloride and sodium content and found that the skull contained significantly greater amounts of Ca, P, CO3 and Na and lesser amounts of water, N, Cl, K than did the ilium. Composition of ribs was intermediate between skull and ilium.

It is generally agreed that the apatite lattice of the bone mineral approximates the structure of hydroxyapatite, CaIO(PO4)6(OH)2. Two types of mechanisms are proposed to account for the variability of bone crystals and the hydroxyapatites: a) Absorption because of the minute size of the crystals; b) Substitution between the various ions within the lattice.

Zelma Milnar (1960) mentions that the dimensions of the crystals in different species probably do not differ much. The growth of the crystal may be influenced by the remodelling process.

Molecular Excursion of Bone.

Mineral phase of bone is very well discussed by Neuman and Neuman, according to who the crystals of bone are minute tablets, 25-50A thick, approximately 400A long and 300A wide. In the intact bone, these crystals are found to be closely associated with the collagen, lying between the bands of the fibers (Fig. 2, 3) with the long axis and the c-axis parallel to the longitudinal direction of the fiber.

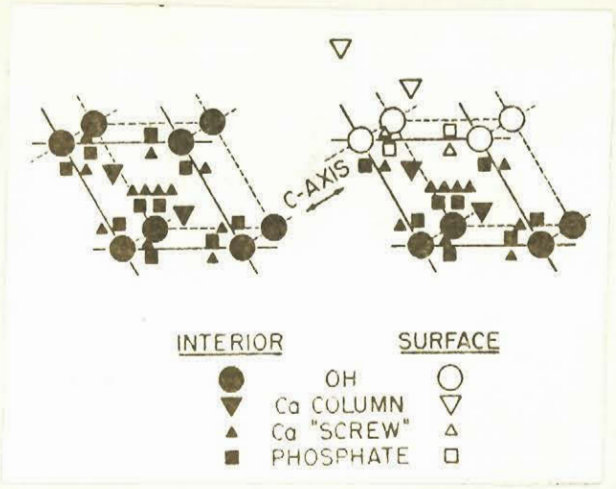
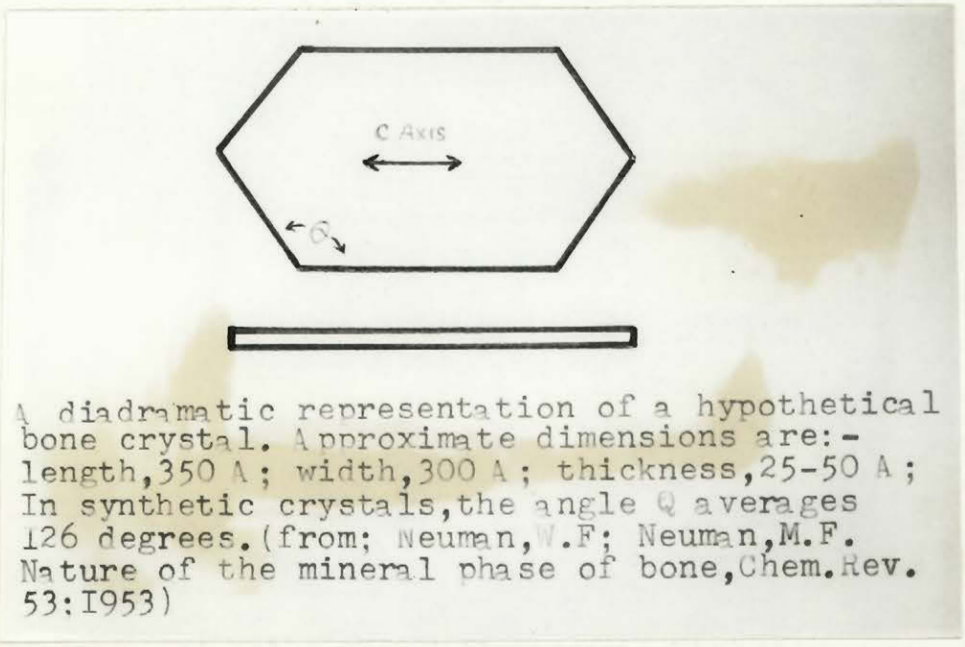


Fig. 2.
A diagramatic illustration of units cells from the crystal interior and surface. (From Neuman and Neuman).



A diadramatic representation of a hypothetical bone crystal. Approximate dimensions are:- length,350 A; width,300 A; thickness,25-50 A; In synthetic crystals,the angle Q averages 126 degrees.(from; Neuman,W.F; Neuman,M.F. Nature of the mineral phase of bone,Chem.Rev. 53:1953)

Fig. 3.

(Robinson, 1952, 1960; Fitton-Jackson, 1956 and Watson, Robinson, 1953). These crystals are composed of calcium, phosphate and hydroxyl ions arranged in a hexagonal lattice structure which diffracts x-rays to give a pattern characteristic of the apatite material. This lattice structure is not of fixed composition but may undergo some isomorphic substitution, particularly at the surface.

The specific surface area of bone mineral is enormous because of the minute size of the bone mineral, (confirmed by Carlström, 1955). Due to ionic exchange, the surface ions are in equilibrium with the solution bathing the crystals, By heteroionic exchange many nonlatice ions are bound by the crystal; hydronium, sodium, fluoride, carbon dioxide, and citrate. The crystals are highly hydrate in aqueous medium. The extreme thinness of the crystals permits an interchange of the ions within the crystal with the ions in solution (recrystallization).

More details of the mineral phase and their relation to strontium ion will be discussed in the chapter IV dealing with strontium deposition.

The organic component of the bone tissue is called bone matrix. It has to be present before mineralization occurs (Robinson, 1960).

Bone matrix has an average composition of 96% collagen, 3.5% polysaccharide, 0.5% non-collagenous protein. The collagen fibrils are of the native 700A variety (Sheldon and Robinson, 1957). Robinson (1958) showed that the band regions of the collagen fibers are associated spatially with the earliest evidence of mineralization by the apatite. When the first crystal seeds are sufficient in quantity per unit volume of matrix the characteristic x-ray diffraction pattern is produced.

Studies on mineralization have been carried out under the direction of Dr. Leblond at McGill University. Other investigators in this field include Engström, Robinson, Bloom and Bloom, Glimcher, Walker, McLean, Urist, Sobel, Howard, Henrichsen, Watson, Belanger and many others.

Leblond and Lacroix (1959) studied the events during osteogenesis in dogs by injecting glycine-H3. The radioautographs showed radioactivity within the osteoblasts as well as in the prebone (osteoid) in close contact with them and by 6 hours, radioactivity was almost exclusively present in the prebone and 15 days after, it was found in the calcified matrix. The study also suggests that the glycoprotein and other substances appearing at the frontier line (outermost portion of the prebone) may play a critical role in initiating calcification, by inducing "nucleation" of the crystals in hydroxyapatite.

Radioautographic studies with Ca45 showed the entry of this ion into the superficial layers of bone, dentin and enamel during growth but never into the cells themselves. Other labelled substances: bicarbonate-14C, glucose-14C and methionine-14C, resulted in early appearance of radioactivity in the matrix close to the specialized cells. This seemed to implicate them in the formation of the matrix. Studies with glycine-H3 showed radioactivity within osteoblasts and odontoblasts and later in the prebone. It was suggested that protein material synthesized in the cells was secreted to the outside to become collagen. Another view on collagen formation is that collagenous fibers arose within the cells, close to the cell membrane and were then released to the outside. Electromicroscopy studies show no fibrils in the cytoplasm (Watson and Avery, 1954). Perhaps collagen or its precursor first appears in the cytoplasm in a form other than recognisable fibers. Schmitt, Gross and others have postulated the existence of minute, acid-soluble rods called tropocollagen and it may be this precursor in the cytoplasm seen in radioautograms after injection with glycine-14C.

The collagen fibrils and the polysaccharide of the connective tissue matrices are both highly hydrated (Blumberg, 1954, Rougnie and Bear, 1953). Robinson, (1957 - 1958) has shown that as the

mineralization of the bone matrix occurs, the apatite crystals displace the water and not the organic solids of the osteoid. As crystallization proceeds, the bone matrix does not shrink or expand, but becomes dehydrated so that the bone is fairly dehydrated when fully calcified.

The relationship that exists between bone matrix mineralization and the collagen fibril has been the subject of investigation by Neuman, Neuman, Glimcher, Hodge, Schmidt, Cartier, LaCroix, Leblond, Sobel, Solomons, Irving, Heller-Steinberg and many others. It is suggested that ATP may play a role in the initiation of calcification in vivo. Irving (1960) used histochemical investigations to study the early stages of calcification and concluded that mucopolysaccharide is concerned specifically with the initiation of calcification; this is a confirmation of the findings of Belanger (1954) and Boyd & Neuman (1951). Sobel has recently advocated the 'nucleation theory'. Sobel (1960) and personal communications (1961) suggests that although collagen induces nucleation, the complete system is more complex and probably includes 1) a specific form of acid insoluble collagen 2) sulfated mucopolysaccharide or mucoprotein 3) enzyme systems such as glycolytic or citric acid cycles 4) energy sources such as ATP and/or UTP 5) a system concentrating calcium and phosphate ions.

CHAPTER III.

RADIOACTIVITY.

Introduction.

A fundamental knowledge of radioactivity is necessary in this work.

Whether the beginning of the atomic age will date from the discovery of the Curies, the researches of Rutherford, the first fission of the atom by Hahn and Strassman or from the detonation of the first bomb will probably never be determined to the satisfaction of everyone (Brown, 1959).

Atomic Time Table. (From: Quimby, Feitelberg and Silver. Quoted by Boyd, 1961).

1808 John Dalton (England) presented experimental basis for atomic hypothesis.

1811 Amadeo Avogadro (Italy) distinguished between atoms and molecules.

1895 Wilhelm Roentgen (Germany) discovered x-rays.

1896 Henri Becquerel (France) discovered radioactivity of radium.

1897 J. J. Thompson (England) showed electrons present in all atoms.

1898 Marie and Pierre Curie (France) discovered radium.

1905 Albert Einstein (Switzerland) suggested equivalence of mass and energy.

1911 Ernest Rutherford (England) discovered the atomic nucleus.

1919 Ernest Rutherford (England) produced nuclear transmutation.

1932 James Chadwick (England) discovered the neutron.

1939 O. Hahn and F. Strassman (Germany) discovered the nuclear fission (U-235).

1939 Enrico Fermi (Italy) suggested possibility of chain reaction in nuclear fission.

1942 (USA) Nuclear chain reaction in a uranium-graphite "pile".

1945 Atomic bomb exploded on July 16th in New Mexico, U.S.A., August 6th and 11th over Hiroshima and Nagasaki, Japan.

Atom and its Basic Structure:-

An atom is an empty space with a tiny dense nucleus, consisting of protons and neutrons forming a core, around which rotate small particles called "electrons". Atoms differ from each other by reason of variations in the number and arrangement of nuclear particles and orbital electrons (Behrens, 1959).

The diameter of the nucleus is approximately 10^{-13} cm. while the diameter of the atom as a whole, i.e. the electron orbit, is about 10^{-8}. Thus the ratio of the size of an atom to the size of its nucleus is $\frac{10^{-13}}{10^{-8}} = 10^{5}$. If we were to draw an atom to scale using a

circle one inch in diameter for the nucleus, the diameter of the electron orbit would be about 1.6 miles (Beierwaltes, Johnson, Solari, 1957).

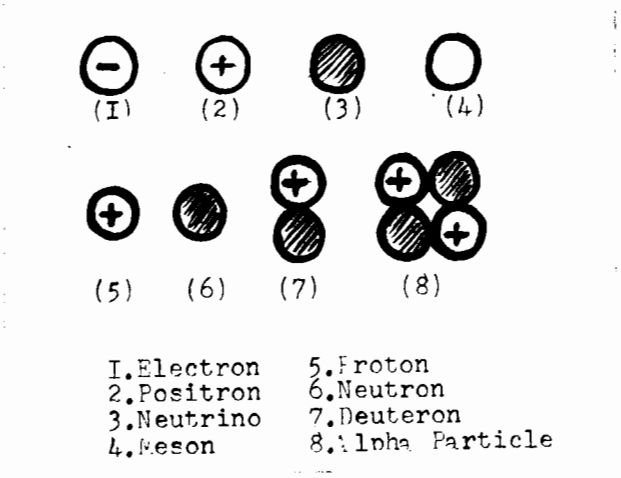


Fig. 4.

Schematic representation of subatomic particles and some of their combinations. Most of these particles have important biologic and therapeutic potentialities (From Glasser, O., Quimby, E.H., Taylor, L.S., Watherwax, J.L., Physical Foundations of Radiology, Ed. 2. New York, Paul B. Hoever, Inc., 1952).

The proton and the neutron are within the nucleus. (Rutherford, 1920, Chadwick, 1932). The proton weighs about 1.7×10^{-24} grams

and has a positive electric charge. The neutron has approximately the same weight but no electrical charge. The electron weighs only 1/1840 times as much as the proton or neutron but it has a negative electrical charge, equal in magnitude to that of a proton, but of opposite sign.

The stable atom is electrically neutral, the number of electrons equaling the number of protons. An atomic number is given to each atom according to the number of protons contained in its nucleus. Thus the atomic number indicates the sum of the protons, while the atomic weight represents the sum of the protons and neutrons. If the nucleus contains an excess of either neutrons or protons, it is no longer electrically stable and when this occurs, there is redistribution of the particles with emission of energy in the form of radiation and this form of the atom is termed radioactive. An isotope is formed by varying the atomic weight by the addition of a neutron. Thus with the addition of a neutron to an atom the atomic number remains the same, but the atomic weight increases and the element is now termed an "isotope". (Behrens, 1959).

Isotope.

"Isotope" is derived from two greek words (isos and topos)

meaning the 'same place' i.e. the same place in the periodic table. Therefore, isotopes are atoms of a given element which differ from each other in the number of neutrons in the nucleus.

Isotopes may occur in nature or may be made in an atomic pile reactor through bombardment into neutrons. A natural element is generally a mixture of isotopes. Some isotopes are stable, while the rest are unstable and radioactive. The unstable, radioactive atoms emit radiation from the nucleus, designated alpha, beta, gamma. (Glasser, 1944).

Alpha radiation or alpha particle is a combination of two protons and two neutrons. Its emission leaves the original nucleus with 4 less particles (2 protons and 2 neutrons) thus decreasing its mass number by 4 and the atomic number by 2. The change in the atomic number results in a change from the original atom to another element, e.g. uranium is transformed into thorium by the emission of an alpha particle.

Beta particle is an electron which comes from a neutron in the nucleus. The neutron changes to a proton and an electron, and the electron is then ejected from the nucleus. The emission of this electron from the nucleus does not change the mass

number (the total number of particles in the nucleus) but does increase the atomic number by one. Radioactive phosphorus, P^{32} decays to sulfur by beta emission.

Along with the beta particle, a particle called the "neutrino" is also emitted. It has a small mass (undetermined) and no charge. There is no direct physical method for detecting the neutrino at the present time.

Gamma radiation or rays are electro-magnetic waves, a term which includes radio-waves, infra-red waves, visible light, ultra-violet rays, and roentgen rays. These all travel with the speed of light (186,000 miles a second) and differ from each other only in their wave-lengths and frequencies. Gamma ray emission changes neither the atomic number nor the mass number; the original atom and the residual atom differ only in their energy state and are called isomers. Therefore, an isomer of an atom is one which contains the same number of protons and neutrons as its parent atom, but differs in its energy state, by nature of a loss of electro-magnetic waves. The neutrons and protons can be considered as existing in a certain configuration with an inherent amount of energy. They then shift into a different configuration of lower energy. The difference in energy between these two states is released as a gamma ray (Beierwaltes, Johnson, Solari, 1957). A given isotope may emit

more than one type of radiation. Insome cases the resultant atom is also radioactive, and this too will decay. The original isotope is considered the parent and the isotope resulting from the decay is the daughter isotope. In turn this daughter isotope is the parent of the isotope to which it decays.

Units:-

Half-Life: Through the process of decay, the amount of the original radioactive isotope continually decreases with time. It has been found that a radioactive isotope decays at a rate specific for that particular isotope (Glasser, 1944). The time required for 50% of a radioisotope to decay is called the half-life. Half lives are known to an accuracy of about 5%.

Curie: Curie is widely used as a unit for artificial radioactive isotopes. Originally (1910) it was defined as the amount or activity of radon in equilibrium with 1 g of radium, the curie was extended (1930) to other members of the uranium series of naturally radioactive elements (Glasser, 1944). The curie has been extended to all natural and artificial radioactive isotopes. When so employed, a curie is an activity unit, and denotes a source in which 3.7×10^{10} atoms disintegrate per second, regardless of the type of radiation emitted.

Millicurie (mc) = 3.7×10^7 disintegrations per second.

Microcurie (uc) = 3.7×10^4 disintegrations per second.

Fission Process:-

Radiations from unstable nuclei show the greatest energy when the decay period is shortest, and the least when it is longest, in accordance with a definite ratio.

For many years, no practical way to influence the rate of decay was known. The situation altered when Hahn and Strassman (in early 1939) in Germany found that neutron bombardment of Uranium caused that element to split into two major fragments. This aroused intense interest not only on its own score, but because it brought up the possibility of chain or continuous reaction. Since neutrons occasion fission, it was realized that if enough neutrons were emitted and if enough suitable atoms were available, a spreading type of chain reaction should be possible under proper circumstances.

Chain reactions once passed the critical level built up to destructive levels with extreme rapidity unless controlled, because nearly all fission neutrons are of prompt type emitted within a milli-second. Control mechanisms function by absorbing neutrons and so reducing the fission rate (Behrens, 1959).

Fission of uranium is practically limited to the U235 isotope which is present in natural uranium in only a small percentage. Natural uranium is composed of 99.3% of U 238, 0.7% U235 and a trace of U234. Fissioning of each U235 nucleus releases about 200 Mev of energy which produces expansive pressure estimated at about a million times that of TNT. It is theoretically possible for approximately 5 pounds of U235 to equal 20,000 tons of TNT.

IONIZATION.

Concept of Ionization:-

When an electron is ejected from an atom, it leaves the electrically unbalanced residue of the atom as a positively charged ion and this is the essence of ionizing radiation. The detached electron may become attached to some other atom, which also loses its electric neutrality, and thus becomes charged, ionized or 'excited'.

Sources of Ionizing Radiation:-

They include:

1) Cosmic rays

2) Radioactive materials occurring in nature, e.g. Uranium and Radium.

3) X-rays (Therapeutic and diagnostic).

4) Isotopes (natural or artificial).

Physical Characteristics of Ionizing Radiation:-

There are two types of radiation, electro-magnetic and particulate. Ionizing radiation includes gamma, x-radiation and the particulate radiations. (Alpha, beta, neutrons, protons). By virtue of creating ions indiscriminately through the medium that they pass through, they damage the cells (Wright, 1956).

Alpha rays are rapidly moving nuclei of helium atoms. They are continuously emitted by certain radioactive elements (radium, polonium) and also arise during certain nuclear transformations. In spite of their large kinetic energy (4.19 Mev to 8.78 Mev) at the instant of liberation, yet they possess little ability to penetrate tissues. The large size of the particle ensures its early collision with atoms on its path, and its initial energy is quickly lost along a short but densely clustered ionization track.

Beta-rays are streams of rapidly moving electrons which are emitted from many radioactive elements or may be created by the passage of high tension currents through cathode-ray tube. Their kinetic energy varies widely (0.050 Mev to 3 Mev) according to their source. Though, of much smaller size and kinetic energy than the alpha particle, most beta rays have greater powers of penetration, and their capacity to ionize the molecules in their path is correspon-

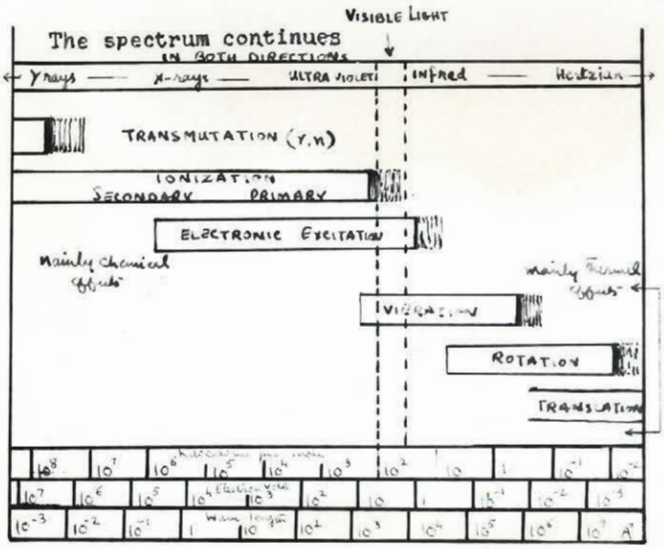
dingly less, and this lower density of the clustering of the ions formed in the track modifies correspondingly the chemical and hence the biological effects of this type of radiation.

Neutrons are electrically neutral particles that are present in the nuclei of all atoms of mass number 2 and upwards (of all atoms of deuterium and larger elements). They are generated from uranium and plutonium in atomic piles and bombs. In passing through matter, they may be "captured". The initial velocity of neutrons, their energy and penetrating power varies greatly according to the source. Because they have no electrostatic charge, they can readily penetrate the electron cloud of the atom and so reach its nucleus. Such nuclei of certain elements lead to the local emission of more potent form of radiation (it was such radiations responsible for the damage in Hiroshima and Nagasaki).

Protons are the positively charged nuclei of hydrogen atoms, which can be accelerated to very high velocities in the cyclotron or similar apparatus. They are relatively large in size and can be made to possess high kinetic energy. They are unable to penetrate far into the tissues, but along their short tracks, they produce dense clusters of ions as compared to alpha rays.

The Electromagnetic radiations comprise a continuous spectrum which extends from the long electrical waves, through the infra-red,

visible and ultraviolet lights, down to x and gamma rays (wave lengths of 10,000 meters or more for longer to one ten-millionth of a millimeter or less for shorter wave-length). With such extremes of wave-lengths, the ionization effect is restricted to only a small fraction of this enormous spectrum, x and gamma rays.



The spectrum and the effects produced(From Sparrow,A.H., and Rubin,B.A.:In Survey of Biological Progress,Volume II. New York,Inc.1952).

At the right,the infrared portion of the spectrum produces mainly thermal effects.As ultraviolet merges into x-ray, ionization occurs and chemical effects are produced.The wavelengths are given below.Note how little of the spectrum is visible to the naked eye.
(Radiation and Radioactive Isotopes,Chapter 8,Pathophsiology in Surgery,Hardy,J.D.1958)

Fig. 5.

At the right, the infra-red portion of the spectrum produces mainly thermal effects. As ultraviolet merges into x-ray, ionization occurs and chemical effects are produced. The wavelengths are given below. Note how little of the spectrum is visible to the naked eye.

Their effect is primarily mediated by the detachment of electrons from atoms encountered along their tracks and these then constitute beta radiation of varying energy levels and pursue independent paths through the tissue, bringing about many ionizations in nearby molecules. The energy level of these ejected ionizing electrons increases as the wavelength of the incident radiation shortens, so that the destructive effects of gamma rays are more prominent than those of x-rays. X and gamma rays have immense power of penetration.

Fall-out.

"Fall-out" means radioactive dust, falling out of fission products out of atmosphere, either due to natural causes or as a consequence of atomic explosions or use of radiant energy in any form (Behrens, 1959).

Fig. 6.

Showing relatively uniform distribution of fall-out on the ground in relation to buildings, trees and personnel. Wavy, shaded area represents gamma rays, stipling represents beta rays. Alpha rays are usually not present in fall-out to any great extent. (From: Behrens, 1959; Atomic Medicine).

Arakawa, (1960) states that when a nuclear explosion occurs, temperature rises to millions of degrees and a rapidly expanding fireball forms which ascends into the stratosphere, pushing the surrounding medium away with great force. 50% of the total energy is released in this manner, 35% as thermal radiation, 10% as residual nuclear radiation and only 5% of the total energy is released during the first minute as initial nuclear radiation, consisting of alpha, beta, neutrons and gamma rays. The visible flash of light at the time of detonation attains a flash many times brighter than the sunlight.

The updraft, in the case of low level burst in which the fireball touches the ground, sucks up large amounts of debris and again subsurface bursts hurl up large amounts of material. The substances concerned are rendered radioactive by the neutron fluxes and contamination with fission products.

A. E. C. report (Adm. Strauss) pictures a cigar shaped area some 140 x 20 miles where fall-out might be lethal with a lesser but dangerous contamination extending over 240 x 40 miles. This, of course, is subjected to variations depending on the type of bomb, its power, point of detonation, nature of the soil and the weather. In the case of surface burst, with newer weapons which would produce a dangerous fall-out over an area of approximately 5,000 square miles or more, the picture has become very gloomy.

Cytochemistry of Ionizing Radiation:-

The effect of radiant energy on biological systems is still not completely elucidated. However, at the present state of knowledge it appears that its important site of action is its effect on the living cell.

Absorption of energy by a cell represents the first step in the chain of events which lead to morphological and functional changes of a cell. The cell represents the biological unit of life. Damage to living cells is effected by a transfer of energy from the radioactive agent to the cells by the process of ionization, which is the loss or the acquisition of an electron by an atom, resulting in ionization of the molecular structure of the cells (Behrens, 1959).

Morphological Effects:-

Morphological effects consist of pyknosis of the cell nucleus (coagulation of the chromatin), karyorrhexis (disintegration) of cell-nucleus and their fragments are distributed in the cytoplasm, liquefaction of the cell mass with vacuolation. All these changes depend on the radiation dose and are characteristic, but not specific for radiation, as they occur during the necrosis of cells from many different causes.

Biological Effects:-

According to Regand, the bioligic effects of ionizing radiation are:

1) Diffuse cytocaustic effect.

2) Selective cytolethal effect, resulting in
   a) Immediate death of the cells
   b) Damage resulting in delayed growth
   c) An effect appreciable only in the cell descendants.

Mechanism:-

Although the definite effect of radiant energy on the cell is not completely understood, several hypothesis have been forwarded. It is generally believed that the primary process occurs in the large proton molecules and the water of the cells (Behrens, 1959).

Direct or Target Hypothesis:-

Radiation produces bioligic change because of a direct hit on a very small biologically sensitive structure. The cell nucleus contains chromosomes with their genes, while the cytoplasm contains mitochondria and centrosomes. Any of these may be hit by the electron. The resultant effect is due to the high energy delivered to small volumes of the cell. If an enzyme molecule is damaged, the biological result may be small, as other similar molecules may carry on the function. However, if a gene is destroyed, the effect may be serious, resulting in a mutation. Boyd (1961) quotes that a

dose of 1000 roentgens of whole body radiation, which would kill a man in a few days, results in the ionization of only about 1 molecule out of every hundred million in the body.

Indirect or Toxic Hypothesis.

Radiation produces a certain concentration of toxic material which by one means or another results in a biologic change. These hypothetical toxins may be primarily, H^{+} or OH^{-} radicals which form hydrogen peroxide or various forms of "active water", arising by the action of radiation on water present in all living material.

Enzyme Hypothesis.

Some believe that the effects are produced from a disturbance in cellular physiology, secondary to inactivation of enzymes, denaturation of essential proteins, alteration of colloidal properties or changes in protoplasmic viscosity, all of which can actually be produced in isolated systems. Other workers believe that the effect on cells is primarily an alteration of cell membranes, and cellular water balance.

Among the dominant constituents of both nucleus and cytoplasm are nucleic acids, of which two groups, ribosenucleic acid and desoxyribose nucleic acid (RNA, DNA) are known to play the

most important part in the metabolism of the cell. DNA is confined to the nucleus and the chromosomes. RNA is mainly present in the mitochondria and the nucleolus.

Ciba Foundation Symposium on Ionizing Radiation and cell metabolism (1956) includes the investigations of Brachet, Krebs, Alexander, Dale, van Bekkum, Gray, Hollander, Alper, Cohn, Davidson, Forssberg, Gale, Haddow, Hevesy, Holmes, Koller, Lajtha, Laser, Latarjet, Loutit, Mitchell and many others. Though the studies on the role of nucleus, mitochondria, the nucleic acids, the enzymes and the pacemakers, and many intricate mechanisms were reported, no definite conclusions were drawn except for appreciation of the unawareness of the facts and that further investigations in this field of biochemistry were necessary.

Radiosensitivity of Cells.

It is generally agreed that the most actively dividing cells are the most radiosensitive, and that radiosensitivity in general is related to high division rates. Law of Bergonie and Tribondeau states that immature cells and cells in active state of division are more sensitive to radiation than are those that have acquired adult morphological and physiological characteristics.

In the animal kingdom the more complex the organism, the more easily it is affected by the radiation. When a small portion of the animal is irradiated, it could tolerate much greater dose than when large volume is exposed. (Spear, 1953).

Ionization is one of the early links in the chain of events between the absorption of the radiant energy and the change in the biological behavior of the irradiated cells. (Spear, 1953).

The ability to survive a given dose reflects recuperative powers as well as susceptibility. The median lethal dose for different cells and organisms differ widely. Bacteria, viruses and some cold-blooded creatures could survive doses in excess of 100,000 r. Human lymphocytes are damaged by 100 r, but nervous tissue could withstand 5,000 r (Anderson, 1948).

A dose of radiation which is not lethal and does not produce an immediate gross alteration may, however, still have a definite delayed deliterious effect on the cell.

Different living tissues react differently to radiation. Tissues composed of differentiated cells (nervous system, bone, muscle) have low radio sensitivity and the effect on these tissues are to a large extent the indirect result of the resulting impaired vascularity and fibrosis. Tissues with undifferentiated cells (epidermis,

spermatogenic cells), on the other hand, are in continuous state of growth and are very radiosensitive.

Radiosensitivity of various human organs is as follows:

1) Hemopoitic and lymphoid tissues, skin, gut, mucosa, gonads (radiosensitive).

2) Liver, kidney, spleen, (intermediate).

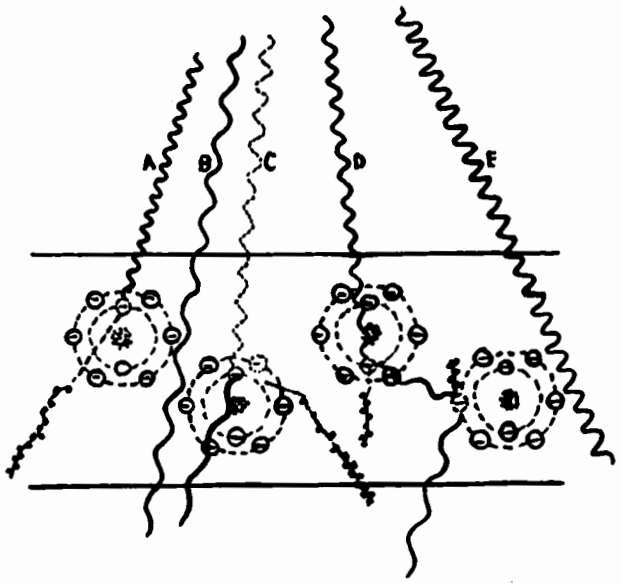
3) Nervous tissue, bone (least radiosensitive).

Warren has shown that the effect of radiation on a given tissue is proportionate to the amount absorbed. The radiant energy (photon) may traverse without absorption. It may, if it collides with an electron, be entirely expended (photoelectric effect) or it may be removing an electron from an atom, expend only part of its energy and continue in a changed direction, in which case the wave length will become longer (Compton's Effect). Hence the size of the field is the most important factor as to the result of the secondary radiation. (Fig. 7).

Radiation and Cellular Growth Pattern:-

With varying amounts of radiation, growth of the cell may be unaffected temporarily, permanently inhibited and/or altered in pattern. Examples of the latter are tumour induction and irradiation-induced somatic mutations. The apparent stimulating effect of radiation is a physiologic artefact in which the increased mitotic rate after irradiation represents a recovery phase. This

is a purely compensatory response, following supression of mitoses. After doses, small enough to permit complete recovery, the increased mitotic rate which follows the initial depression is sufficiently high to allow the irradiated cells to "catch up" with the controls in regard to the number of cell divisions. The compensatory increases become progressively less marked as the dose increases. Consequently in severe radiation injury, the compensatory increase in mitosis may be absent. (Behrens, 1959).



This diagram indicates how radiation(physical energy) is transformed into chemical energy through its capacity for altering the chemical characteristics of the atoms and molecules of tissue cells. That is,it is now possible in many instances to explain physiologic effects of therapeutic radiation in terms of familiar chemical reactions.(From Glasser,O.,Quimby,E.H.,Taylor,L.S.,Weatherwax,J.L.:Physical Foundations of Radiology,Ed.2,New York.Paul B.Hoeber,Inc.,1952)

Fig. 7.

This diagram indicates how radiation (physical energy) is transformed into chemical energy through its capacity for altering the chemical characteristics of the atoms and molecules of tissue cells. That is, it is now possible in many instances to explain physiologic effects of therapeutic radiation in terms of familiar chemical reactions.

Radiation and Physiochemical Processes:-

In extreme radiation injury, the cell may become completely permeable with total disruption of its physiochemical processes and dissolution. With milder degrees of damage, permeability may appear to be altered selectively. Altered distribution of calcium and potassium in irradiated skin has been interpreted as an indication that the permeability of cells and tissues to the respective cations had been altered (Behrens, 1959). Similarly, an increase in cytoplasmic nucleic acid and a decrease in nuclear, nucleic acid content have been interpreted by some (Ingram, Mason, Whippleand Rolland; 1952) as an indication of altered nuclear permeability.

Other physiochemical changes in irradiated cells include liquifaction and coagulation of the cytoplasm. Heilbrunn postulated that cytoplasmic coagulation reflects increased permeability of the injured cell to calcium and hence is analogous to blood clotting. Mitosis is accompanied by changes in cytoplasmic viscosity and especially by a marked increase in viscosity during metaphase and early telephase. It is quite possible that increased mitotic rate following irradiation may also stem from altered viscosity.

In clinical practice then, one should be aware of the radiosensitivity of any structure and the changes that may result in that

tissue or organ from irradiation before using radioisotopes that are concentrated in that organ or tissue. Ingram et al (1952) have published a clear and consice presentation of these observations based in part on a review of 5000 references in this field.

Genetic Hazards of Radiation:-

In all organisms, ionizing radiation can produce gene mutations and chromosome breaks. Predictions as to their genetic effects is only an informed guess. The effect of radiation, whether natural, diagnostic, therapeutic, or due to 'fall-out' has been summarized by the head of the Biology Board, Atomic Energy of Canada: "The frequency of the conditions which are mutation-maintained will increase with any rise in the level of ionizing radiation. Present levels of exposure resulting from the medical uses of x-rays average about 3 roentgens per generation per person in the population, and might eventually increase the load of hereditary defects by as much as IO %. The genetic effect of 'fall-out' from nuclear weapon testing is probably about one-thirtieth of this". (Newcombe and James, 1959). About 4% of all individuals born will suffer, at some time, in their lives from hereditary defects due to naturally occurring mutations (Quoted by Boyd, 1961).

In A.E.C. (1958-1959) report about radiation problem relating to society, Dunham states that NAS Committee has estimated that in the normal course of events in the next 30 years, 100,000,000 children will be born in U.S.A. and there will be among them some 2,000,000 with tangible genetic defects. If 40 r is taken as the radiation dose per generation necessary to double the present 'spontaneous' mutation rate, the 10 r dose per generation would add in U.S.A. alone 50,000 tangible defects in the first generation and eventually after 20 or 30 generations, about 500,000 per generation, i.e. about 16,000 per year.

Clinical Observations on Effects of Whole-body Radiation:-

The principal data on human reaction to harmful doses of whole body irradiation come from observations in Hiroshima and Nagasaki (1945), detonation in the Marshall Islands (1954) in the Pacific and after the Los Alamos reactor accident.

Hollingsworth (1960) reported the delayed radiation effects in survivors of the atomic bombings. The symptom pattern is made up of three essential phases, the latent period, the period of acute symptoms, and/or death and/or the recovery period.

a) Latent Period:-

The larger the dose and the smaller the person, the shorter

the latent period.

b) Hyperacute Reactions:-

These occur in persons dying within the first 30 minutes, mostly from central nervous system damage, usually with convulsions and usually after receiving more than 3,000 r of whole body radiation. Radiation dosage of 25,000 to 70,000 r produces death within the first two hours probably with an LD_{100}.

c) Initial Reaction:-

With smaller doses of radiation (2,000 to 3,000 r) the latent period is longer, the onset of symptoms is less acute and the clinical picture is characterized primarily by fatigue, nausea, vomiting and diarrhea, fever, intense thirst. Leukopenia may be found (especially lymphopenia). Thrombocytopenia and an increasingly severe anaemia appear, but usually not until after the second week. Coma and delirium may develop in patients dying in the first few weeks after irradiation. At autopsy, these patients show the effects of radiation upon gastrointestinal tract, lymphoid tissue, bone marrow and gonads. Persons dying during this first week have usually received 800 to 3,000 r whole body radiation.

d) Acute to Subacute Reaction:-

Patients dying during the 3rd to 6th week show direct damage from ionizing radiation and aplastic anaemia, consequent

upon destruction of the bone marrow. Bacterial infection is largely responsible for the development of necrotising lesions commonly found associated with the aplastic anaemia. Later thrombocytopenia may contribute to purpura. The initial decrease in platelets is noted at 3 days while the maximum drop is noted at 20 to 28 days (the maximum drop in lymphocytes occurs at 4 - 6 days and the maximum drop in granulocytes occurs at 32 - 36 days).

The sequence of events in the acute to subacute reaction may be as follows. Nausea, vomiting and other symptoms may occur as in the initial reaction group, but usually in a milder form. Then there is usually remission of symptoms with a period of relative well-being. 1 to 2 weeks after irradiation, epilation occurs, with increasing malaise, daily ascending unremittent fever, then pharyngeal pain with the development of the clinical picture of agranulucytic angina. Bloody diarrhea may be a prominent symptom associated with ulcerative gastritis and enteritis. Leukopenia and anaemia appear. If the patient does not die, the recovery is marked by disappearance of the pharyngitis, platelet and white cell counts improve.

e) Chronic Radiation Syndrome:-

If persons are exposed to one sublethal dose of radiation

or repeated small doses, they develop diseases as a result of the radiation exposure, which for all practical purposes are identical with many diseases ordinarily occurring in the absence of known exposure to radiation. In a summary of the Japanese data, Miller (1956) found the following:-

1) Leukaemia:- Leukaemia rate was 240 cases per 100,000 persons within 1000 meters of the hypocentre of the bomb explosion as compared to the incidence of only 60 per 1000 in persons outside this range. Younger persons were more susceptible than older persons, yet people past the age of 40 years had a higher incidence of granulocytic leukaemia than non-radiated individuals.

2) Radiation Cataracts:- The posterior subcapsular region (vacuolar) was completely involved in 10 patients. 70% of the epilated patients developed polychromatic granular plaque on the posterior capsule. This capacity was eight times more frequent in the exposed population than in the non-exposed population. In older persons there was twice the incidence of visual defects in those within 1500 meters of the hypocenter as compared with persons of comparable age at a greater distance than this from the hypocenter.

3) Pregnant Females exposed during 0 - 6 weeks of gestation

delivered infants with microcephaly with mental retardation as the worst defect or microcephaly with normal intelligence as the next most serious defect.

4) No unusual incidence of degenerative diseases have yet been shown to occur in persons who were less than 10 years of age at the time of bomb explosion.

5) Miller found neither an increased incidence of aplastic anaemia or any other type of blood disorder (excluding leukaemia) as a late effect of irradiation.

6) Other abnormalities found, include infertility and hypoplasia of dental enamel in the children exposed while still in utero.

7) One of the most widely accepted chronic effects of irradiation is the acceleration of physiologic ageing. The exact mechanism for this has not yet been elucidated, but is is postulated that the ageing process is speeded up at the time the irradiation is administered.

8) Increased incidence of tumours of lung, ovary, uterus, breast, skin, bone and pituitary have been described as late effect of irradiation (Hollingsworth, 1960).

9) In rats, 100 r of roentgen irradiation given in one dose produces 3% reduction in life span. If 5 r is given every day the life span is reduced 36%. Neel in summarizing the most important deliterious genetic effects of radiation reminds us that any quantity of ionizing radiation increases the incidence of mutations. Unplanned mutations are usually harmful to subsequent generations (Beierwaltes et al, 1957).

CHAPTER IV

STRONTIUM AND ITS METABOLISM

Introduction.

Strontium occurs in traces in most biological materials but there is no evidence that is is an essential element for either plants or animals. It normally represents a trace element in the human body with an average concentration of 4.5 parts per 10,000 parts of calcium in the bone (Thurber, Kulp, Hodges, Gast, Wampler: 1958). Strontium content of the human bones is reported to represent 0.016% of the ash for fetal specimens and 0.024% for adults (Nut. Rev. 16.58).

Radiation Hazard.

Currently, there is wide spread interest in radioactive strontium. Radioactive isotopes with long half-life present the greatest problem from public health point of view (Lisco, Finkel, Brues, 1947, Wasserman, Comar, 1957). Their chemical similarity also introduces a nutritional aspect of the fall out problem (Wasserman, Comar, 1957). Sr90 constitutes the most important nuclear fission product. Its long half-life, bone seeking property and very prolonged incorporation make it the dangerous isotope (Hamilton, 1948). Other fission products have been consideredless potentially hazardous because of lower fission

yield, shorter half-life and smaller incorporation and more rapid turn-over into the biological systems (Schulert, Peets, Laszlo, Spencer, Charles, Scmachson, 1959).

From soil to man.

The incorporation of Sr90 in the biosphere generally occurs as follows (Comar, Russell, Wasserman, 1957):

Large weapons (megaton) deposit a large fraction of their Sr90 in the stratosphere which slowly passes back into the troposphere with an average residence time in the stratosphere of about 10 years.

Small weapons (kilotons) deposit their Sr90 content in the troposphere. From here it is relatively quickly deposited on the surface of the earth, primarily by precipitation. Sr90 falls out upon the surface of the soil and foliage, is incorporated in plant tissue by foliar absorption and mixed with plant calcium. In the soil it mixes with soil calcium and is absorbed by the plant roots. The reservoirs include the human and animal skeletons, milk, vegetation, upper layers of soil and water. (Comar, 1956).

Strontium in lactation.

It has been reported that Sr90/Ca ratio of milk is from 0.09 to 0.16 of that of the ratio being consumed by the animal (Harwell, 1956).

The Oak Ridge Institute Report (1956) gives a detailed report of strontium and calcium metabolism in goats. Calcium was preferentially secreted into milk by a factor of 10 to 12 times that of strontium. Calcium moved from plasma to milk by a factor of 2.5 of that observed for strontium. It is evident that considerable discrimination against strontium in favour of calcium occurs in the passage of these alkaline earths from diet of dairy animals to milk. Sr90 content of human population would be 5 times greater than it is now, if it was not for the differential metabolism. There is also a differential behaviour of calcium and strontium in various steps of the food chain. These differential behaviours normally provide a factor of protection against Sr90 in the soil and vegetation that may be as high as 25 for the new born and is most likely not less than 6 for adults depending on food habits. (Comar, Russell, Wasserman, 1957).

Strontium and bone.

It is generally accepted that strontium is rapidly accumulated in the bone (Norris, Kisieleski, Leblond, Greulich, Comar, Gotz, Boyd, Kidman, Rayner, Tutt, Waughan, Engstrom, Bjornestedt, Climedson, Nelson, Hamilton, McLean, Urist, Neuman, Neuman, Arnold, Jee, Johnson, Jowesy, Owen, Vanghan, Tomlin, Henry, Kon, Bohr, Bauer, and many others).

Strontium content of human bones was found by Hodges (1950) by Emmission - Spectrographic technique to be 220 ppm for bone ash but Tipton found a lower value of 120 ppm using the same technique. Turekian and Kulp (1956) studied the Sr content of 277 human bones from world-wide sampling. The average ratio was 0.60 ($\%Sr/Ca\%X10^{3}$). If it is converted to parts per million Sr on the assumption of pure calcium phosphate ash, this value is equivalent to 234 ppm. which compares closely to that found by Hodges.

When large variation occurs, the main source of variation appears to be related to regional influences. It could be attributed to a more mobile population or to regions with diverse geologic and geochemical environments. In some areas, diet may play an important role. It has been established that there are marked regional differences in strontium content of almost all types of rocks. Waters draining from these rocks, and plants growing in these areas would take a strontium complexion of the locale, thus explaining strontium content variations. This has been confirmed in the examination of human bone tissue from a known high Sr/Ca area. The Waukesha (Wisconsin) water supply (U.S. Geol. Survey 1959) has 30 - 50 ppm Sr and 50 - 90 ppm Ca which is a very high Sr/Ca ratio, and human bone examined from this area likewise had a high Sr/Ca ratio.

Metabolism of Strontium.

a) Similarity to Calcium.

It has been demonstrated that there is close similarity between the behaviour of radioactive calcium and strontium (Jones, Copp, 1948, Norris, Kisieleski, 1948, Hamilton, Kidman, 1950, Pecher, 1941). It is generally assumed (Laszlo, Daniel, Spencer, 1955 and Pecher), though not uniformly (Comar, Whitney, Lengemann, 1955) accepted that strontium is metabolised and incorporated in bone ash, in a manner somewhat similar to calcium.

b) Absorption.

The absorption of strontium from the gastrointestinal tract varies markedly (Laszlo et al, Spencer et al, 1956) and is affected by the calcium intake before, during and possibly shortly after the intake of strontium (MacDonald, 1956, Ray, 1955). Two factors affect absorption of strontium from gut; age of the animal and adequacy of calcium in the diet.

c) Strontium retention Vs. age.

It is of interest (Cordery, Christie, MacIntyre, Palmer, 1960) that whereas the chemical studies showed no significant change in the percentage of calcium, phosphorus or residue in mice of varying ages, the radioassays showed a significant alteration in strontium retention in animals with an age difference of no more than 2 weeks.

d) Route of Administration:-

It has been demonstrated that parenteral administration of radioactive strontium eliminates to a large degree the effects resulting from difference in absorption of Strontium 85 from the gastrointestinal tract (Comar et al, 1955). No significant difference was demonstrated in the results obtained when strontium was administered as a chloride, lactate or gluconate salt (Pecher, 1941). After parenteral administration of radioactive strontium the isotope is excreted in both the feces and urine. In mice (Pecher, Vaughan, Tutt, Kidman, 1952) the larger fraction is excreted in the feces.

e) Metabolism in Adult, Young and Rachitic Rats:-

Strontium, when given intraperitoneally (Jones and Copp, 1948) to normal adult rats, showed maximum concentration in blood after 15 minutes. Strontium was removed from the plasma much more slowly in the adult animal than in young growing, or in rats maintained on a low calcium diet. After parenteral administration, uptake of strontium by adult bone is continuous for the first two hours and reaches maximum within 2 - 4 hours. The skeletal uptake is much more rapid (5 times) in young animals, reaching a maximum within 30 minutes. The deposited isotope appears to be retained in the bone. In rachitic rats, the rapid

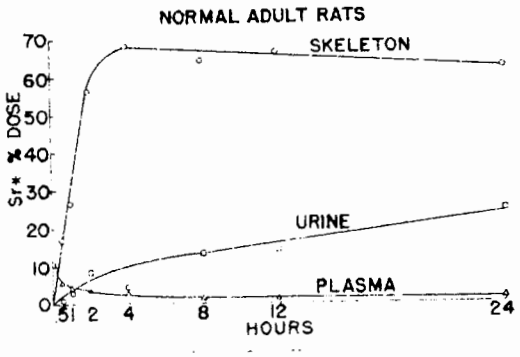
initial uptake was similar to that in the normal young animals but was followed by active loss from the skeleton, thus only one third was left at the end of 24 hours. This suggests that the strontium was in a labile state in the bone. A large part of the dose of strontium was excreted in the urine by the rachitic rats within the first 24 hours. Normal crystalline matrix is not formed in the rachitic animals, therefore radiostrontium cannot be incorporated in to this, although ion exchange with existing bone salt may take place as in the adult. Rapid initial intake is a labile combination from which strontium is readily released in rachitic animals. (Fig. 8).

In the young animal very little strontium was lost in urine, in contrast to the rachitic rats, in which a large part of the dose was eliminated by this route with 24 hours.

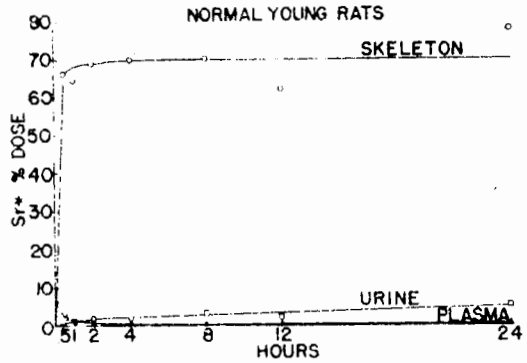
The renal plasma clearance was similar in both young and adult normal animals with approximately 1% of the blood plasma 'cleared' per minute. In the rachitic rats, the plasma clearance was 10 - 15 times greater than in the normals indicating a direct effect of this condition on the excretion of strontium by the kidneys (Jones, Copp, 1951).

Similar investigations by Bohr (1961), who studied the uptake of strontium and calcium in normal and rachitic rats were

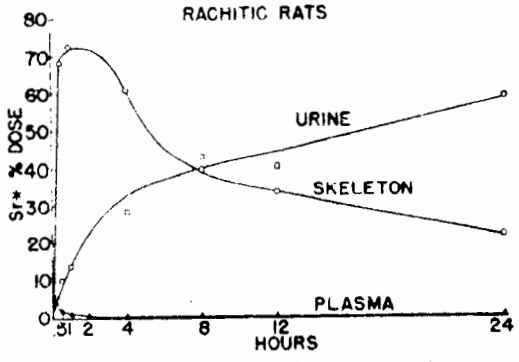
confirmed by using external counting method in the living rats. The results show that beside the more diffuse uptake in the bone tissue itself in both normal and rachitic bone, a heavy accumulation takes place in the metaphysial side of the epiphyseal line in the normal bone which has almost no corresponding phenomenon in the rachitic bone.



Distribution and excretion of radiostrontium in adult female rats after intraperitoneal administration.



Distribution and excretion of radiostrontium in young female rats after intraperitoneal administration.



Distribution and excretion of radiostrontium in young rachitic female rats after intraperitoneal administration.

Fig. 8.
(From Jones, Copp, 1951).

Loeser and Konwiser (1929) found in rats that strontium when administered subcutaneously is eliminated by the intestines, while urine contained only traces of it. However, McCance and Widdowson, (1939) studied the strontium excretion in themselves. The urinary/fecal excretion ratio was 1.4 to 1 and 4.3 to 1 respectively, thus showing that up to 90% may be excreted by the kidneys.

Fay et al (1942) found in dogs that strontium is excreted mostly in feces when ingested and in the urine when injected. When strontium was fed for 20 - 40 days, 28 - 87% was excreted mostly in the feces. On discontinuation of the feeding, it disappears rapidly from the feces, but more slowly from the urine.

Mineralization Vs. Viability.

Ray, Violette, Buckley and Mosiman (1958) concluded that viability of the cells is not essential in the process of inorganic salt uptake by the bone, provided the organic matrix is present and normal. Mobilization of the inorganic salt, on the other hand, is facilitated by the presence of the living cells. Uptake of radioactivity is therefore probably not an accurate index of bone cell "viability". Their studies also showed that radiographic density

in aseptic necrosis of bone is probably the result of failure of mobilization of inorganic salts and continued uptake by the necrotic bone from the tissue fluids and that it may be that the bone seeking radioisotopes probably become relatively fixed in the skeleton when radiation damage is sufficient to cause necrosis of the cells.

Metabolism in Man:-

Spencer, Laszlo, Brothers (1957) reported strontium 85 and calcium 45 metabolism in man. They used oral route in 6 patients and intravenous route in 4 patients. The main route of excretion for strontium 85 is via kidney, irrespective of the route of administration. The urinary excretion differs in different subjects depending upon the state of metabolism of the skeleton. About 10% was excreted through the G.I.T. Their studies also show poor absorption from the gastrointestinal tract with 80% being unabsorbed. Body load was 4 - 6 times higher after I. V. than oral route. They showed that oral calcium has the higher absorption while strontium has a higher excretion rate.

The behaviour of radiostrontium in man was found to be similar to that in animals. When adults ingested strontium 90 in a water solution, about 74% was excreted via the feces and 6% via the urine.

The absorption occurred mainly within 4 hours after ingestion (Comar et al, 1957).

When radiostrontium and radiocalcium are fed simultaneously in the milk, with each meal for several days, there is a discrimination as to retention against strontium by a factor of about 2. This is due to preferential absorption of calcium and preferential urinary excretion of strontium (Comar, Wasserman, Ullberg, Andrews, 1957).

Studies using external counting techniques after introduction of suitable isotopes in the humans have been reported by many authors (e.g. Miller, Munro, Renchler and Wilson, 1954, Bauer, 1954, Van der Werff, 1954, Dudley, Markowitz and Mitchell, 1956, Desgrez, Guerin and Guerin, 1958, Bauer, Carlsson and Lindquist, 1958, Bauer, Ray, 1958, Bauer, Wendeberg, 1959).

Bauer and Wendeberg studied 75 patients in order to localise and delineate skeletal lesions after administration of Ca47 and Sr85. They found, by external counting method that the activity was high in skeletal lesions like fractures, metastatic carcinoma, eosinophilic granuloma, chondromas and Paget's disease. The high uptake is interpreted as "evidence of increased rate of bone tissue turn over".

Comparative Metabolism:-

Pecher (1941) was the first to demonstrate similarity in the

biological behaviour of radiocalcium and radiostrontium, confirmed by Copp et al, 1947, Hamilton, 1947, Kidman et al, 1950. The two compete with each other (Wasserman, Comar, 1957), and since on a long term basis, the human body burden will result from Sr90 contamination of dietary calcium, it becomes necessary to study their comparative behaviour.

Alexander, Nusbaum (1959) and Alexander, Nusbaum, MacDonald (1956) studied the relative retention of strontium and calcium in various animals and in human bones. They introduced the term 'bone retention factor' to describe the ratio of the concentration of an element with respect to calcium for bone tissue divided by the ratio for the diet. The bone retention factor for strontium in mice was 0.35, in rats 0.27, guinea pigs 0.22. Among Nevada desert animals, the bone retention factor for jack rabbits was 0.20, cotton tail rabbits 0.22 and kangaroo rats 0.16. They also examined samples of human rib, milk, vegetable, dairy cattle feed, which were collected from 5 different regions in the United States and analysed for Sr 90 and Ca 45 by emission spectrographic techniques. A retention factor between cow's milk and feed was determined at 0.13 - 0.03 and the human bone retention factor of 0.18 - 0.05 was determined by comparing Sr - Ca ration observed for the human rib samples and the ratio estimated for the respective diets.

a) Dietary Calcium and Radiostrontium Retention:-

Due to its chemical similarities to calcium, strontium will appear in biological materials in most of the positions normally occupied by calcium. Since these two compete with each other in metabolic reactions, the possibility of reducing radiostrontium assimilation by increasing the dietary calcium was studied (Palmer et al, 1958). Groups of young rats were given a diet with three different levels of dietary calcium, 0.5, 1.0 and 2.0%. These diets were fed for 15 days in one series and 45 days in another. During the last 7 days of each experiment the drinking water for fortified with strontium 85. At the termination of the experiment the percentage of the ingested radiostrontium retained was determined. In the short term experiment, animals fed on 2.0% calcium retained only one third as much radiostrontium as did animals fed 0.5% calcium. In the second series (45 days series) increasing the dietary calcium from 0.5 to 2.0% reduced carcass radiostrontium from 17.7% of the ingested dose to 3.8%. This demonstrates clearly that increasing the dietary calcium intake will reduce the assimilation of radiostrontium. The practical implications are obvious. In areas where there is exposure to large quantities of strontium 90,

it may be advisable to recommend increases in dietary calcium. Such calcium should be uncontaminated with strontium 90 and could be obtained from deep mineral deposits.

b) Effect of Calcium on Deposition of Strontium 90 and Calcium 45.

Palmer, Thompson, Kornberg (1958) studied the effect of calcium on deposition of strontium 90 and calcium 45 in rats. 3 groups of 16 mature female rats were maintained for 30 days on diets containing 0.5% phosphorus, 0.1, 0.5, 2.0% calcium. After this strontium 90, calcium 45 and insoluble Cr203 labelled with Cr51 were added. One femur and blood samples were assayed for strontium and calcium 45. Total Ca and P were determined in the other femur. Food consumption was estimated (Sr 90 and Ca 45 intake) by Cr51 measurements in total feces and gastrointestinal tract. Food consumption was practically the same in the three groups. For Sr 90 and Ca 45 concentrations in femur, within each dietary group, there was an evident trend towards lower values for Sr 90/Ca45 ratio, as time on the labelled diet increased. Sr90/Ca45 ration is a function of dietary calcium level. While the content of both Sr90 and Ca45 in bone varies inversely with dietary calcium level, the effect is quantitatively different for

the two isotopes and is not simply proportional in either case. Reducing calcium five folds from 0.5 to 0.1% of the diet, increased the percentage reduction of Ca45 by a factor of approximately 4, at all time periods, but increased Sr90 retention by a factor of only 2 to 3. Increasing calcium 4 fold from 0.5 to 2.0% of the diet decreased the percentage retention of calcium 45 about 2 fold, while having no significant effect on Sr90 retention. Blood levels of Sr90 and Ca45 at different times of sacrifice were reasonably constant within a given dietary group. Between dietary groups, the blood levels of Sr90 and Ca45 varied in essentially the same manner as their concentrations in the femur. In general, for a given group of animals the ratio of Sr90/Ca45 in the blood was not significantly different from the ratio of Sr90/Ca45 in the bone. This suggests that the major discrimination between dietary calcium and strontium does not occur in the specific processes of deposition in the bone.

Langemann (1957) studied the comparative metabolism of Sr89 and Ca45 in vitro. Calcium was preferentially abosorbed from the gastrointestinal tract and strontium was excreted to a greater extent than calcium by the kidney. Bone exhibits an overall discrimination against strontium in favour of calcium. Their data

shows that strontium is favoured in initial uptake by bone by a ratio of 1.08 and during removal processes more strontium was lost than calcium by a factor of 1.2. Their results show that 2 hours after administration, bone contained 1.08 times as much Sr89 as Ca45. When exposed to constant levels of Sr89 and Ca45, the ratio equilibrated at 0.03 within 7 days. Sr89 was released at a ratio of 1.2 times that of Ca45. Addition of carrier strontium markedly inhibited the long term deposition of calcium in bone, but the discriminatory mechanisms were unaltered.

Radioactive Strontium and Carcinogenesis:-

Ionizing radiation has a blastomatogenic property. Radiation from radioactive elements entering the organism can lead to malignant growth of that organ or tissue subjected to their influence. Many elements possess the capacity of depositing and maintaining themselves for a long time in the bone. Bone tumours induced by the radioactive substances fixed in bone are most easily induced in rats. 40 to 80% of rats develop osteogenic sarcoma in 6 - 8 months. 80% rats developed tumours upon introduction of Sr89, 90 (Litvinov, 1956).

Radioactive strontium upon introduction in the body of the organism deposits itself at sites of reformations of bone substances.

In growing rats it is the areas of endochondral ossification. Also, the areas of maximum growth, proximal tibia and distal femur accumulate maximum uptake. The most marked morphological changes culminate in disturbances of the process of endochondral bone formation and resorption of parts of the reformed bone substances with the development of cellular-fibrous tissue containing a large amount of osteoclasts. Subsequently the growth of the bone in length is slowed down and distorted. Formation of pathological bone substance which does not undergo further remodelling occurs.

In the metaphysis, around the remaining nonresorbed bone substance and newly formed pathological bone substance, there occurs the growth of the cellular-fibrous tissue with formation of atypical immature bone substance, differing in appearance. With passage of time, the process of bone formation is increasingly disturbed. Atypical osteogenic cells appear. They quickly multiply without producing basic bone substance. Then these tissue formations, having a tumor-like appearance, spread at the side of the epiphyseal-chondral plate, filling the cavity of the metaphysis and through the bone marrow to the diaphysis. Usually the tumorous masses penetrate through the destroyed cortical substance of the

metaphysis and penetration outside the bone takes place early. The external tumorous growth often reach large proportion. Hemorrhage and ulceration of the skin are encountered. Histologically the cellular elements consist of productive osteoblasts and fibroblasts and less often cartilage cells.

Thus radioactive substances produce a model experiment for the production and study of tumours of bone. The tumours develop at the site of most marked morphological changes, e.g. in the metaphyses of long bones. Malignant growth is preceded by a long latent period of distorted bone formation with disturbances of the mutual relationship between resorption and reformation of the bone, and development of cellular-fibrous tissue which undergoes characteristic changes.

Kuzma, Finkel, Brues, Owen, Sissons, Vaughan and many others report on the carcinogenic potentialities of radioactive strontium.

Late effects of Sr89 administration in rats were investigated by Skoryna and Kahn in the Department of Experimental Surgery, McGill University. Sr89 was administered intraperitoneally in doses of 0.9 uc per gm. body weight as the initial dose, followed by 5 injections of 0.7 uc per gm. body weight at monthly intervals. 63% of the animals survived the minimal latent period of 188 days.

Neoplastic changes on microscopic examinations were found in all animals. 89 rats out of 100 had gross palpable tumours.

Radioactive Isotope and Bone:-

In the evaluation of in vivo distribution of any radioactive material, consideration must be given not only to the sites that concentrate the substance most avidly, but also to the excretion or release of the material. The avidity of the bone has been noted by Anthony et al, 1947, Asling, Heller and Copp, 1949, Chaikoff, Fishler, Entenmann, Hamilton, Jones et al, Richmond, 1958.

Within 24 hours the majority of the retained isotope is deposited in the bone. Singer and Armstrong (1957), studying large rats, employed radioactive calcium and determined specific activities of the femoral diaphyses, femoral epiphyses, humeri, lumbar vertebrae, and total skeleton. The values were different on the first day, but these differences decreased thereafter until nearly identical values for all bones were obtained on the fifty-second day. These findings indicate that an intraskeletal redistribution of the exchangeable isotope occurs during the same period.

Since strontium is rapidly deposited in bone, the excretion curves can be evaluated by considering the isotope as being distributed in a two compartmental system, skeletal compartment which constitutes 99% of the retained material, and kidneys and intestines

which clear a fractional amount of the isotope. This becomes available through equilibration of the isotope in the skeleton with that in the plasma.

In younger animals there is decreased retention of the isotope. Although the initial uptake is high in younger animals, the rapid rate of remodelling of the growing skeleton permits the strontium taken up by bone to become available for excretion shortly after injection. (Copp et al, Jones and Copp, 1951). In the mature animals, 36 days after injection (Cordery et al, 1960) the retention of the isotope in 5 months old animals was approximately 30% less than that in 5 - 1/2 months old. At the same period of the experiment the retention was decreased an additional 20% in 6 months old animals. A difference in age of as little as 10% resulted in significant differences in excretion.

Mechanism of Deposition of Radioactive Isotopes:-

It has been shown that a high uptake of Sr90 occurs both in vivo and vitro in low mineralized areas of bone (Engfeldt, Bjoruerstedt, Clemedson, Engstrom, 1954).

There are two main views concerning the mechanism of deposition of this isotope. Reversible exchange theory is led by Neuman while accretion and resorbtion theory is led by Carlsson

and Bauer.

The distribution is not uniform throughout the skeleton, but varies in different bones and in different parts of the same bone. There is greater concentration in areas of active growth and in the most recently deposited mineral. In established bone the isotope deposition, though considerable, is much less than that in areas of growth. The age of the animal determines the pattern of distribtuion. Radioautographic studies show a diffuse general, transient distribution in the young animal and a spotty distribtuion of discrete loci of intense radioactivity in the adult. The loci correspond to haversian systems, which are of recent origin. In both the young and adult animals the resorptive processes and reconstruction of the haversian systems are important in the isotope deposition (Neuman, Neuman, 1953).

Bone crystals have a hydration layer which provides an intimate contact between crystal and the organic phase without exposure of the crystallite surface, and also efficiently isolates the adult bone matrix from the circulating fluids. It is the surface of the crystal that equilibrizes with its surrounding fluids. Surface phenomenon include :

1. Ionic Exchange;

a) Isoionic: a process by which ions from the solution phase exchange with similar ions in the surface of the solid phase

with no net change in the composition of the two phases calcium and phosphorus.

b) Heteroionic: when an ion in the crystal surface is being displaced by a different ion from the solution, uranyl, strontium, sodium and hydronium ions may displace surface calcium. Carbonate ion may displace surface phosphate or hydroxyl ion and fluoride may displace hydroxyl and bicarbonate ions.

2. Recrystallization: is a slow equilibrization of the crystal interior with the solution phase. Bone hydroxyapatite crystals undergo spontaneous recrystallization in an aqeous medium.

"Foreign ions" like sodium, magnesium, carbon dioxide and citrate constitute the physiologic group while the unphysiologic group includes fluoride, strontium, cerium, zinconium, plutonium, uranyl, barium, lead and yttrium (Fig. 9). These may be grouped in 3 categories:

1. Those cations which deposit in the mineral crystals of bone by heteroionic exchange for surface calcium. They show a pattern of skeletal deposition, mobilization and redistribution quantitatively very similar to that of calcium, but they may differ with respect to their distribution in the soft tissues.

II. Cations which deposit in the organic matrix of bone, plutonium, yttrium, barium, zinconium, cerium, gallium.

III. A small number of anions which have been found to concentrate in bone, CO_2, fluoride and citrate appear to be bound to the bone mineral by a process of surface exchange.

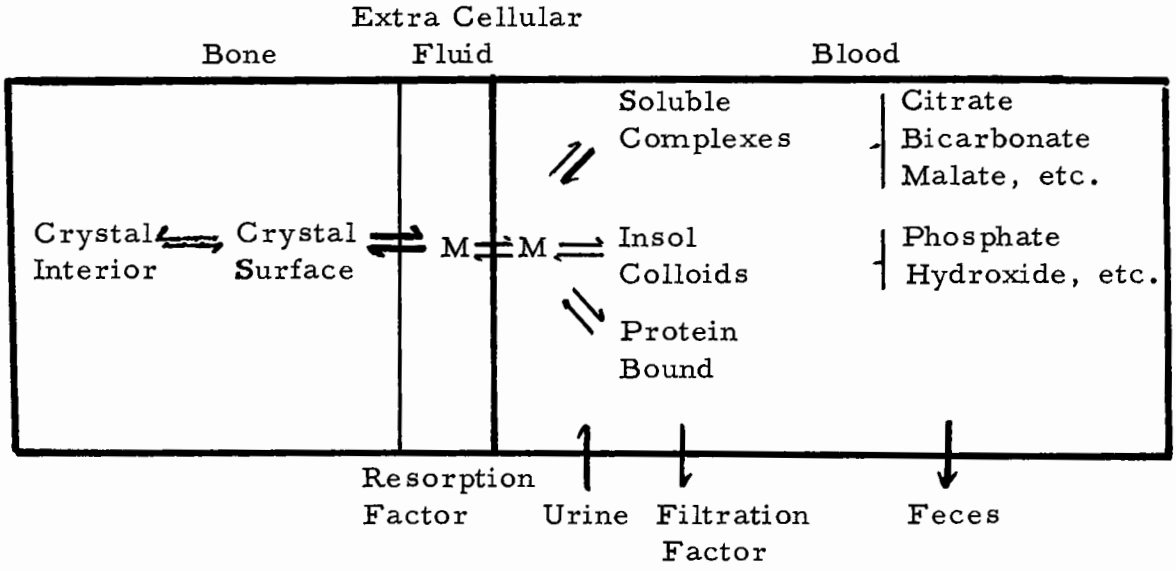


Fig. 9.

Diagram illustrating the principal factors governing the distribution and excretion of foreign cations (Drawn from 'Nature of the mineral phase of bone', Neuman, W.F., Neuman, M.W., Chem. Review, 53, 1953).

MacDonald et al (1951) carried out x-ray diffraction studies on stable strontium and lead. According to their findings the cations could be chemically bound to one or more of the organic components of the bone, deposited as discrete crystals, physically absorbed on the crystallite surface, or assume a position within the crystallite structure of the inorganic salt. They concluded that surface Ca^{++} ions comprise about 6% of the total number of Ca^{++}

ions present in a crystal of the order of 10^{-6} cm in size. Mouse femur ash contained 8% of Sr and so on the basis of 1:1 replacement;this corresponds to a substition of 10% of the total number of Ca^{++} ions by Sr^{++} ions. Some Sr and Pb was found in the interior of the crystal, though a large portion was fixed by exchange with Ca^{++} ions near the crystal surface. It may be that organic binding, surface absorption or exchange constitute the initial processes and that incorporation with the lattice of the bone salt proceeds at a much slower rate.

Mobilization:-

The following is the summary of the discussion of mobilization of the isotopes by Neuman, Neuman, (1953).

While incorporation is rapid, mobilization is very slow. The rate of exchange is a function of the differences between the specific activities (SA) of the two fractions interacting. According to ultramicroscopic location, 3 fractions of a given ion pool may be designed:

a) The ion in the extracellular fluid,

b) The ion in the surface of the available bone crystals,

c) The ion in the interior of the available crystals.

The rate of net transfer of the isotope from one fraction to another is represented as:

$$R_{a-b} = K(SA_a - SA_b)$$

and

$$R_{b-c} = K(SA_b - SA_c)$$

Shortly after the administration of the isotope, the rate of incorporation will be rapid because SA differences are necessarily larger. After a few days or weeks, equilibrium state is established where $SA_a = SA_b = SA_c$ and no net transfer of the isotope can occur. The only reason the isotope leaves the bone at all is that the animal consumes a variable amount of non-isotopic ion in the food, which lowers the SA of the circulating fluid, causing the direction of isotopic transfer to reverse. Since the SA differences are small, the rate of reversal of the skeletally fixed isotope is also small.

The retention of the isotope in young growing animals is nearly complete. Though the isotope is retained, the specific activities of the bone falls as more and more non-isotopic material derived from the diet is added to the growing skeleton. In the adult, greater percentage is excreted. After SA equilibrium is reached, the blood SA keeps falling as it is diluted by the non-labelled ion. Since the adult is not in positive balance, then there is a larger excretion of the ion by the kidney. In its passage through the body, the non-labelled dietary material draws the

isotope from the bones as it reverses the SA relationship.

Physiological growth affects the overall retention of the isotopes. The new crystals formed shortly after an injection will 'bury' the isotope and this can be released only very slowly. In relation to the calcified cartilage of endochondral bone formation, as this is replaced by bone, the isotope buried in the crystals is released into the circulating fluid and redistributed and redeposited into crystals.

CHAPTER V.

FRACTURE HEALING.

Introduction.

As Moore (1959) has stated "Bones - healthy bones - do not break easily". It requires the application of a sudden strong force to produce a major fracture in a healthy individual.

Urist and Johnson (1943) state that since Hippocrates' time, more than 4,000 works have been published on the problem of the healing of fractures.

Repair of bone is an active process. It is not a local mechanism but rather the whole skeleton that participates in fracture repair. The general reaction includes nervous, vascular and hormonal factors constituting a stress-like reaction.

The repair of bone is dependent upon the participation of living cells whose survival and ability to carry out their specialized functions depend upon a proper supply of blood to the affected site. Importance of blood supply is shown by the absence of any evidence of repair in fragments of bone that have been deprived of their blood supply.

Repair Process.

A general characteristic of biological tissues is that injury,

within limits, serves as a 'stimulus' to cellular proliferation and differentiation. The term 'stimulation' is used to describe clinical and experimental efforts to activate the cells, associated with bone in order to form new bone.

Bone healing is a continuous process. Forces put into action with damage of the blood vessels and extravasation of the blood at the time of fracture continue for months and years, leading to complete removal of all traces of injury and bony overgrowth by which repair was accomplished (Bennet, 1944). According to Gallie, Robertson (1919-20), Pollack, Ghormoley (1941) Urist, Johnson, (1943), Bennet (1944), Marshak et al (1945), Copp, Greenberg, (1945), Urist, McLean (1959 - 1953), Mosiman (1950), Phemister (1951), Urist, Mazet, McLean (1954), Yamagishi, Yoshimura (1955), Bohr (1955) Ham, Harried (1956), Bridges, Pritchard (1957) and many others, the basic concept of fracture healing is as follows: -

a) Hematoma formation

b) Inflammatory reaction

c) Fibrous Callus

d) Fibrocartilaginous callus (variable)

e) Bony callus

f) Reconstruction.

As the steps in fracture healing are well accepted, no specific review of these will be undertaken. Only those aspects of the process which are pertinent to this study will be mentioned.

Mineralization of the Callus.

Nilsonne (1959) studied the mineralization process in diaphyseal fractures of rats and dogs by autoradiographic and x-ray diffraction techniques. Dog skeleton normally has a more inhomogenous distribution of bone salts than that of rat. In rats and dogs the mineralization starts mainly in the periosteal region. It affects a greater part of the fractured bone in the rat, while it is more limited to the fracture region in the dog. Mineralization is a continuous process, characterized by a locally inhomogenous distribution of mineral salt in the periosteal callus and in the intermediary callus area.

First trace of mineralization in the callus is observed as early as the 4th day after the fracture. It is found in the periosteal callus and becomes more evident and spreads along the whole length of the fractured bone. The periosteum grows thicker and at the same time becomes raised from the surface of the bone towards the fracture ends and spreads outwards over the callus area. After one week, the periosteal cuff is still sparsely mineralized, but after three weeks, the mineralization is greatly increased, particularly

near the fracture ends, although it still shows a variable degree of mineralization.

The intermediate callus at first has low mineralization. At the 3rd week it develops into irregularly shaped bone trabeculae, lacking any orderly arrangement, with varying degrees of mineralization. Mineral content is higher near the centre. The part near the fracture is bordered by a thin bubble-shaped structure of cartilage-like appearance which in places are relatively well mineralized.

As the process proceeds, the periosteal cuff spreads farther over the callus area, showing a higher mineral content in its outer layer than in the side facing the callus.

This unequal distribution of bone salt continues for a very long time, even after a mineralized continuity between the fracture ends has been achieved and the rebuilding of the fracture area is in the final stage.

Bone Repair in Man Vs Animals.

Generally in man, the repair is a slow process, there is much less provisional callus, ensheathing the site of the fracture and there is less tendency for cartilage to appear in the early stages of repair. Both these differences may be associated with the lesser opportunity

that an animal has of resting, and so immobilising the fractured bone and its surrounding soft parts. It is interesting to observe in this connection, that it is in fractures of the ribs, which are necessarily in constant movement, that bone repair in man resembles that in animals most closely. Wright (1956). The significance of this feature is that in animals with a definite cartilage stage, mineralization takes place when the cartilage is to be replaced by bone. This mineralization of the cartilage is greater in animals than in man.

Functional Reconstruction.

During callus formation, bone is produced in excess as a protective measure. As Paget remarked 'safety is provided first, shape later on'. The volume of the callus is greatest on the concave side of the angulation. After bone consolidation is achieved, the surplus bone is removed by resorption, and finally original shape and outline is re-established. Functional reconstruction of the callus is a slow process.

In interpreting the present experiment it is important to remember that during the process of remodelling resorption of the excess bone is accomplished by release of the mineral material of that bone.

PART II.

CHAPTER VI.

EXPERIMENTAL STUDY.

The aim of the present study is two-fold:-

A. To study fracture healing and the various factors influencing it in male, R. V. H. strain rats.

B. To study osteogenesis utilizing Sr89 as a marker.

Experiment A.

Study of normal fracture healing in male, 4 weeks old rats (R. V. H. strain) and the effects of various procedures (315 rats).

Group I (T). Fracture right femur to establish the normal pattern of repair (50 rats).

Group II (TP) Fracture right femur, immobilized with external plaster spica (30 rats).

Group III (TG) Fracture right femur, with excision of 3 mm fragment of diaphysis to produce a gap (30 rats).

Group IV (PRT) Fracture right femur, with excision of periosteum from each fractured end, for 3 mm distance (30 rats).

Group V (TM) Fracture right femur, with section of all the musculature of the thigh (30 rats).

Group VI (TE) Fracture right femur, with brushing of the endosteum from each fractured end (30 rats).

Group VII (PRTM) Fracture right femur, with excision of periosteum and section of the thigh musculature (30 rats).

Group VIII (MT) Fracture right femur, with repeated fracture at the same site by manual manipulation once a week for 4 weeks (30 rats).

Group IX (LCT) Fracture right femur in rats kept on low calcium and Vitamin D deficient diet*, no immobilization (30 rats).

Group X (P) Excision of periosteal cuff, 1 cm wide from the diaphysis of right femur. No fracture. (35 rats).

*Calculated analysis:

Protein (crude)	28.6%
Fat	1.9%
Fibre	4.6%
Calcium (contained in vegetable ingredients)	0.153%
Phosphorus (total)	0.44%
Available phosphorus	0.132%
Salt	0.25%
Productive energy, based on poultry table	850 Cal/lb
Vitamin A (added)	7.087 U.I./lb

This diet consists of :Corn gluten meal, soyabean oilcake meal, brewer's yeast, yellow corn meal, common fine salt, manganese, iodine, iron, copper, zinc, cobalt, vitamin concentrate, choline chloride, vitamin B_{12}.

Experiment B:-

Study of Osteogenesis utilizing Radioactive Strontium 89 as a Marker (800 rats).

This includes the following groups. A total of 800 rats were used in this study:-

Group BI Study of uptake of radioactivity at the fracture site as compared to the diaphysis of the contralateral femur, from post-fracture day 1 to 289.(Uptake study).

Group B II Study of uptake of radioactivity at the fracture site in comparison to uptake at proximal and distal metaphyses on the fractured femur and diaphysis and the two metaphyses in the contralateral femur (Correlative Study).

Group B III Study of 'turn-over' of radioactivity at the fracture site and its relation to 'turn-over' at other sites (contralateral diaphysis and the metaphyses), at various intervals after injection of Sr89. This study included groups of fractures at different post-fracture

intervals. (Retention or 'Turn-over' study).

Group B IV Study of cumulative effect of small doses (0.1 uc per gm body weight) of Sr 89 (Cumulative study).

Group B V Effect of low calcium and Vitamin D deficient diet in rats with and without fractures of right femur and comparison with findings in rats on standard diet (Groups B I, B II, B III and B IV).

A dosimetric study is in progress, but is not far enough advanced to be reported in this thesis.

CHAPTER VII.

METHODS.

Experimental Animals:-

The rats, male, R. V. H. strain, were 4 weeks old at the time of induction of fractures, with average weight ranging from 75 - 90 gms. Three rats were caged in each cage, fed on water and standard Purina diet. The animals on low calcium diet were put on a calcium and vitamin D deficient diet from the age of weaning, 3 weeks old, for a period of 10 - 15 days before the induction of fracture and were maintained on the same deficient diet all through the experiment. The rats were housed in two rooms, an ordinary animal room where all operated animals are kept in their cages on racks and another radioactive animal room where all animals injected with the isotope are transferred and kept afterwards till the end of the experiment.

Anesthesia:-

Open fracture is made in the right femurs of rats under Nembutal anesthesia. Nembutal was given intraperitoneally in doses of 0.08 ml. per 100 gms. body weight. The dose is significantly reduced for rats on a low calcium diet, approximately 0.04 - 0.05 ml per 100 gms. body weight.

Operative Procedure:- (Fig. 10).

Group I (T). Transverse fracture in right femur diaphysis:-

After anesthesia the rat was placed in the left lateral position, the skin painted with an antiseptic and a one half inch long incision made on the lateral aspect of the right thigh, in line with its long axis. With the scissors, the skin edges were widely separated from the underlying musculature. A white line could be very well visualized on the lateral aspect of the thigh musculature between the anterior group and the posterior group of muscles. This represents the lateral intermuscular septum. With blunt dissection through the lateral white line the whole length of the shaft of the femur was easily exposed without any damage to the muscle or any significant hemorrhage.

The periosteum was incised on the diaphysis in line with the shaft and the shaft fractured at the selected site and with the blades of a strong scissor. In 4 weeks old rats the femur is easy to divide with a pair of scissors.

No attempt was made to reduce the fracture to its anatomical position, though the position was brought to as near a position as possible. Simple skin closure was performed.

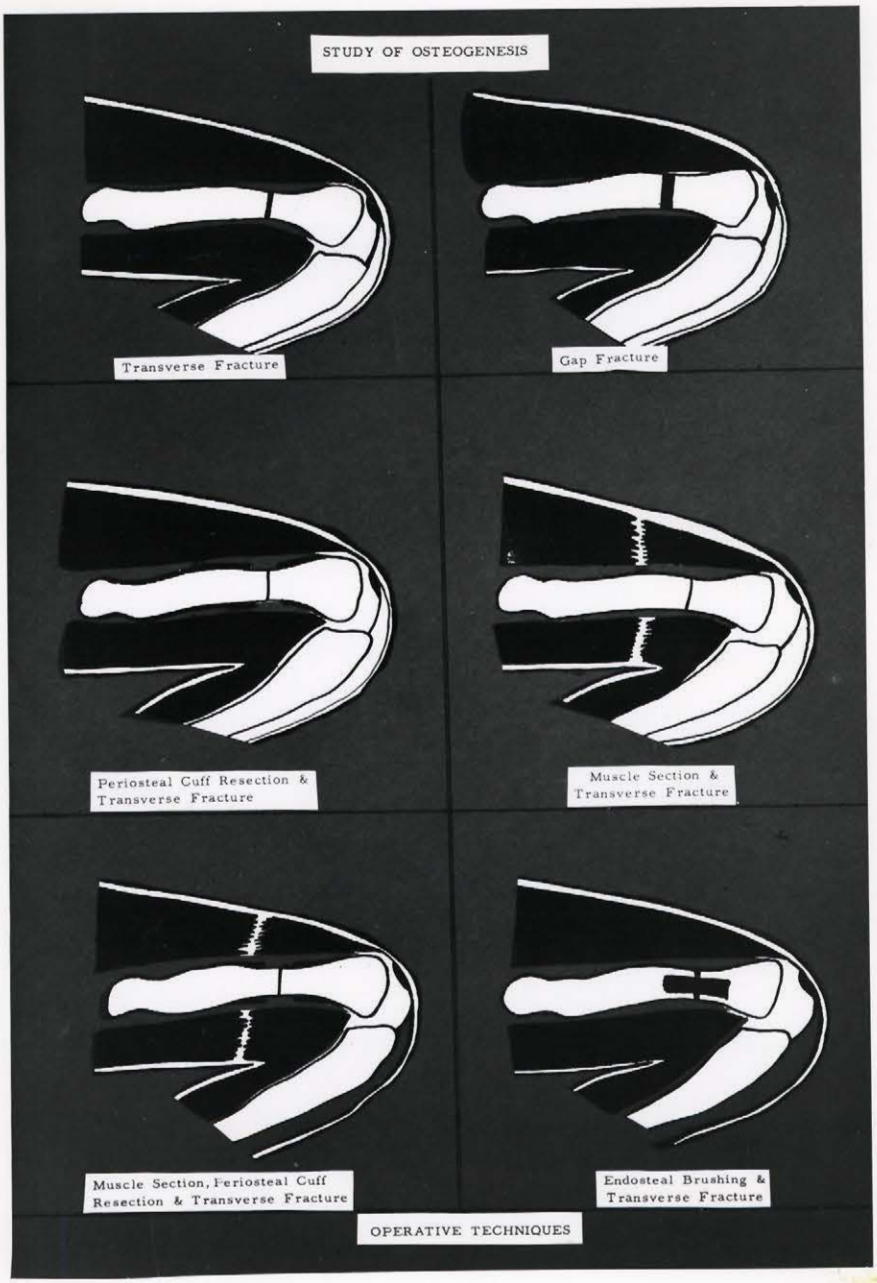


Fig. 10.

Operative Techniques for Producing Fracture.

Right femur was exposed through lateral intermuscular route. Periosteum was incised in line with the shaft and a transverse fracture of the diaphysis was produced. No attempt was made to reduce the fracture to anatomical position. Simple skin closure was done. For details, see the text.

External Immobilization Series:-

Group II (TP). After the fracture was completed and the skin closed, the right limb was immobilized in a plaster hip-spica.

Gap Series:-

Group III (TG). Approximately 3 mm of bone was removed from one fracture end after inducing the fracture. No immobilization was used.

Periosteal Cuff Resection Series:-

Group IV (PRT). After the fracture was induced, the ends of the femur were delivered into the wound and stabilised by inserting a straight needle temporarily into the medullary canal. Then a cuff of periosteum about 3 mm in width was removed from each fractured end of the femur. The ends were replaced into the wound in as close an apposed position as possible, and the wound closed.

Muscle Section Series:-

Group V (TM) The anterior and posterior group of thigh muscles were sectioned after the fracture was produced, taking care not to traumatise the main artery and the nerve. No gross bleeding resulted, except if the artery is divided when the animal died of hemorrhagic shock. Only the skin was closed. No immobilization was provided.

Endosteal Brushing Series:-

Group VI (TE). The fracture ends were delivered into the wound and the endosteum brushed and destroyed for a distance of about 3 - 5 mm from each fracture end. The wound was closed and no immobilization provided.

Muscle Section and Periosteal Cuff Resection Series:-

Group VII (PRTM). After the fracture is induced, a cuff of periosteum was removed and the thigh muscles sectioned. Only the skin was closed and no immobilization provided.

Repeated Fracture Series:-

Group VIII (MT). The fracture was produced as above. At weekly intervals the leg was manipulated under anesthesia and the fracture refractured. It was thus subjected to 4 refractures and then allowed to heal.

Transverse Fracture in Rats on Low Calcium and Vitamin D Deficient Diet.

Group IX (LCT). After 15 days on a low calcium and vitamin D deficient diet, 5 rats were sacrificed for histology and in the remaining group, open fracture was produced as in the other groups. No immobilization was provided.

Periosteal Cuff Resection Only.

Group X (P). No fracture was induced. The femur was exposed and then a cuff of periosteum from the shaft about 6 mm in width was excised and the wound closed.

Experiment B.

Study of Osteogenesis Utilizing Radioactive Strontium 89 as a marker. Animals with fractures produced as in Part A were used for this study.

Uptake Study.

Group B I. This study utilizes rats with fractures of the right femur which had been produced from 1 to 289 days before they were taken into this study. The animals with fractures were selected from the 'fractured stock' which had been kept in the ordinary animal room and transferred to the 'radioactive animal room'. One dose of 0.1 uc per gm body weight was injected intraperitoneally and the animals were sacrificed 24 hours after the administration of the isotope. The uptake at fracture site and contralateral site was compared and studied.

Group B II. Correlative Study of uptake at fracture site, contralateral site and proximal and distal metaphysis of both the ipsilateral and contralateral femurs was studied.

'Retention', 'Turn-Over' Study;-

Group B III. Groups of animals with fractures of varying age were injected intraperitoneally with one dose of Sr 89, 0.1 uc per gm body weight and kept in the radioactive animal room. Animals from each group were sacrificed at different intervals ranging from post-injection day 1 to 192 days or more.

'Cumulative' Study:-

Group B IV. Animals with a fracture, one day old, were injected intraperitoneally with Sr 89, 0.1 uc per gm body weight. The strontium injections were repeated twice a week. Groups of animals after 20, 25, 30, 35, 40, 45, 50, 53 injections were separated off and kept for subsequent follow-up study. Some animals from each group were sacrificed at varying intervals to measure the radioactivity at various sites (as above) and other animals were followed for tumour formation.

'Low Calcium and Vitamin D Deficient Diet Series'.

Group B V. Uptake, correlative and cumulative studies were carried out in the rats who were kept on a low calcium and vitamin D deficient diet from the time they were weaned (3 weeks old) till the end of the experiment. The uptake, correlative and

cumulative studies were made in the same way as in Experimental Groups B I, B II, B III and B IV.

Dosimetric Study:-

Group B VI. Animals with fractures were injected one-day post-fracture with varying dosage of Sr 89, 1 uc, 2 uc, 3 uc, 4 uc, 5 uc per gm body weight. These groups of animals were given only one injection and kept for follow-up study.

Strontium 89:-

The isotope used in this study, Sr 89 was supplied by Atomic Energy of Canada Limited, Commercial Products Division, Ottawa. It was received monthly as Strontium Chloride in HCl solution. Decay Chart was used to calculate the activity on the day of preparation of the standard solution before the injections were made. Half-life of Sr 89 is 51 days. The standard solution was prepared in such doses that 1 ml contains 10 microcuries. The standard dose throughout the experiment was 0.1 uc per gm body weight, except in the dosimetric study series where the doses varied, being 1, 2, 3, 4, 5 uc per gm body weight.

Radioactive Laboratory:-

This room is provided with an exhaust chamber, where the isotope is kept, solutions made and stored and an oven and a

precision scale. The set of glass-ware used is washed with 'radiowash' and rinsed with tap water 15 - 20 times before being used again. A Geiger-Muller counter was used for measuring radioactivity of the samples. It is located in a separate room where the average background reading is recorded in the neighbourhood of 25 - 30 counts per minute. Allpossible precautions are undertaken while handling or working with radioactive materials.

Disposal:

All discarded material including rats, test tubes, planchets, paper, gloves, etc. are disposed of in special containers provided for this purpose by the Atomic Energy of Canada according to their instructions.

Assay of Radioactivity at Various Sites in Fractured and Non-fractured Femurs:-

a) Dissection:-

Both femurs were dissected out and all soft tissues removed. The gross specimen was examined in terms of union of the fracture, degree of displacement, reconstruction etc. The head of each femur was excised flush with diaphysis. The greater trochanter was removed and the lower end cleaned of the articular surface. The remaining femur was divided into three parts, fracture and the two ends. (Fig. 11). Fracture site included the callus mass and adjacent

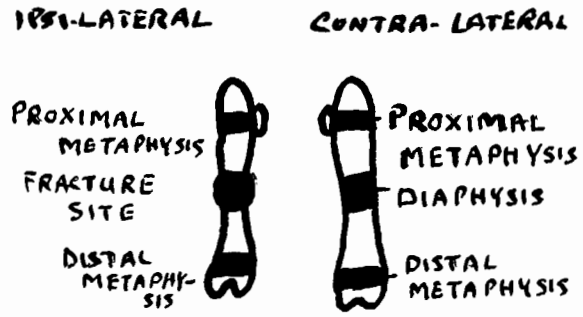


DIAGRAM SHOWING VARIOUS SITES WHICH WERE MEASURED FOR RADIOACTIVITY.

Fig. 11.

1/2 cm of shaft on each side. The callus mass whenever possible was separated from these adjacent areas and measure of radio-activity in the callus and the fracture ends performed.

b) Weighing of the Components:-

Each femur has 3 components to be investigated. They were weighed (wet weight) on a precision scale and put into numbered test tubes for identification.2 cc of concentrated hydrochloric acid were added to the test tube and left at room temperature in the 'radioactive laboratory' for 24 hours. Then it was neutralised with 30% sodium hydroxide solution.

c) Dilution:-

The contents of the test tube after neutralisation were made up to a volume of 10 cc by the addition of distilled water, so that all

samples were of equal volume after neutralisation. 2 cc of this diluted solution was transferred to an aluminum planchet.

d) Drying:-

These planchets were dried in an oven for 24 hours.

e) Procedure for Determining Radioactivity:-

1. Instrument: - Geiger-Muller counter, provided with type TGC 2 Geiger tube, with window thickness of 1.8 MG/CM^2.

2. Background: - This is checked before the start, during and after the counting session. It is recorded as counts per minute and is subtracted from the counts of the sample per minute.

3. Planchets:- Aluminum planchets containing the dried samples are placed in the counter and three readings a minute each carried out, and the average count per minute recorded.

4. Correction:- The background count was subtracted from the sample count. In the uptake series (Group B I) allowance for 4% decay from the time of administration to the time of counting (72 hours) was made to arrive at a corrected count per min.

f) Methods of Calculation:-

1. In uptake and correlative studies, counts per minute per mgm body weight are compared with similar counts at corresponding sites in the contralateral femur and the ratio of fractured to non-fractured sites was determined.

2. In order to calculate the increased percentage of uptake at the fracture site, the following formula was used:

$$\text{Increased } \% = \frac{R_{F_f} - R_{F_{cl}}}{R_{F_{cl}}} \times 100$$

where:

R_{F_f} = Corrected count per minute at the fracture site in the femur.

$R_{F_{cl}}$ = Corrected count per minute at the contralateral site in the diaphysis of the opposite femur.

CHAPTER VIII.

RESULTS.

The results of this study can be classified into two major groups:

1) Results of the study of normal osteogenesis and the effect of various procedures on the pattern of healing (Experiment A).
2) Results of the study of osteogenesis using Sr 89 as a marker (Experiment B).

Experiment A.

Study of normal fracture healing in male rats (R. V. H. strain) and the effects of various procedures (310 rats):

Group I (T). Fracture right femur, no immobilization (Fig. 12).

Mortality rate:

Anesthesia: - 0 - 5%

Operative: - 2 - 4%

Post-operative Course:- After the effect of the anesthesia disappeared, the rats became active and moved about in their cages, as if nothing had happened. Weight-bearing on the fractured limb was restricted (Fig. 13) and the animal assumed a 'broad base' position of the hind limbs. This was probably due to angulation at the fracture site under stress of partial weight-bearing on the

limb. Limping was no longer noticable after the 4th - 5th day (Fig. 14).

The histology of the fracture repair process was studied from one-half hour after fracture to 179 post-fracture day. Radiological investigations were carried out from day 2 to day 260 after the fracture. Fig. 15 shows radiograms after 181 days and 260 days after the production of the fracture.

The sequence of events observed in the repair phenomenon is in agreement with the well-established and generally agreed histological changes. Average time required for bony union in this series of displaced diaphyseal fractures of the femur without any immobilization was 100 days. There was some variation from animal to animal. The earliest union was obtained on the 28th post-fracture day while one rat examined at 289 day had only cartilaginous union of the fracture.

Fig. 16. (A, B, C, D) shows the gross appearance of the fractures and the non-fractured contralateral femur at 2, 15, 21 and 28 days after fracture.

Fig. 17. (A, B, C, D) shows the gross appearance of the fracture and the non-fractured contralateral femur at 61, 75, 91 and 137 days after fracture.

Fig. 18. (A, B, C) shows the gross appearance of the fracture and the non-fractured contralateral femur at 144, 153 and 232 days after the fracture.

Fig. 19. shows a fracture one-half hour after operation. There was evidence of fresh hemorrhage and gross displacement of the fragments.

Fig. 20. shows a fracture site 2 hours after the operation. The extent of the hematoma and submuscular spread can be seen. There was some evidence of encapsulation of the hematoma.

Fig. 21. shows a fracture one day after the operation. Note the marked angulation at the fracture site.

Fig. 22. shows a fracture 3 days after the operation. There is gross displacement of the fracture and periosteal proliferation and the medullary plugs are well seen.

Fig. 23. shows a fracture 7 days after operation. The callus was easily palpable and the animal was fully active. Note the appearance of both cartilaginous and bony callus under periosteum and in the medullary canal.

Fig. 24. shows a fracture 10 days after the operation. The callus is further advanced.

Fig. 25. shows a fracture 15 days after operation. There is abundance of callus. The periosteum along the whole

length of the femur contributes to periosteal cuff.

Fig. 26 shows another fracture 15 days after operation. Here the repair has lagged behind that shown in Fig. 25 because of persistence of marked displacement of the fragments.

Fig. 27. shows a fracture 20 days after operation. Various components of the callus can be easily differentiated.

Fig. 28. shows a fracture 30 days after operation. The cartilaginous callus is being replaced by immature bone.

Fig. 29. shows a fracture 40 days after operation. Note the good alignment and the far advanced healing.

Fig. 30. shows a fracture 91 days after operation. Bony union and reconstruction is far advanced.

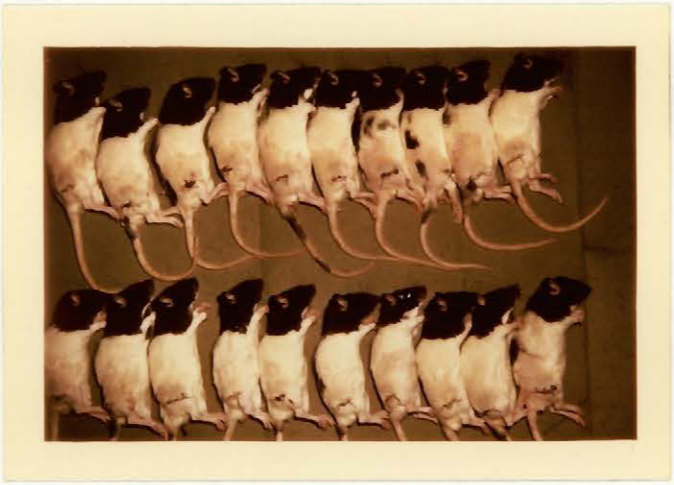


Fig. 12.

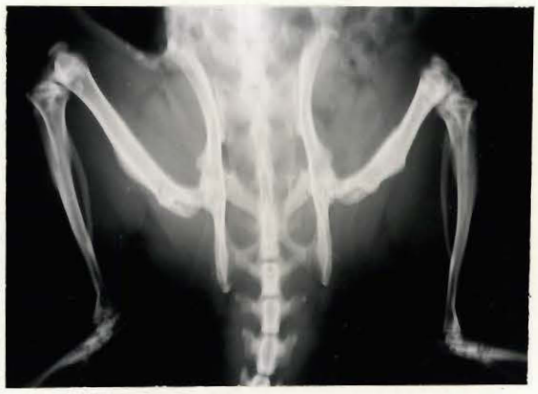
R. V. H. strain, male, 4 weeks old rats. Diaphysis of the right femur was fractured. These rats have not yet recovered from the effect of Nembutal.

Fig. 13.

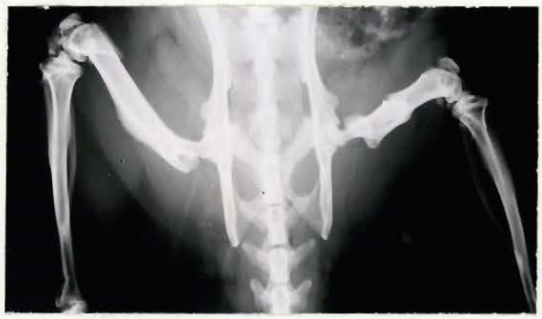
"Guarded weight bearing" and restricted activity was observed post-operatively.

Fig. 14.

Full activity was resumed. Post-operative limping was no longer noticed after the 4th - 5th day.



A. Radiogram showing bony consolidation of the diaphyseal fracture of right femur 181 days after the production of fracture. Note the shortening of the right femur.



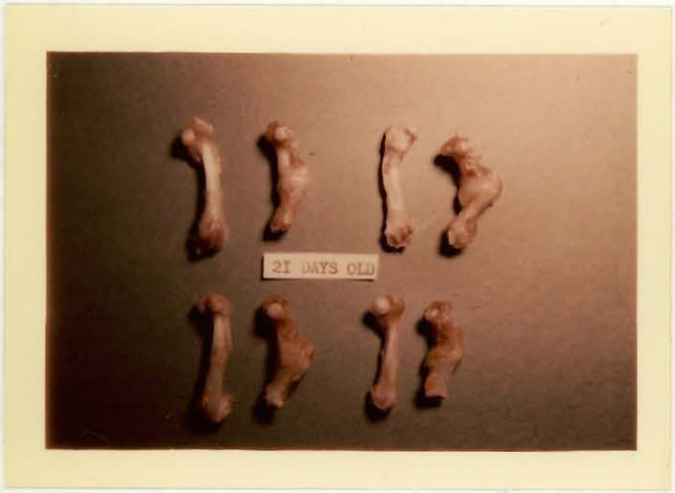
B. Radiogram showing lack of bony union, 260 days after production of fracture. There is marked shortening of the femur.

Fig. 15.

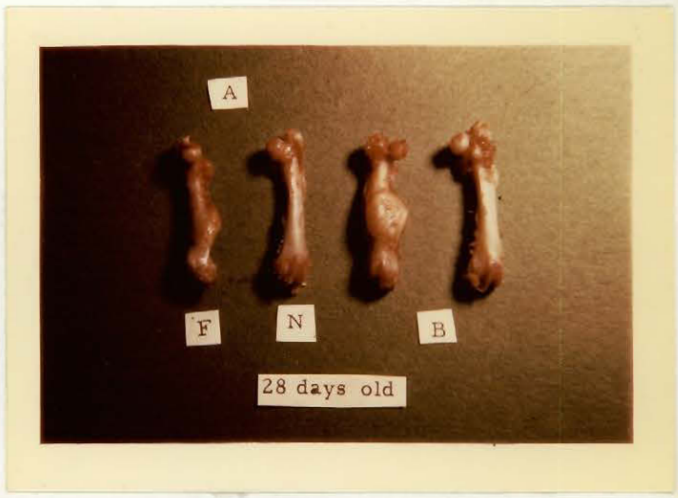
Experiment A, Group I (T).

A. 2 Post-fracture days.

B. 15 Post-fracture days.



C. 21 Post-fracture days.



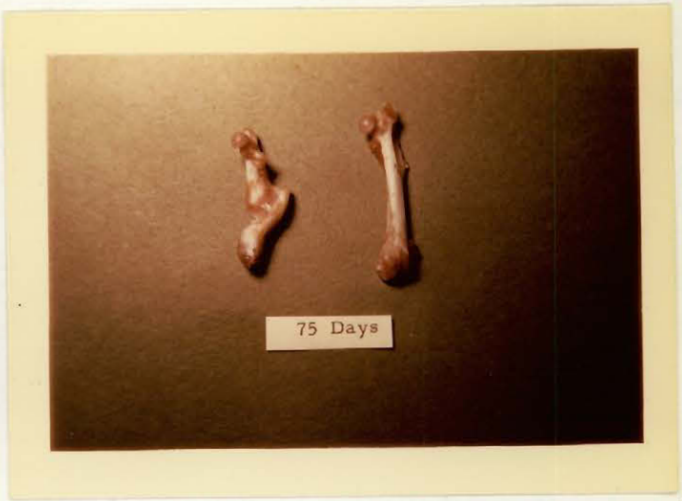
D. 28 Post-fracture days.

Fig. 16.

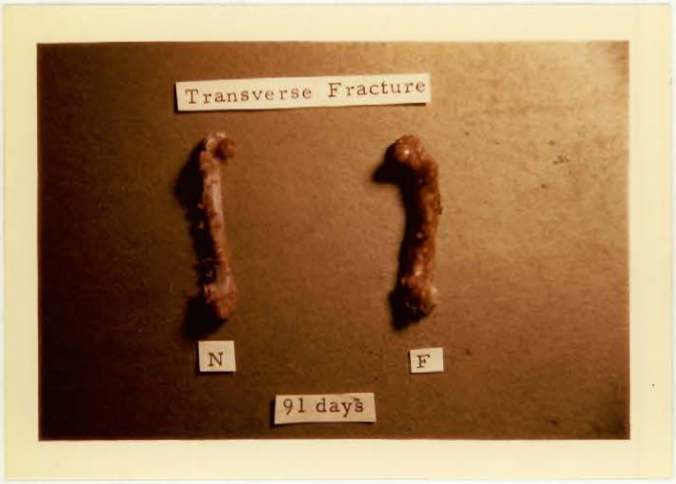
Experiment A, Group I (T).

Gross appearance of the fractures. 2 days post-fracture to 28 days post-fracture. Non-fractured contralateral femur is shown adjacent to fractured femur.

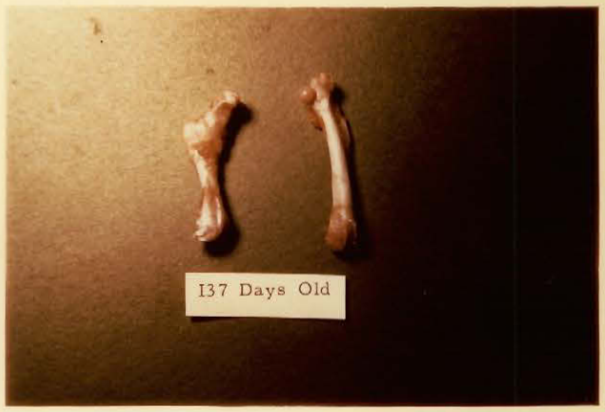
A. 61 Post-fracture days.



B. 75 Post-fracture days.



C. 91 Post-fracture days.



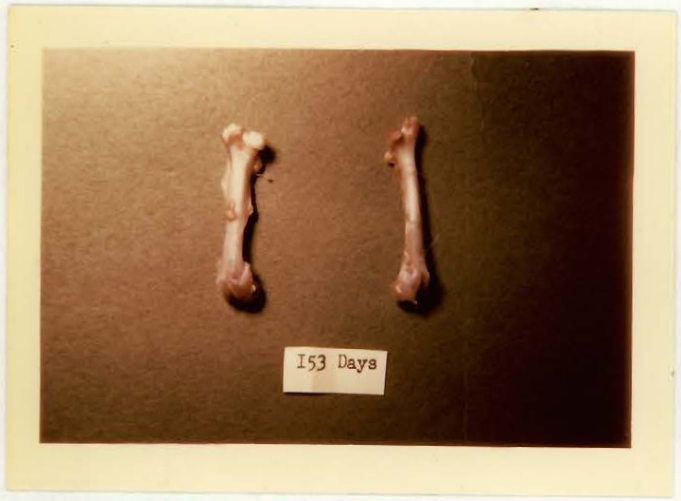
D. 137 Post-fracture days.

Fig. 17.

Experiment A. Group I (T).

Gross appearance of the fractures. 61 post-fracture day to 137 post-fracture days. Non-fractured contralateral femur is shown adjacent to fractured femur.

A. 144 Post-fracture days.



B. 153 Post-fracture days.

C. 232 Post-fracture days.

Fig. 18.

Experiment A. Group I (T).

Gross appearance of fractures. 144 post-fracture days to 232 post-fracture days. Non-fractured contralateral femur is shown adjacent to fractured femur.

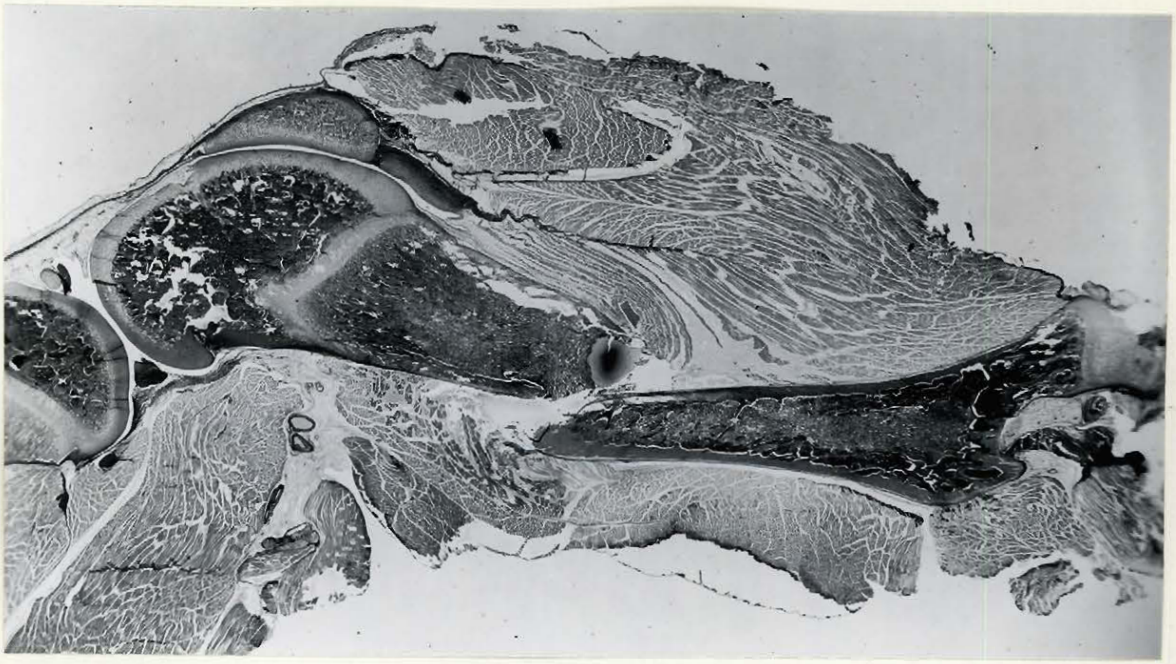


Fig. 19.

Shows the appearance of the fracture (X 20) one-half hour after the operation. Note the degree of displacement.

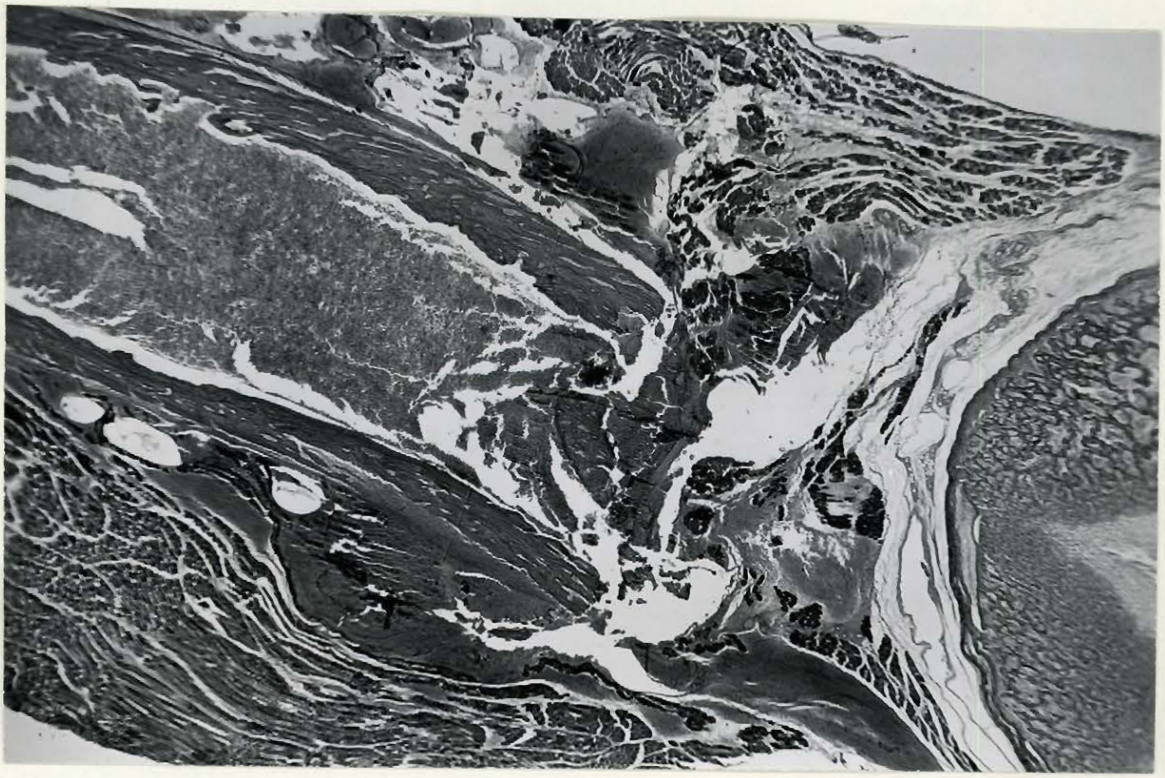


Fig. 20.

Shows a fracture site (X 30) 2 hours after the operation. The extent and spread of the hematoma can be seen.

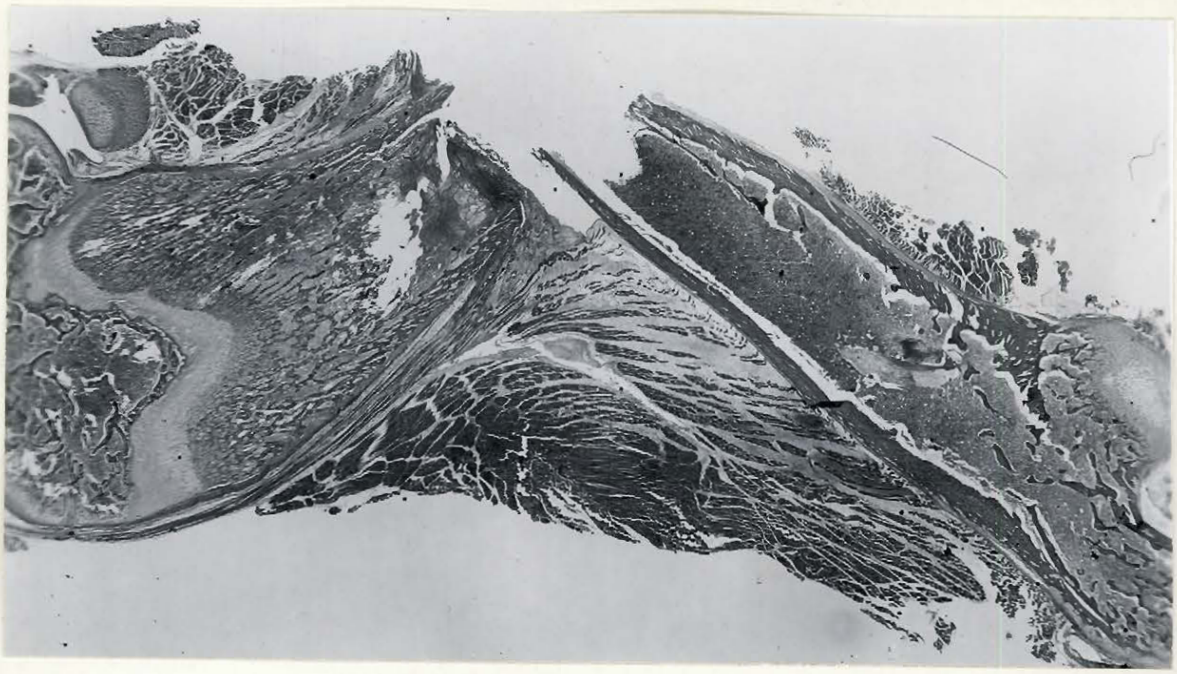


Fig. 21.
Shows a fracture (X 20) one day after operation. Marked angulation at the fracture site is seen.

Fig. 22.
Shows a fracture (X 25) 3 days after operation. Note the degree of displacement. Periosteal proliferation and the endosteal plug are well seen.

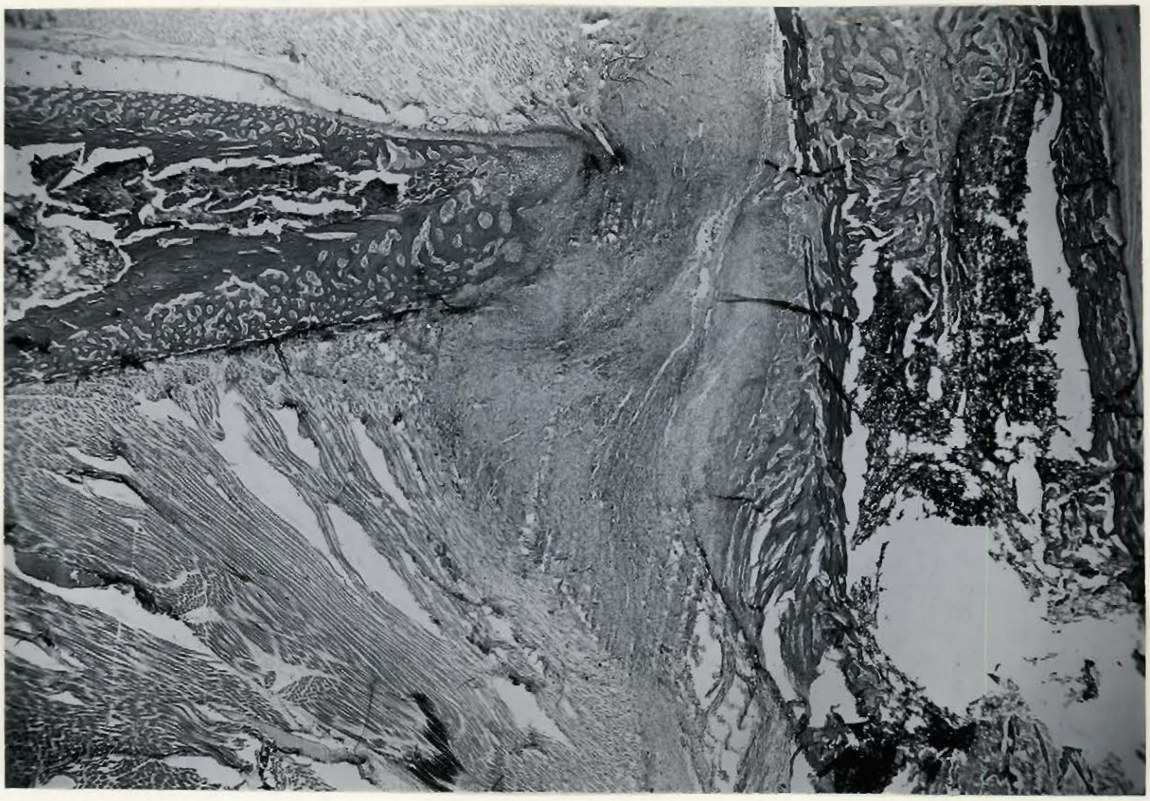


Fig. 23.

Shows a fracture (X 20) 7 days after operation. Note the appearance of both cartilaginous and bony callus under the periosteum, and in the medullary canal.

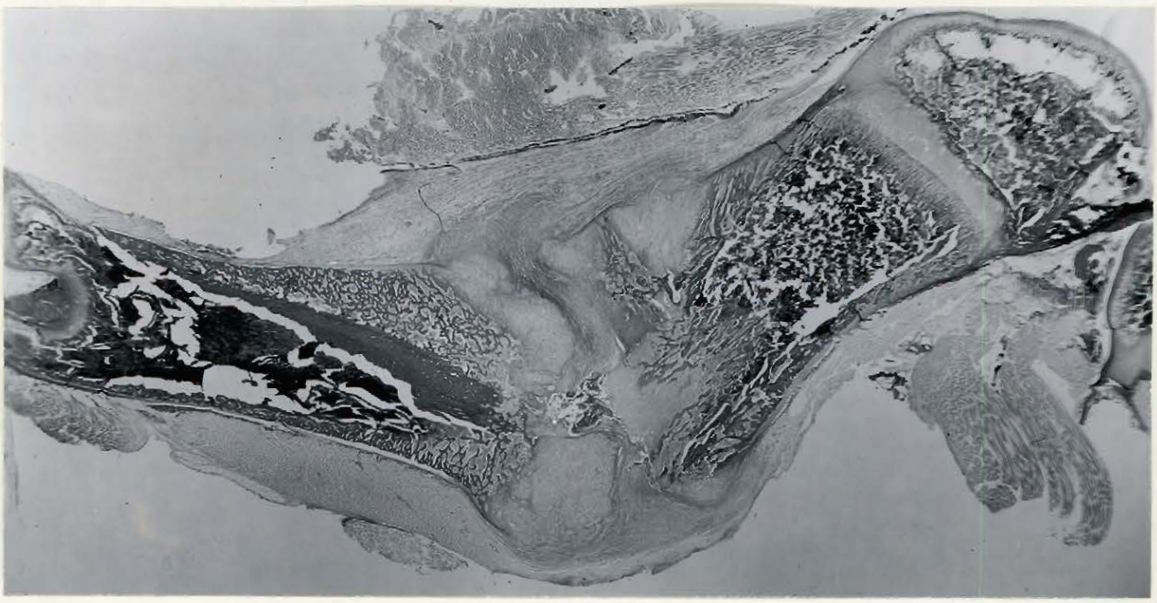


Fig. 24.

Shows a fracture (X 20) 10 days after operation. The callus is further advanced. Note the periosteal cuff and lesser degree of displacement.

Fig. 25.

Shows a fracture (X 20) 15 days after operation. There is abundance of callus formation. Periosteum along the whole length of the femur contributes to the periosteal cuff.

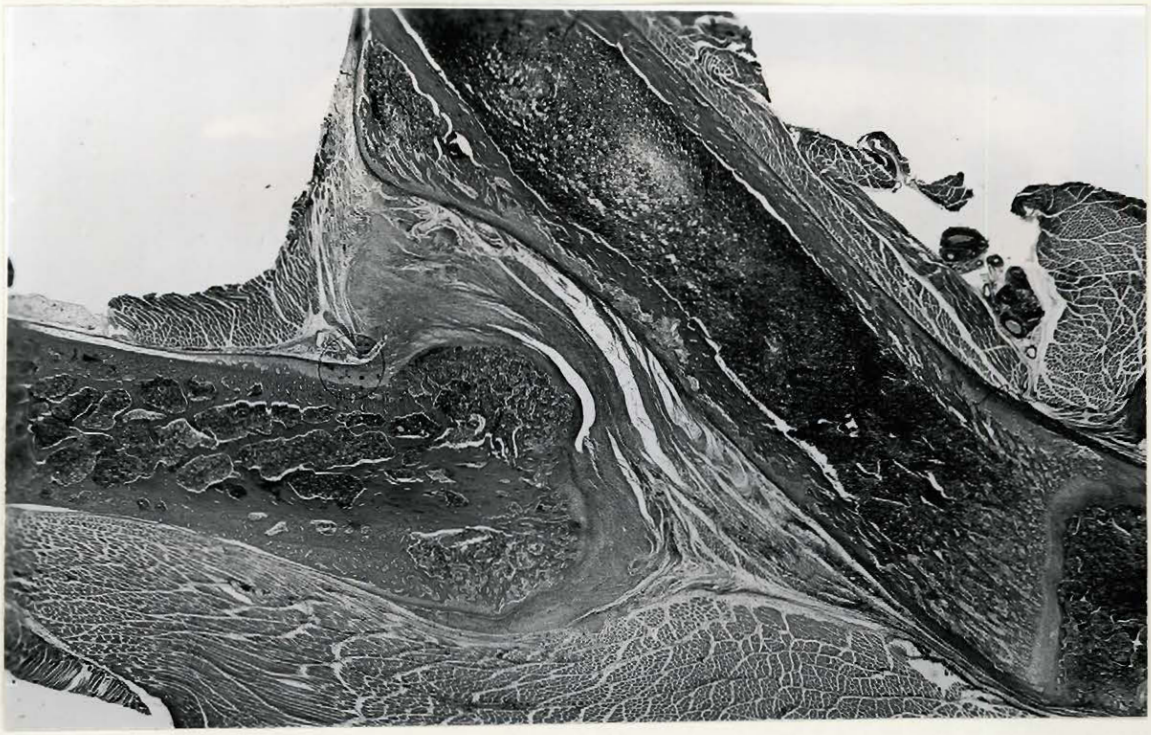


Fig. 26.

Shows another fracture (X 20) 15 days after operation. Here the repair has lagged behind that shown in Fig. 25 because of persistence of displacement of the fragments.

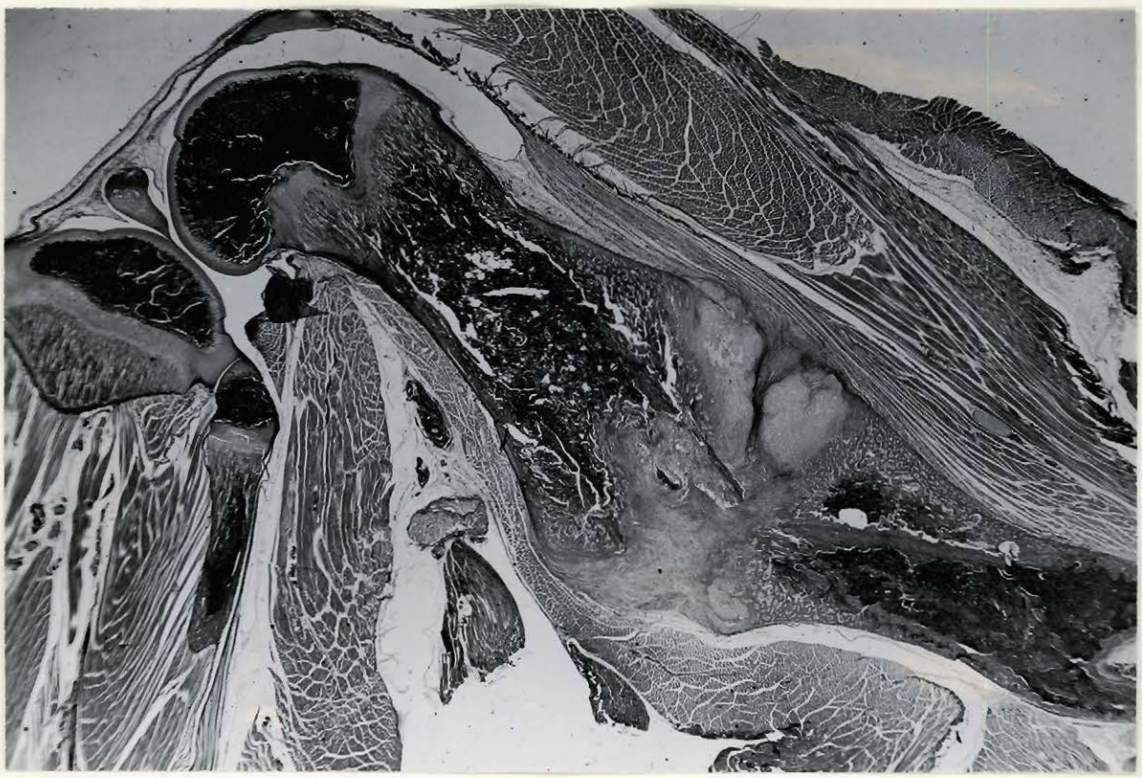


Fig. 27.
Shows a fracture (X 20) 20 days after operation. Various components of the callus can be easily differentiated.

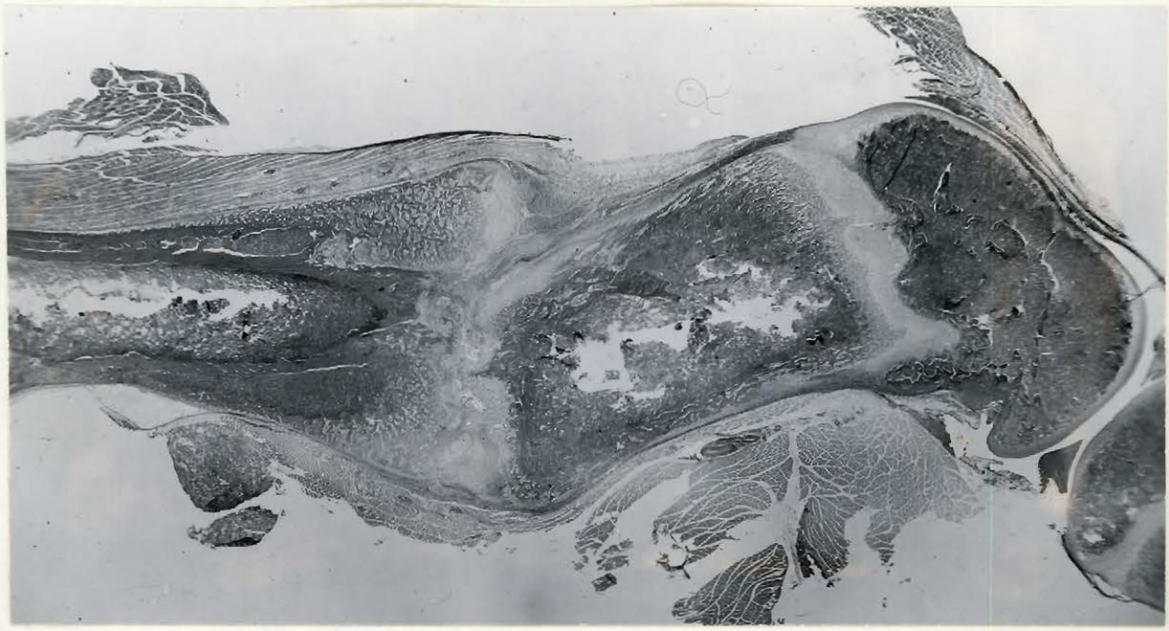


Fig. 28.
Shows a fracture (X 20) 30 days after operation. The cartilaginous callus is being replaced by immature bone.

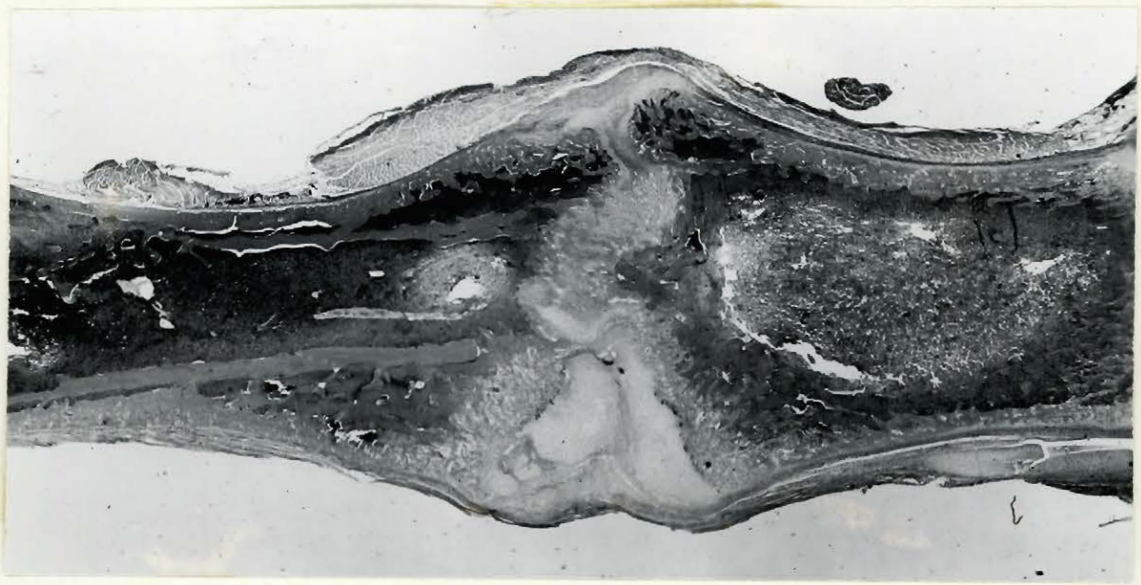


Fig. 29.

Shows a fracture (X 20) 40 days after operation. Note the good alignment and the far advanced healing.

Fig. 30.

Shows a fracture (X 15) 91 days after operation. Bony union and reconstruction is far advanced.

Experiment A.

Group II (TP). Fracture right femur, immobilized with external plaster hip spica.

Mortality same as reported for the group I (T).

Post-operative Course: The animals did not tolerate external immobilization very well. A rigid spica (Fig. 31) restricts their activity to a great extent. Rats could drag the plastered leg without lifting the trunk by the first day and by the 3rd day they were able to clear their bellies off the floor of the cages. They were sluggish, but could stand on their hind limbs. They attempted to eat each others plaster. As the growth is fast during this age period, the spica had to be frequently changed. The complications seen were swelling, edema and ulceration of the leg and foot. One case led to gangrene and amputation of the leg (Fig. 32). These complications could be avoided by changing the spicas every 7 - 10 days and by including the whole of the foot in the plaster.

Two of the most important complications are muscle atrophy and stiffness of the knee joint. Muscle atrophy of the thigh musculature (Fig. 33) was noticed as early as the 7th day after immobilization and it was progressive in nature. This was observed in all animals during the 92 days of immobilization used in this

experiment. The atrophied muscle lost its normal colour and bulk as compared to similar but unimmobilized group (T). These changes were reversible, if the immobilization was removed and the animals allowed to walk.

Stiffness of the right knee was noticeable as early as the 10th day (Fig. 34). This was also progressive in nature. The knee joint capsule was thickened and fibrosed. Intra-articular adhesions and loss of normal colour of the articular surfaces were noticed.

The histological observations are essentially the same as in the previous group (T) without any immobilization. However, certain phases of the repair process seem to be shortened. Average time for bony consolidation in this group was 73 days.

Fig. 35. shows a fracture 16 days after operation and immobilization. There is reasonably good alignment of the fragments and abundant callus as compared to a fracture of the same duration in control group (T) which was not immobilized.

Fig. 36. shows the histological appearance of a fracture 30 days after operation and immobilization, showing well advanced repair process.

Fig. 37. shows the histological appearance of a knee joint in a rat with a clinically stiff knee, 28 days after immobilization of the fractured limb. Note the almost normal histological appearance of the joint, in spite of almost complete loss of passive joint movement under anesthesia. This indicates that peri-articular changes are more important than intra-articular changes in the development of this loss of joint movement.

Fig. 38. shows another fracture 28 days after immobilization. The various components of the callus are in abundance and are well differentiated.

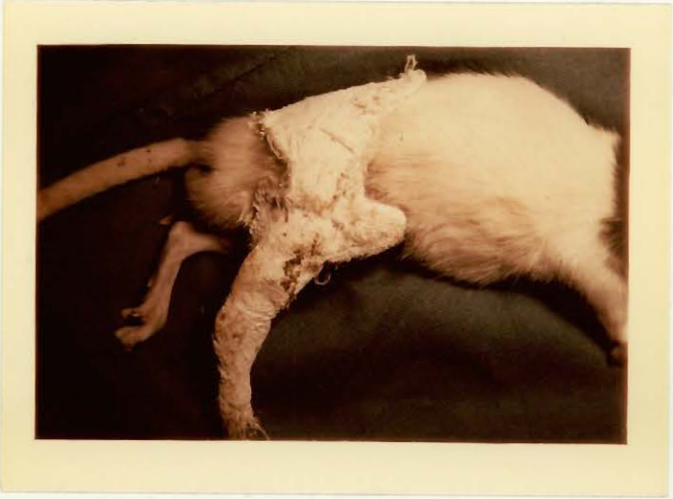
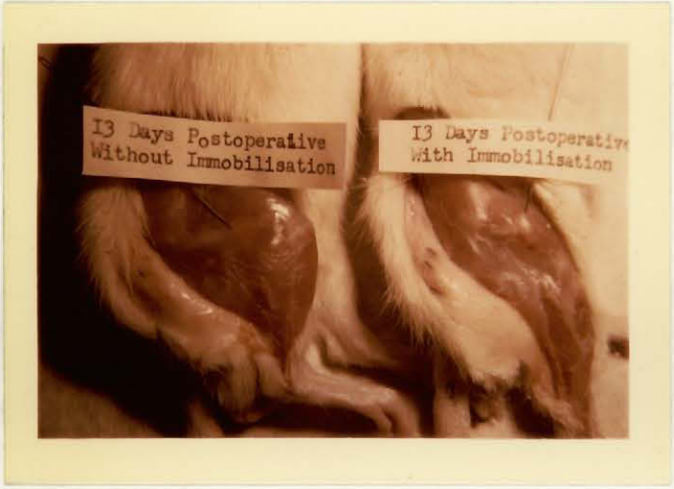


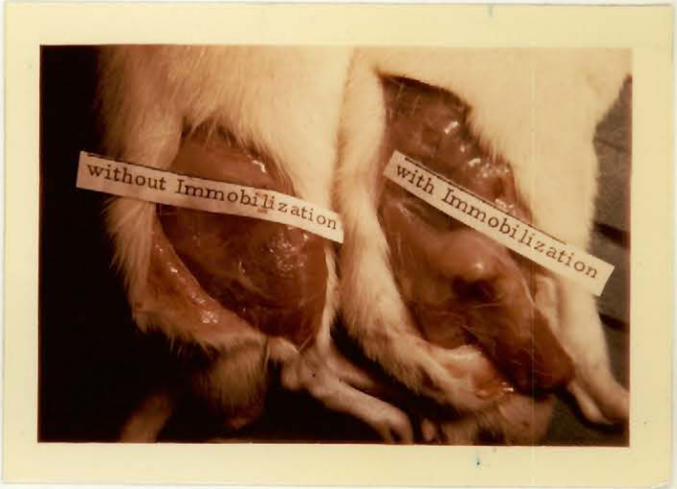
Fig. 31.

Hip spica immobilization of right hind limb. Animals have a tendency to eat each others plaster, necessitating reapplication of plaster spica.

Fig. 32. Gangrene of the right leg and foot due to vascular impairment.



A. 13 days after production of fracture and hip spica immobilization.



B. 21 days after production of fracture and hip spica immobilization.

Fig. 33.

Experiment A, Group II (TP).

Shows muscle atrophy following external immobilization.

Fig. 34. Shows the right stiff knee 28 days after immobilization. Passive stiffness was noticed as early as the 10th day after immobilization.

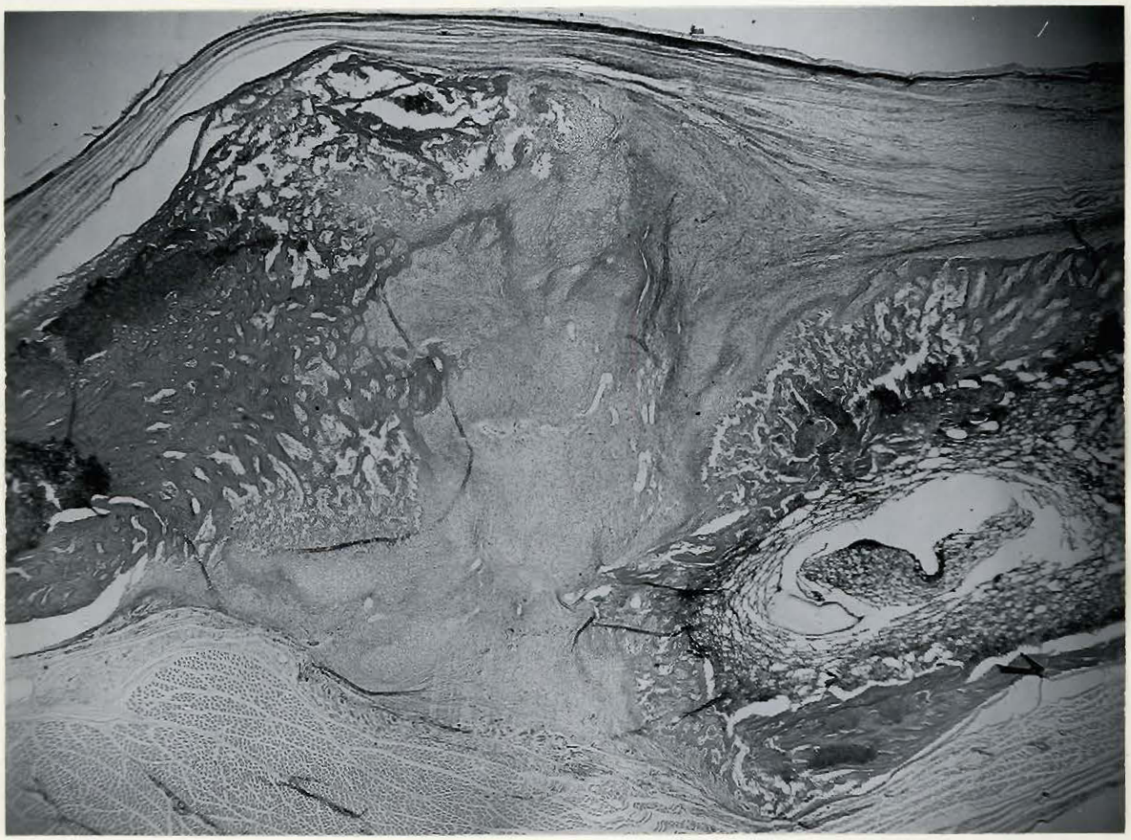


Fig. 35.
Shows a fracture 16 days after operation and immobilization. There is reasonably good alignment of the fragments and abundant callus as compared to a fracture of the same duration in control group (T) which not immobilized. (X 20).

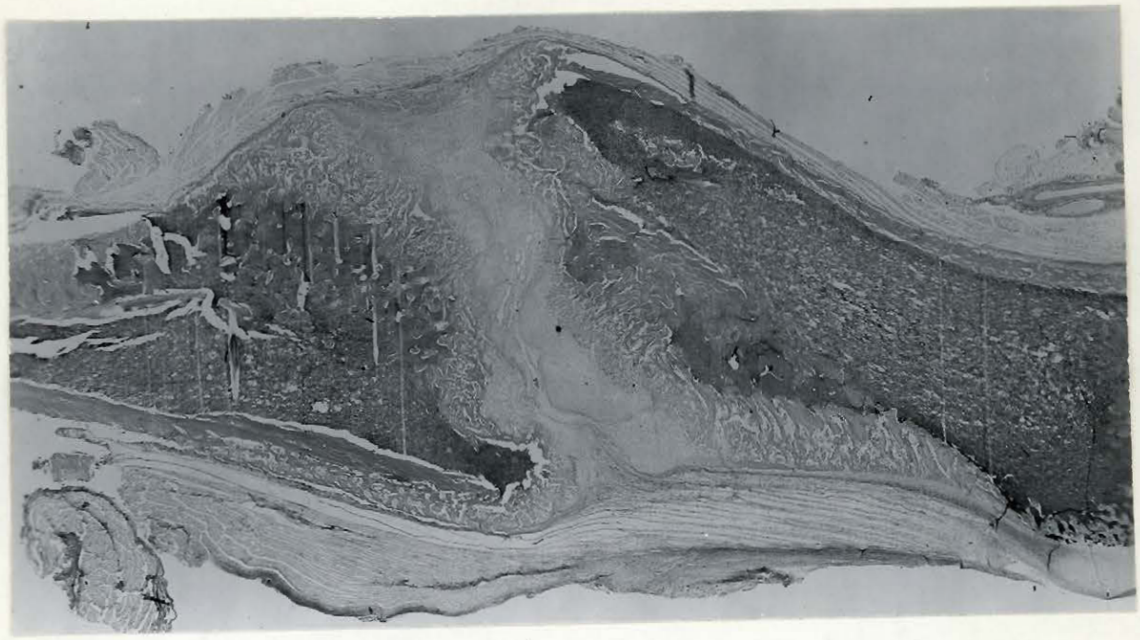


Fig. 36.

Shows a fracture (X 20) 30 days after operation and immobilization, showing well advanced repair process as compared to control group (T).

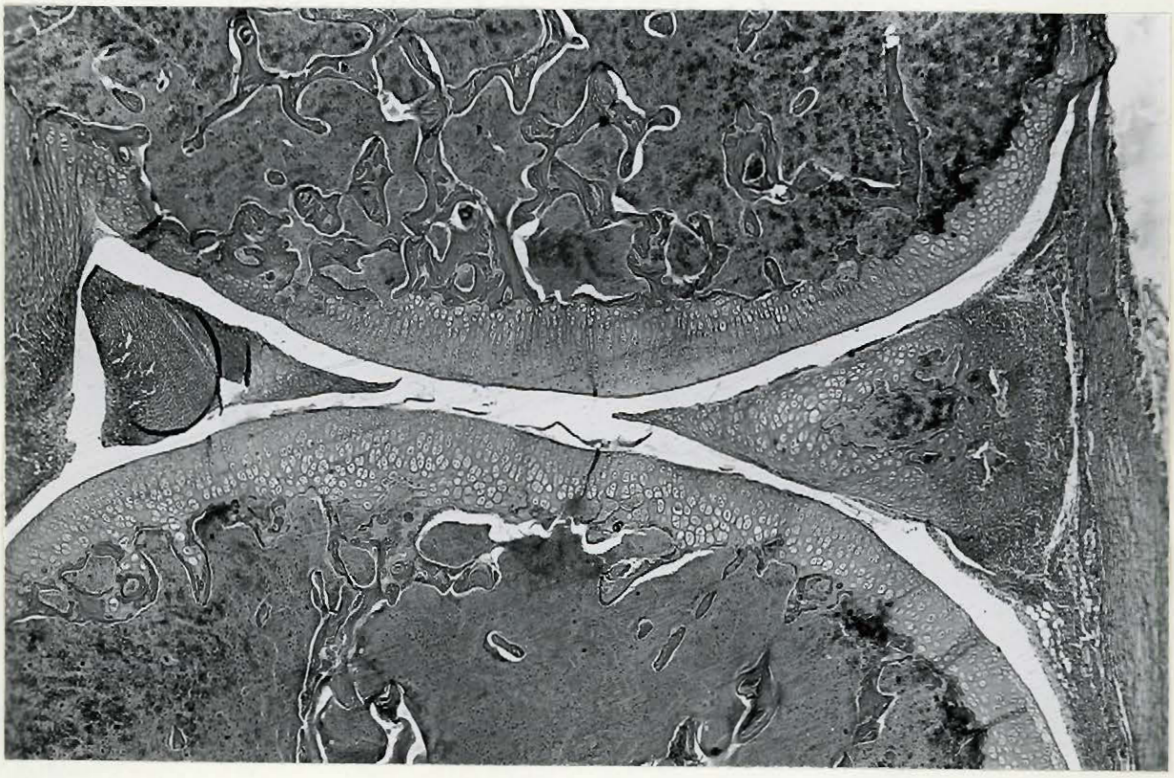


Fig. 37.

Shows the histological appearance (X 30) of a knee joint in a rat with stiff knee, 28 days after immobilization of the fracture limb. In spite of loss of passive movement, the histology of the joint shows almost normal pattern. Peri-articular changes seem to be more important than intra-articular changes in the development of loss of joint movement.

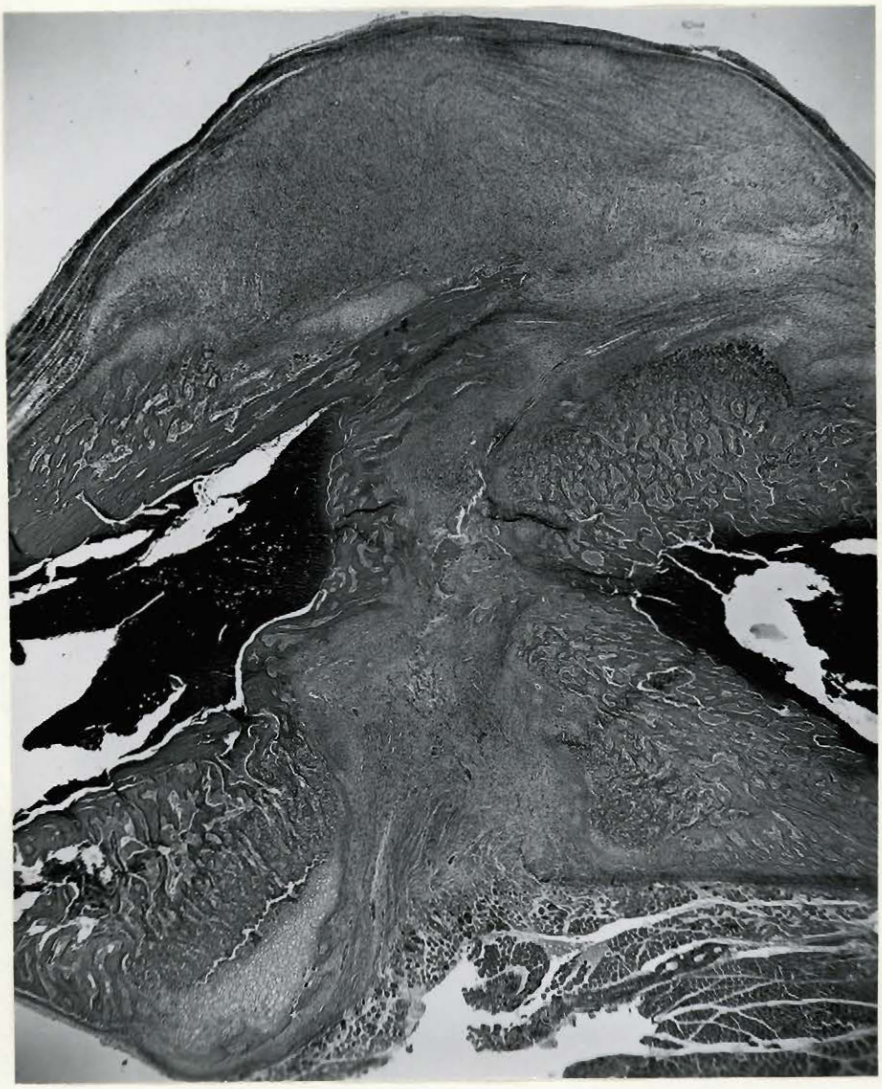


Fig. 38.

Shows another fracture callus (X 25) in a rat femur, 28 days after fracture and immobilization. The callus mass is in abundance. Various components are well seen. The process of repair is well advanced.

Experiment A.

Group III (TG). A Gap was produced at the Fracture Site.

Mortality: same as in the control group (T).

Post-operative Course:- The animals assumed a definite 'broad base' position of the hind limbs due to gross angulation at the fracture site under stress of weight bearing. By the 10th day full weight bearing was resumed.

The pattern of bone repair was essentially the same as in the control series (T). There was an initial lag period in the repair, but bony union was obtained by the 90th day after production of fracture. However, shortening of the fractured femur occurred. All the fractures healed during the study period.

Fig. 39. shows the excised fragments from a group of operated animals. A gap of approximately 3 mm was produced at the fracture site.

Fig. 40. shows the gross specimen with bony union and reconstruction in a femur 63 days after fracture. Note the shortening of the fractured femur in comparison to the non-fractured contralateral femur.

Fig. 41. shows a fracture 43 days after operation. Note the large bulk of cartilaginous callus occupying the 'gap'.

There is a cleft suggesting some degree of pseud-arthrosis.

Fig. 42. shows a fracture 60 days after operation. The alignment of the cartilaginous callus is taking place.

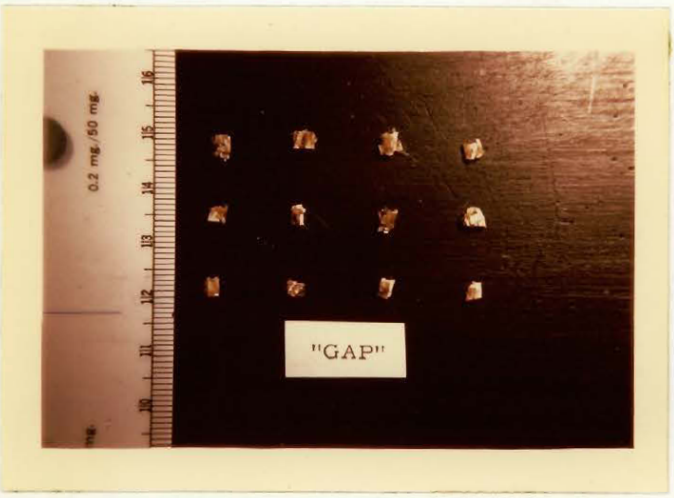


Fig. 39. Shows the excised fragments from a a group of operated animals. A gap of approximately 3 mm width was produced at the fracture site.

Fig. 40. Shows the gross specimen with bony union and reconstruction in a femur (F) 63 days after fracture. Note the shortening of the fractured femur in comparison to the non-fractured contralateral femur (N).

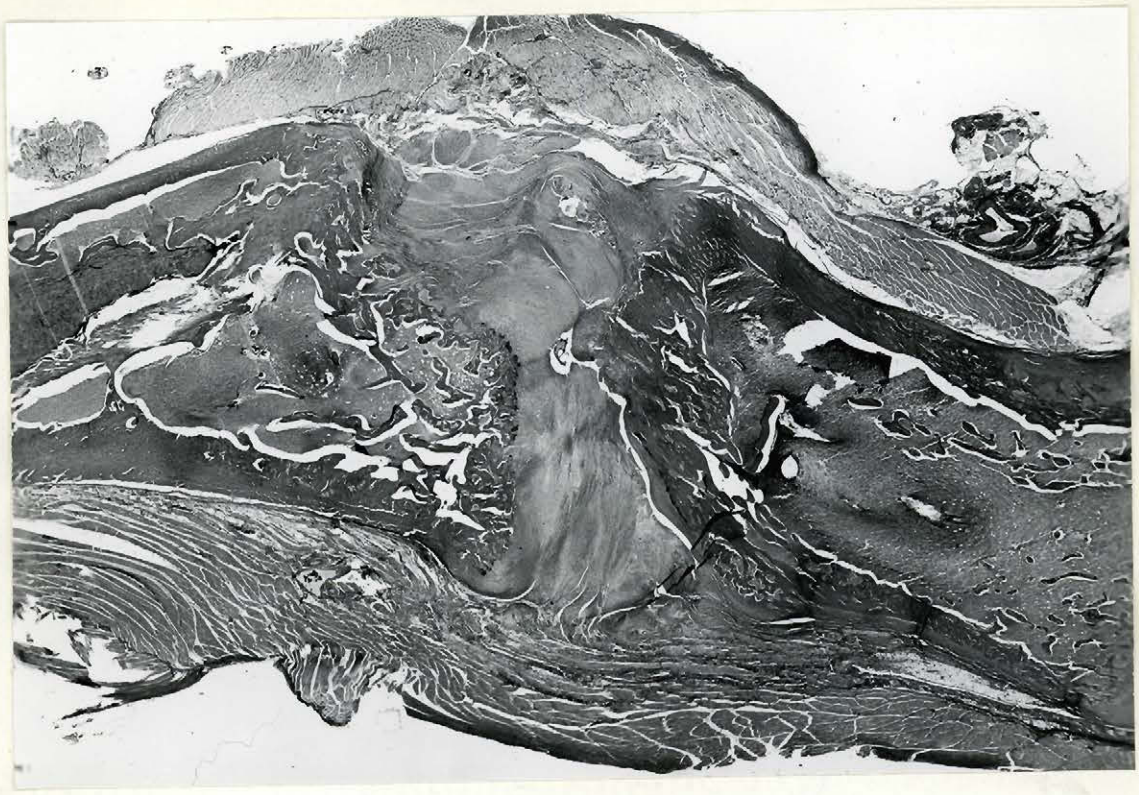


Fig. 41.

Shows a fracture (X 20) 43 days after operation. Note the large bulk of cartilaginous callus occupying the 'gap'. There is a cleft suggesting some degree of pseudarthrosis.

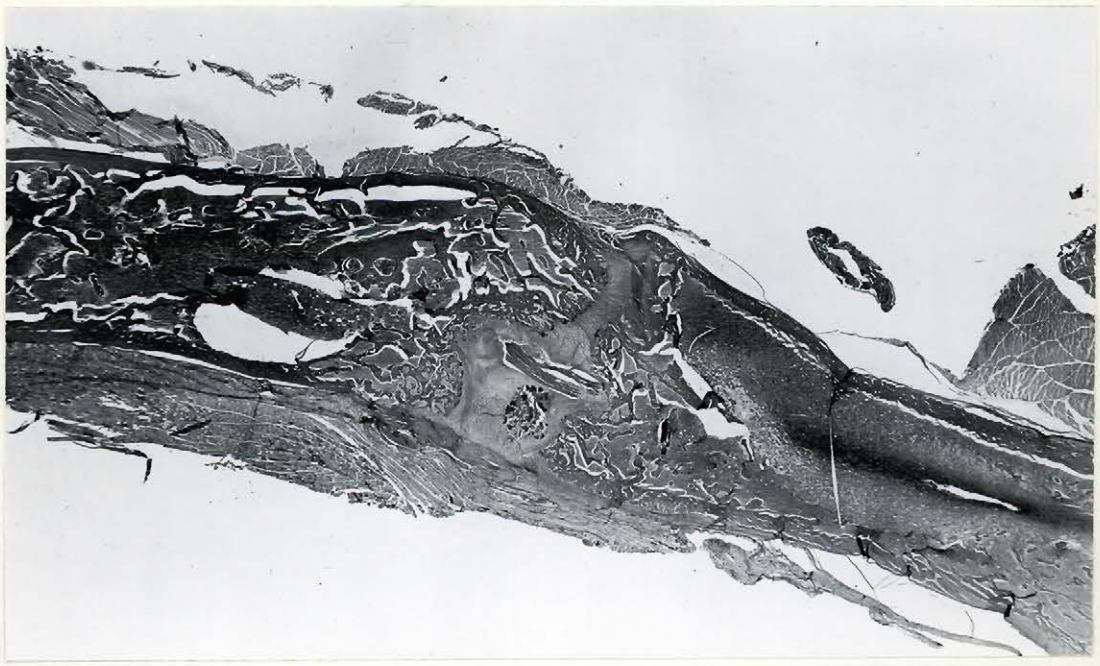


Fig. 42.

Shows a fracture (X 20) 60 days after operation. The alignment is good and bony replacement of the cartilaginous callus is taking place.

Experiment A.

Group IV (PRT). Fracture and Periosteal Cuff Resection.

Operative Mortality - 10 - 15%.

Post-operative Course :- It was essentially the same as in the control group I (T). There was a definite lag in the repair of the fracture in this series. The fracture ends from which the periosteum had been excised underwent necrosis (Fig. 43). However, the end result (bony consolidation) was not ultimately adversely affected to any great degree. Complete bony union was obtained in one specimen by 72 days (Fig. 44). One 88 day old fracture still showed necrosis of the ends while another fracture at 158 days was completely healed and solid. The healed femurs were shorter in length. This was probably the result of the necrosis of the fracture ends. There was also a definite increase in the transverse width of the femur.

Fig. 45. shows a radiogram of a rat with fracture right femur and excision of a cuff of periosteum 239 days after operation. The fracture shows bony union. Note the shortening of the fractured femur.

Fig. 46. Shows the histological appearance of a specimen 9 days after fracture and excision of a cuff of periosteum. Note

the necrotic bone in the middle of the field. The repair process is seen progressing from each fracture end.

Fig. 47. shows a fracture 46 days after operation. The necrosis of fracture ends, absorption and new bone formation are seen.

Fig. 48. shows a fracture 63 days after operation. Healing is well advanced.

Fig. 49. shows a fracture 72 days after operation. Healing is further advanced. New bone formation is replacing the cartilaginous callus.

Fig. 43.
Gross specimen showing necrosis of the fracture ends (28 days after operation). Right femur was fractured and a cuff of periosteum was excised. Necrosis of the denuded fracture ends was noted in all the specimens, in one specimen as late as 88 days after the operation.

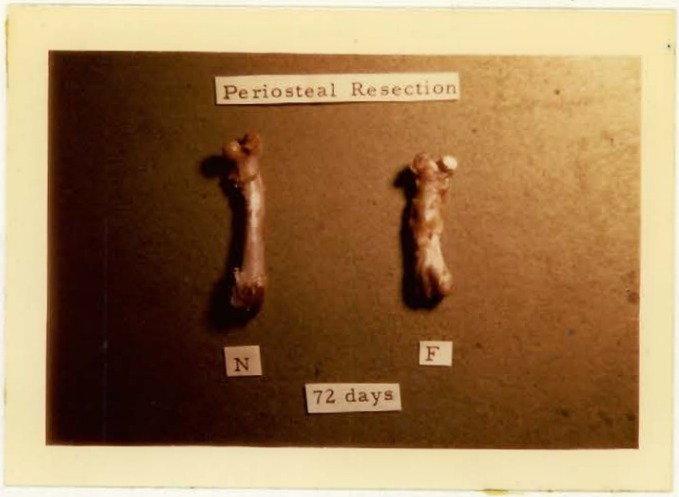


Fig. 44.

Gross specimen showing complete bony union in a fracture with excision of periosteal cuff, 72 days after operation. Note the shortening in the length of femur, probably due to necrosis of the fracture ends. There was a definite increase in the transverse width of the femur.

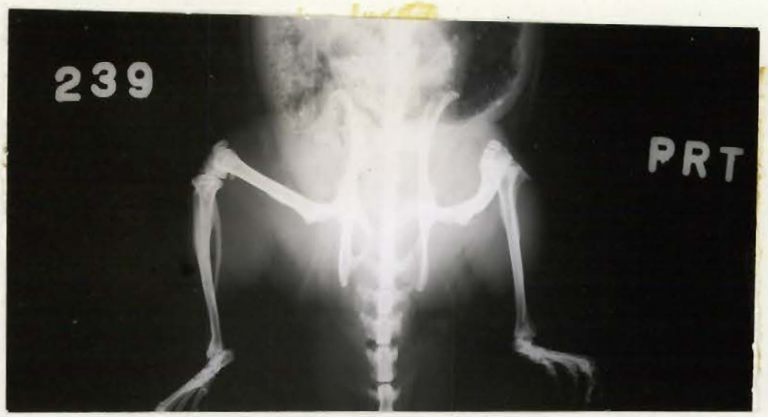


Fig. 45.

Radiogram of a rat with fracture right femur 239 days after operation. A cuff of periosteum was excised at the time of producing the fracture. The fracture shows bony union with resultant shortening of the shaft of the femur.

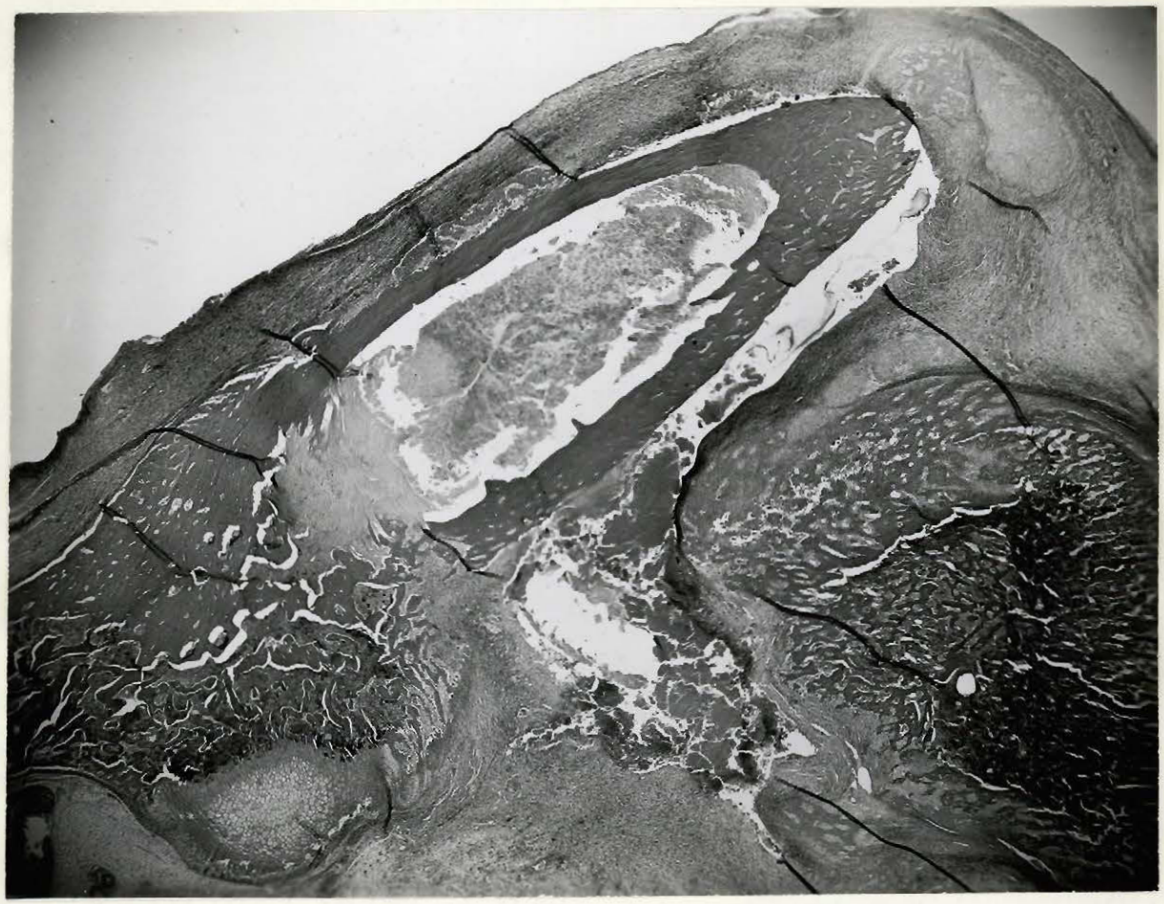


Fig. 46.

Histological appearance of a specimen 9 days after fracture (X 30) and excision of a cuff of periosteum. Note the necrotic bone in the middle of the field. The repair process is seen progressing from each fracture ends.

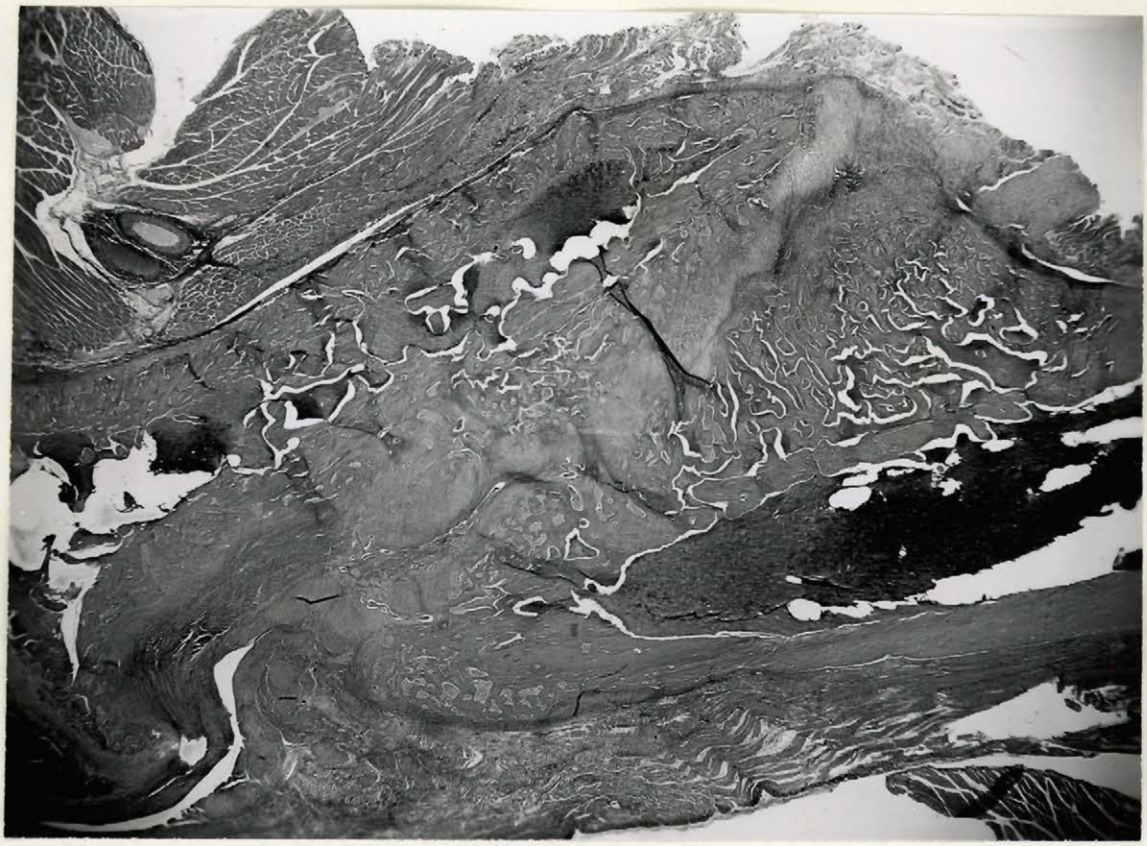


Fig. 47.

Shows a fracture (X 25) 46 days after operation. The necrosis of fracture ends, absorption and new bone formation are seen.

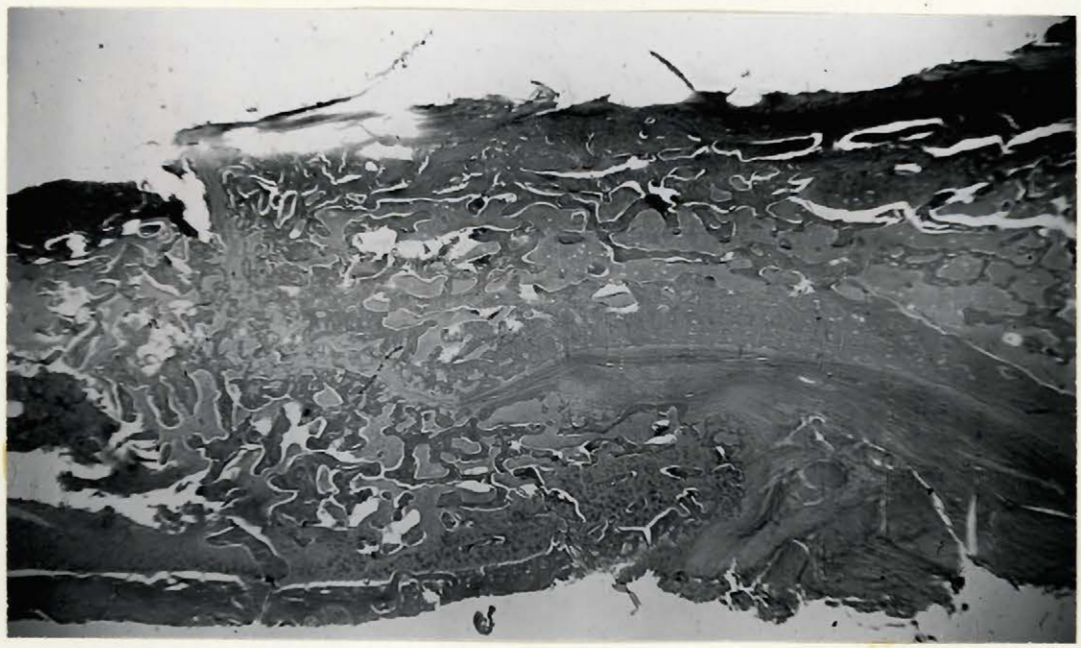


Fig. 48.

Shows a fracture (X 20) 63 days after fracture and excision of a cuff of periosteum. Healing is well advanced.

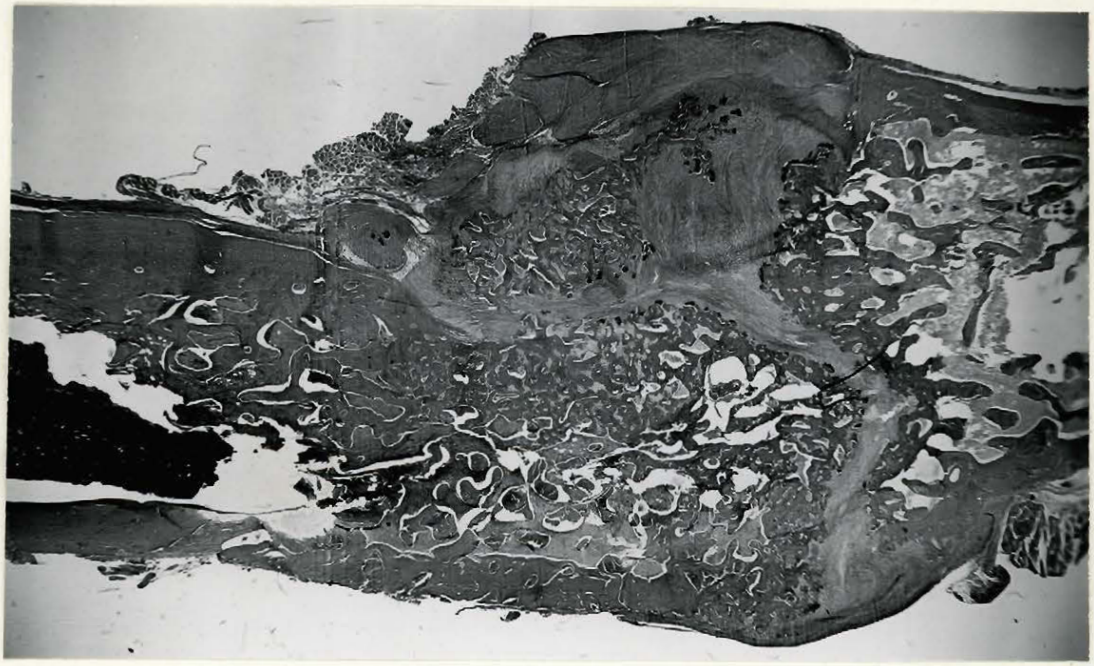


Fig. 49.

Shows a fracture (X 25) 72 days after production of fracture and excision of a cuff of periosteum. Healing is further advanced. New bone formation is seen replacing the cartilaginous callus.

Experiment A.

Group V (TM). Fracture and Muscle Section.

Mortality - 5%.

Post-operative Course: - The use of right hind limb was greatly restricted in this group and the animals were unable to use the affected limb for a variable length of time, from 21 to 28 post-fracture days. Clawing of the toes were observed in a few animals. Swelling of the leg was commonly seen.

There was a marked displacement of the fragments. Delayed union was common. Bone ends were capped and no evidence of union was seen up to the 40th postfracture-day. First specimen with union was found on the 50th day. Although union took a longer time to complete (average 176 days), the ultimate results (bony consolidation) were the same as in the other series.

Fig. 50. Shows the inability of the animal to use the limb post-operatively. Clawing and edema were commonly seen.

Fig. 51. Shows the marked displacement of the fragments and discontinuity of the muscle 21 days after the operation.

Fig. 52. Shows the operated site 40 days after fracture and muscle section. Note the discontinuity in the musculature.

Fig. 53. Shows the operated site 223 days after fracture and muscle section. Muscle is healed by scar. Dissection showed cartilaginous union.

Fig. 54. Radiogram of a fracture with muscle section, 223 days after operation, showing non-union of the fracture.

Fig. 55. Shows a fracture 56 days after production of a fracture and muscle section. Note the marked degree of displacement. There is preponderence of fibro-cartilaginous callus.

Fig. 56. Shows a fracture 98 days after production of a fracture and muscle section. The displacement is persistent, the ends are necrotic and the repair process is slow.

Fig. 57. Shows a fracture 106 days after production of fracture and muscle section. The intermediary callus shows a number of clefts suggesting non-union. The repair process otherwise is similar to previous specimens.

Fig. 50. Shows the inability to use the limb post-operatively. This animal was operated 15 days ago. Clawing and edema of the foot and leg were common.

Fig. 51.
Shows the gross appearance of the thigh 21 days post-operatively. Note the marked displacement of the fragments and discontinuity of the thigh musculature.

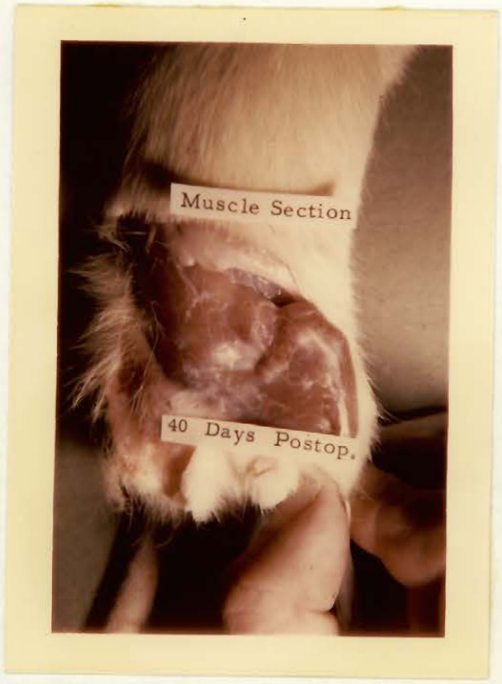


Fig. 52. Shows the operated site 40 days after fracture and muscle section. Note the discontinuity in the musculature.

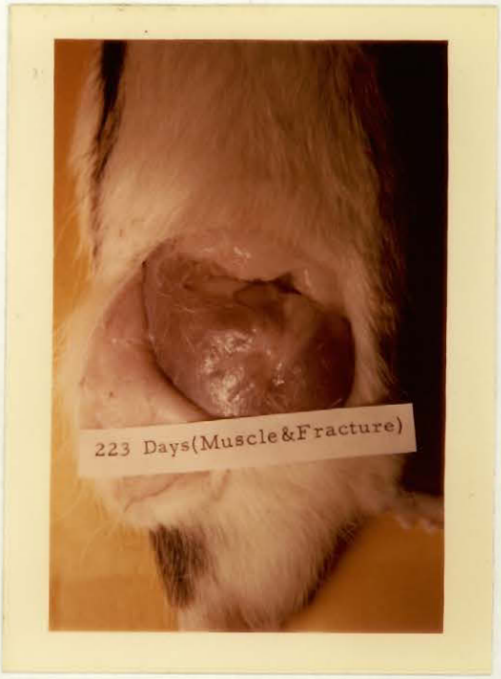


Fig. 53. Shows the operated site 223 days after fracture and muscle section. Muscle is healed by scar. Dissection showed cartilaginous union.

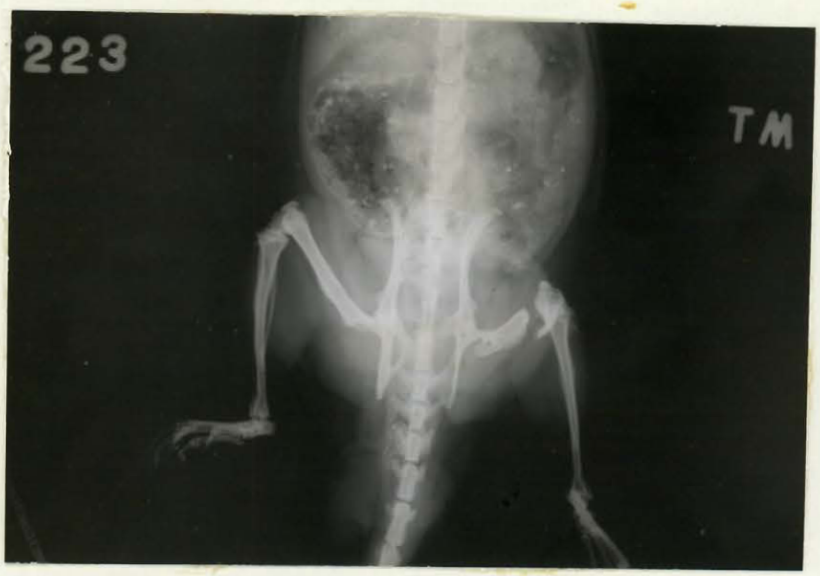


Fig. 54.
Radiogram of a fracture with muscle section, 223 days after operation, showing non-union.

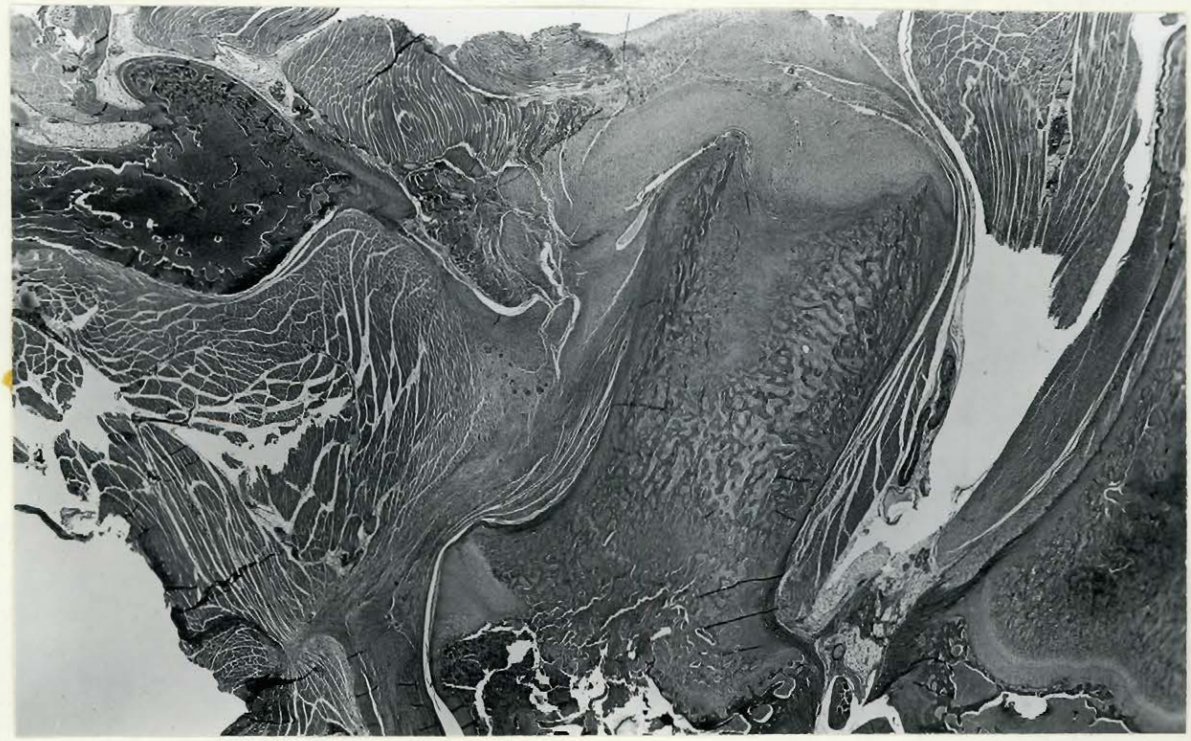


Fig. 55.
Shows a fracture (X20) 56 days after production of fracture and muscle section. Note the marked degree of displacement. There is preponderence of fibro-cartilaginous callus.

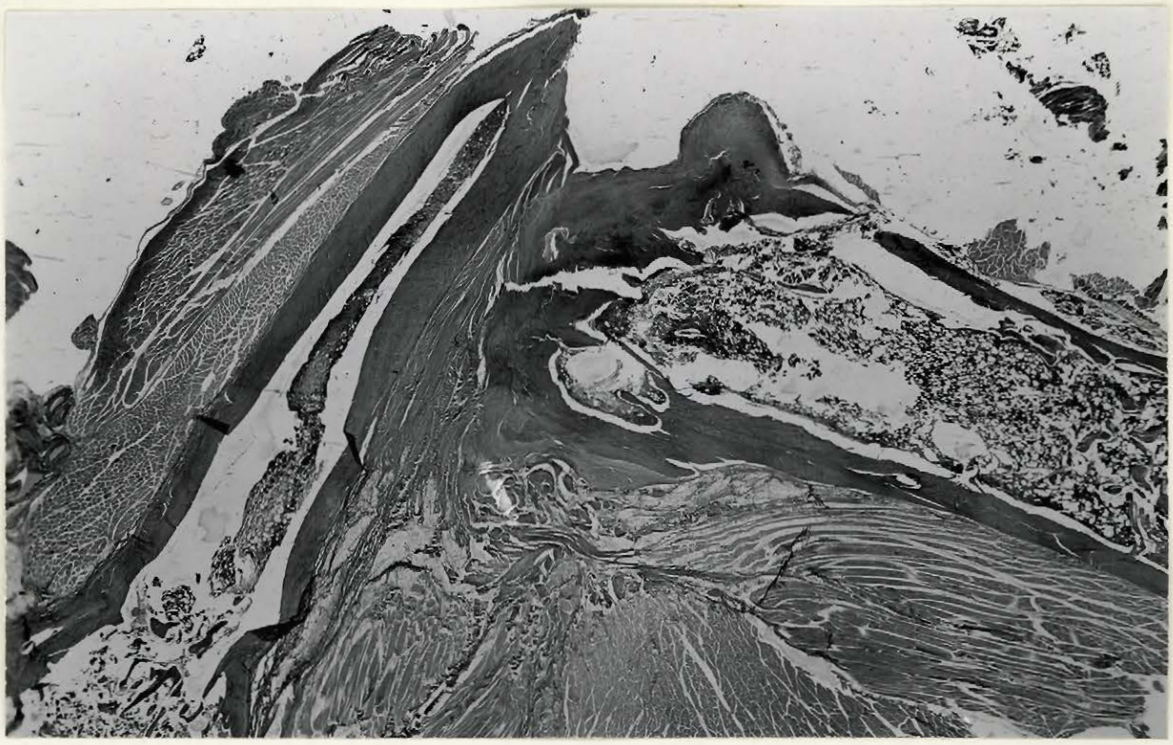


Fig. 56.

Shows a fracture (X 20) 98 days after production of fracture and muscle section. The displacement is persistent, the ends are necrotic and the repair is slow.

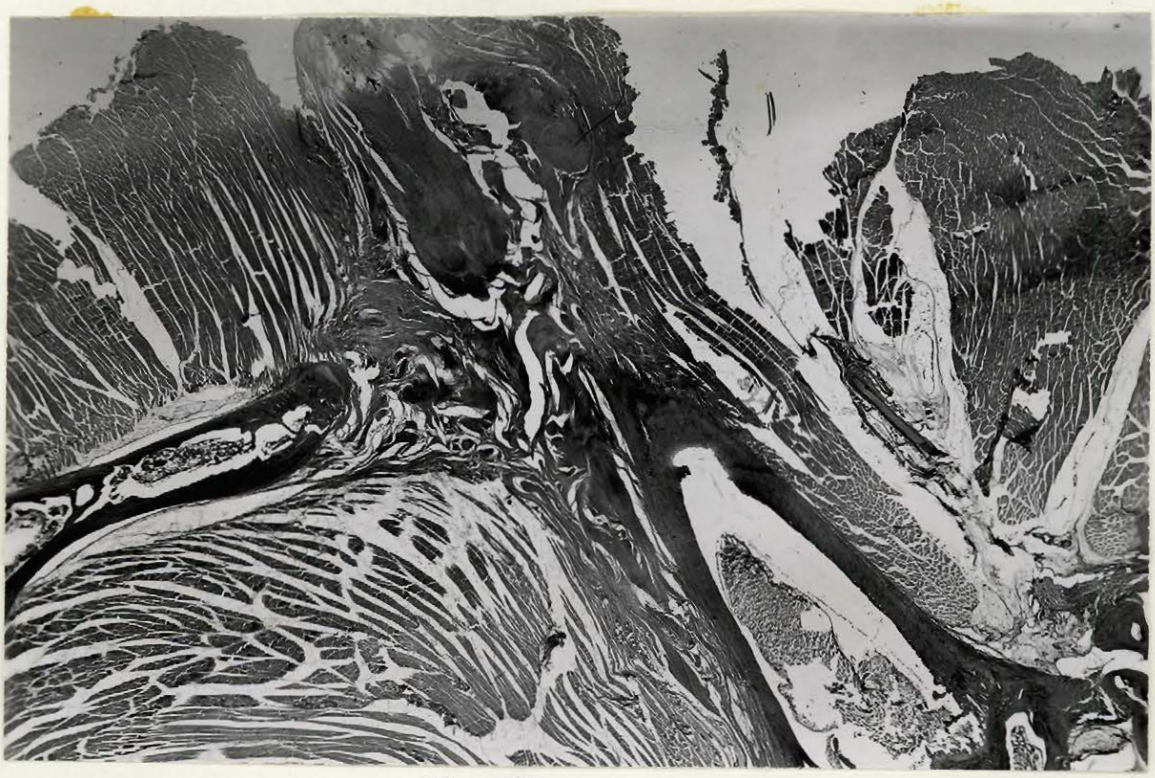


Fig. 57.

Shows a fracture (X 20) 106 days after production of fracture and muscle section. The intermediary callus shows a number of clefts suggesting non-union. The repair process otherwise is similar to previous specimens.

Experiment A.

Group VI (TE). Fracture and Endosteal Reaming.

Mortality - the same as in the control group I (T).

No definite alteration in the pattern of bone healing was observed in this series. There was some evidence that the remodelling capacity of the fracture was slower in this group as compared to group I (T). The average period of time for bony consolidation in this series was 105 days.

Fig. 58. Shows a fractured femur 128 days after fracture and endosteal brushing.

Fig. 59. Shows a radiogram 225 days after fracture and endosteal brushing, showing bony consolidation and shortening of the femur.

Fig. 60. Shows a fracture 15 days after operation. There is marked displacement of the fragments. Cartilage and new bone formation are in progress. Note the widening of the fracture ends.

Fig. 61. Shows a fracture 23 days after operation. The healing process is further advanced. Cartilage is being replaced by new bone.

Fig. 62. Shows a fracture 53 days after operation. The ends are covered with fibro-cartilaginous caps. There is suggestion of pseud-arthrosis.

Fig. 58.

Shows a fractured femur 128 days after fracture and endosteal brushing. Contralateral femur is also shown.

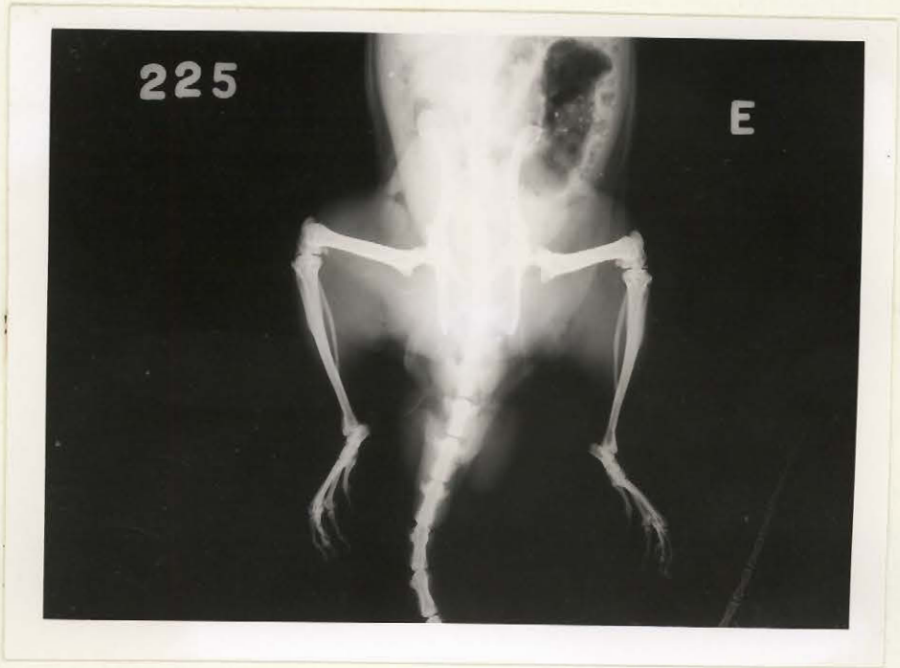


Fig. 59.

Radiogram showing bony union and shortening of the femur 225 days after fracture and endosteal brushing.

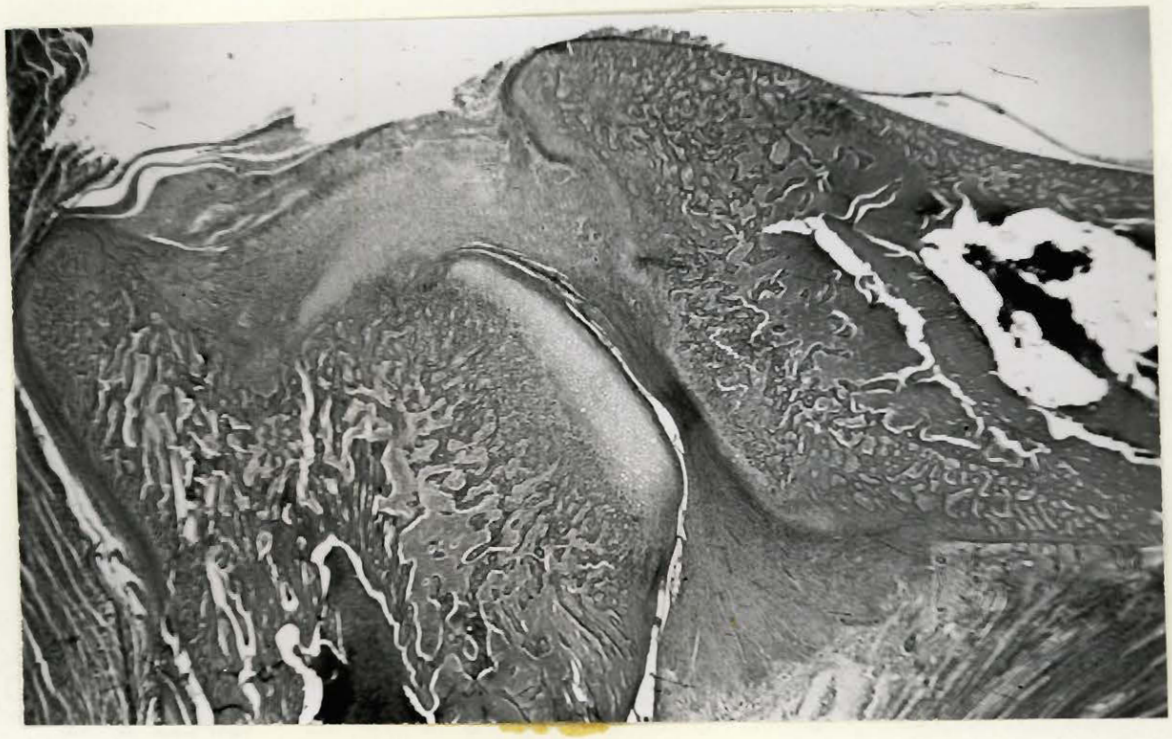


Fig. 60.

Shows a fracture (X 20) 15 days after fracture and endosteal brushing. There is marked displacement of fragments. Cartilage and new bone formation are in progress. Note the widening of the fracture ends.

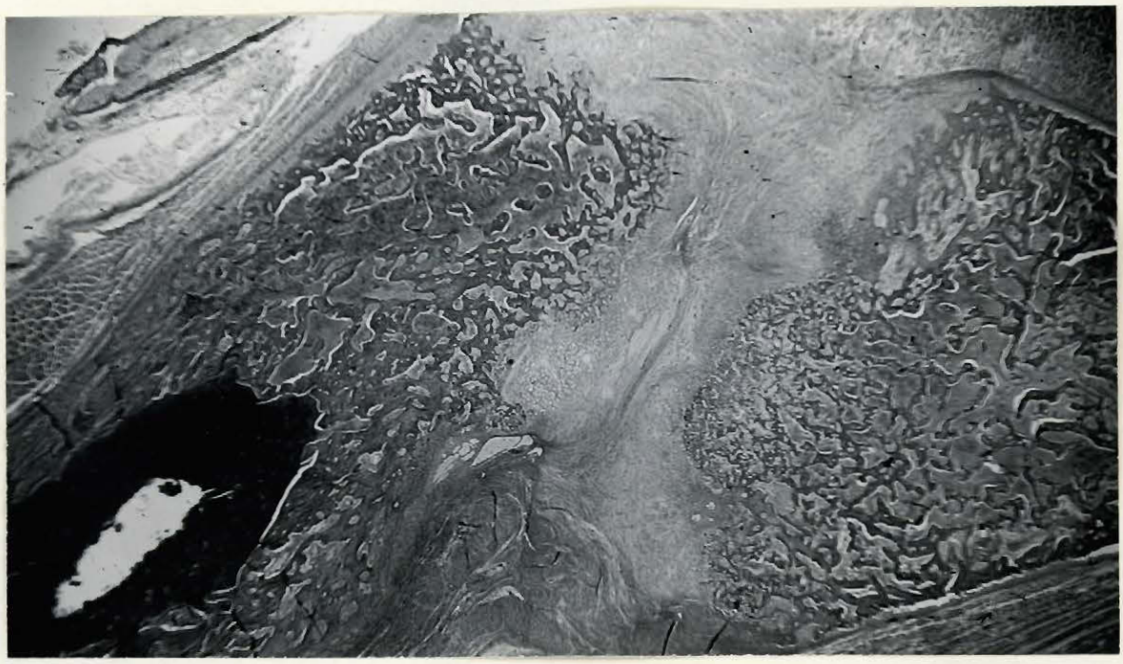


Fig. 61.

Shows a fracture (x 20) 23 days after fracture and endosteal brushing. The healing process is further advanced. Cartilage is being replaced by new bone.

Fig. 62.

Shows a fracture (X20) 53 days after fracture and endosteal brushing. The ends are covered with fibro-cartilaginous caps. There is a cleft between the fracture ends suggesting pseud-arthrosis.

Experiment A.

Group VII (PRTM). Fracture, Periosteal cuff resection & muscle section.

Mortality - 5 - 8%

Post-operative Course:- The post-operative course was a combination of those reported in the two groups separately.

There was gross displacement of the fragments and necrosis of the denuded fracture ends occurred. There was a prolonged initial lag in the repair phenomenon. Complete absorption of the diaphysis occurred in many femurs. One 27 day old femur had only the proximal and distal metaphyseal ends left, the shaft had been completely resorbed. The two procedures (muscle section and periosteal cuff resection) performed in this series had the most adverse affect on the healing as compared to the groups already studied. The average time for bony consolidation in this series was 188 days. The incidence of non-union was high (40%).

Fig. 63. Radiogram 194 days after production of fracture, muscle section and periosteal cuff resection. The fracture is not yet completely healed.

Fig. 64. Shows a fracture 69 days after fracture, muscle section and periosteal cuff resection. There is fibrous union. Necrotic bone is seen. New bone replacing cartilage is also seen.

Fig. 65. Shows a fracture 87 days after fracture, muscle section and periosteal cuff resection. There is evidence of pseud-arthrosis.

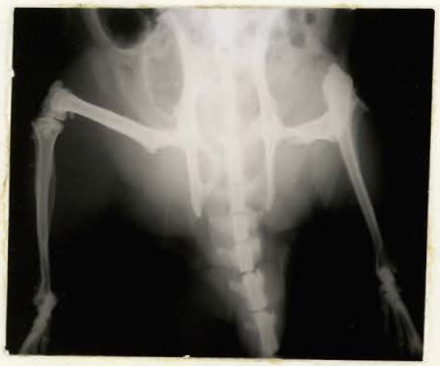


Fig. 63.

Radiogram 194 days after production of fracture, muscle section and periosteal cuff resection. The fracture is not yet completely healed.

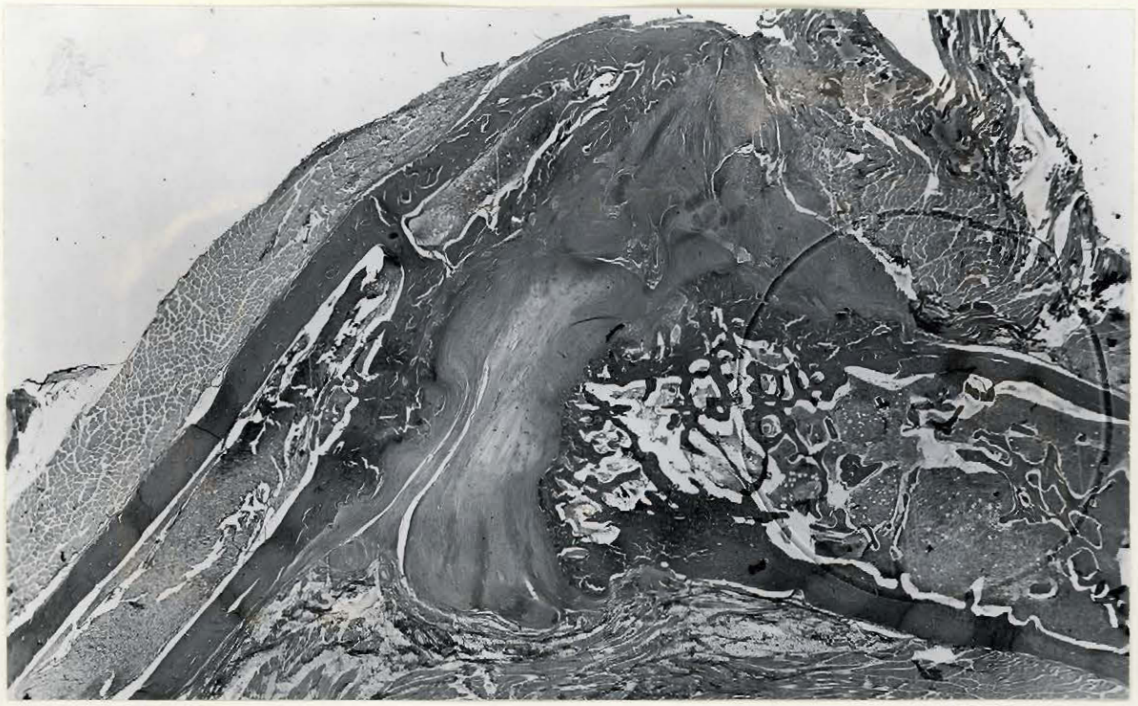


Fig. 64.

Shows a fracture (X 20) 69 days after fracture, muscle section and periosteal cuff resection. There is fibrous union, Necrotic bone is seen. New bone replacing cartilage is also seen.

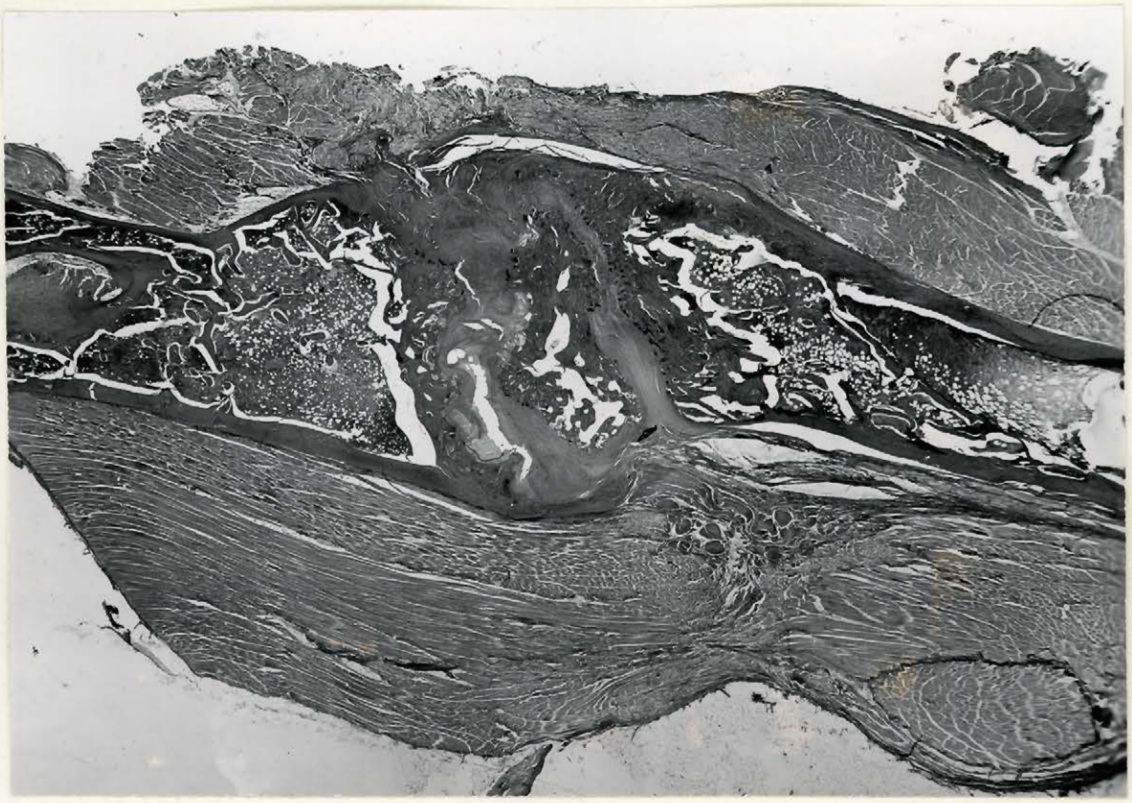


Fig. 65.

Shows a fracture (X 20) 87 days after fracture, muscle section and periosteal cuff resection. There is evidence of pseud-arthrosis.

Experiment A.

Group VIII (MT): - Repeated Manipulative Fractures.

Mortality - the same as in the control group (T).

Post-operative Course:- After the 4th fracture, the pattern of healing is essentially the same as after the first fracture as in group I (T). The fracture healed in the same range of time as in the control group. There was no increase in the incidence of non-union. As many as 85% of the fractures healed in an average period of 120 days. There was no suggestion that previous fractures shortened the healing period.

Fig. 66. Shows the gross specimen (F) 92 days after the last fracture.

Fig. 67. Shows the gross specimen 142 days after the last fracture.

Fig. 68. Shows the histology of a fracture 49 days after the last fracture.

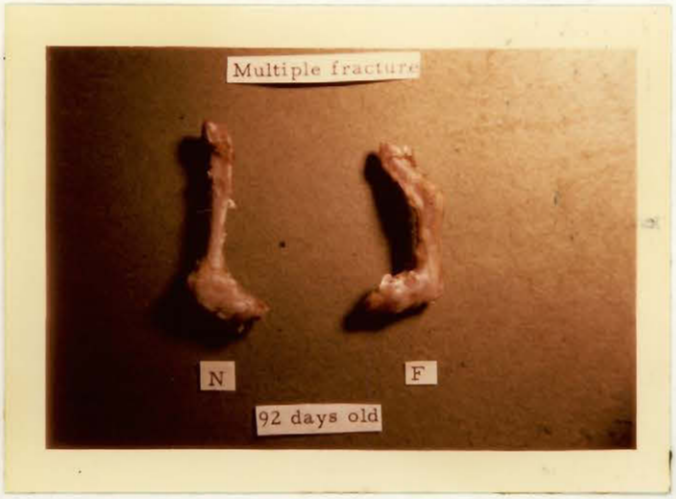


Fig. 66. Shows a gross specimen (F) 92 days after the last fracture. (N) is the contralateral femur.

Fig. 67.

Shows the gross specimen 142 days after the last fracture.
The contralateral femur is shown adjacent to the fractured femur.

Fig. 68.

Shows (X 20) a fracture 49 days after the last fracture.
Periosteal cuff is present.

Experiment A.

Group IX (LCT). - Transverse fracture in animals maintained on low calcium and vitamin D deficient diet throughout the experiment.

Mortality: - 8 - 10%.

Post-operative Course: - The animals were inactive and appeared sluggish and did not resume activity at the same rate as the animals in the other groups.

The repair process was found to be much delayed. The various stages of bone healing were prolonged and the incidence of bony union very low (15%). Cartilaginous union was observed in most of the specimens. The cartilaginous stage persisted throughout the observation period.

Fig. 69. Shows a 4 week old rat (40 gms), one day post-operatively. A rat on a standard diet at the same age normally weighed 80 - 90 gms.

Fig. 70. (A) Shows gross specimen 7 days after operation.
(B) Shows a gross specimen 39 days after operation.
(C) Shows a gross specimen 57 days after operation.

Fig. 71. Shows gross specimens (A) 64 days, (B) 94 days, (C) 156 days after production of the fracture.

Fig. 72. Radiograms (A) 2 days post-operatively, showing the degree of displacement of the fragments. (B) 56 days and (C) 76 days after the production of fracture showing non-union at the fracture sites.

Fig. 73. Shows histological appearance of a fracture 8 days after its production. Note the bulk of the cartilaginous callus.

Fig. 74. Shows histological appearance 15 days after production of fracture. There is a replacement of cartilaginous callus by bone near the fracture ends. A cleft in the intermediate callus is seen.

Fig. 75. Shows histological appearance 44 days after production of fracture with no further advance in the healing process. There is suggestion of pseud-arthrosis.

Fig. 76. Shows histological appearance 55 days after fracture. There is lack of further advance in the healing process.

Fig. 77. Shows a fracture 107 days after production of fracture with persistence of cartilaginous union.

Fig. 69. Shows a 4 week old rat (40 gms), one day post-operatively. A rat on a standard diet at the same age normally weighed 80 - 90 gms.

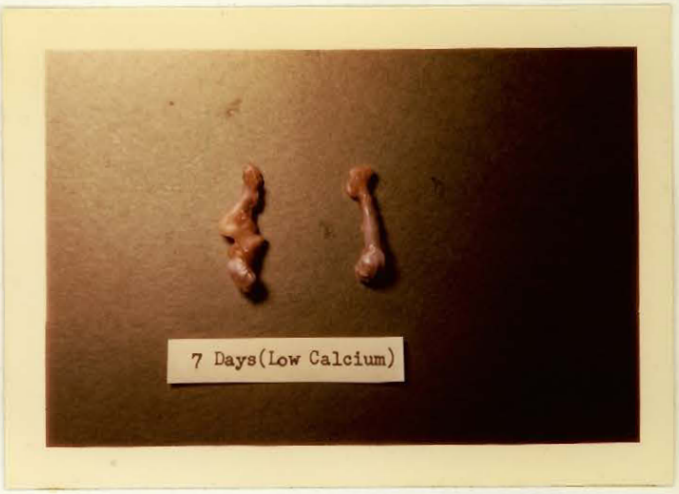


Fig. 70 A. Shows gross specimen 7 days after operation.

Fig. 70 B. Shows a gross specimen 39 days after operation.

Fig. 70 C. Shows a gross specimen 57 days after operation.

Fig. 70.

Shows gross appearance of fractures in animals on low calcium and vitamin D deficient diet. The contralateral femur is shown adjacent to the fractured femur.

Fig. 71 A.

Shows gross specimen 64 days after production of fracture.

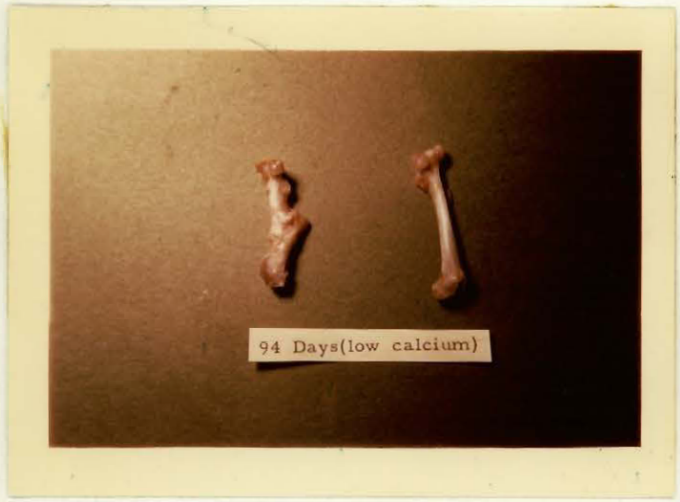


Fig. 71 B. Shows a gross specimen 94 days after fracture.

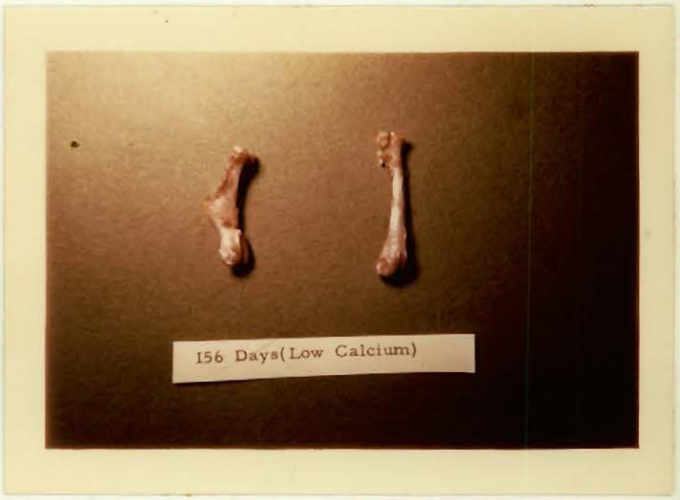


Fig. 71 C. Shows a gross specimen 156 days after fracture.

Fig. 71.

Shows gross appearance of fractures in animals on low calcium and vitamin D deficient diet. The contralateral femur is shown adjacent to the fractured femur.

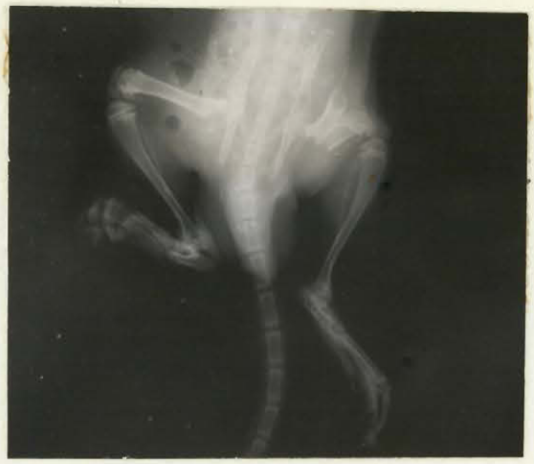


Fig. 72 A.
Radiogram 2 days post-operatively, showing marked degree of displacement of the fragments.

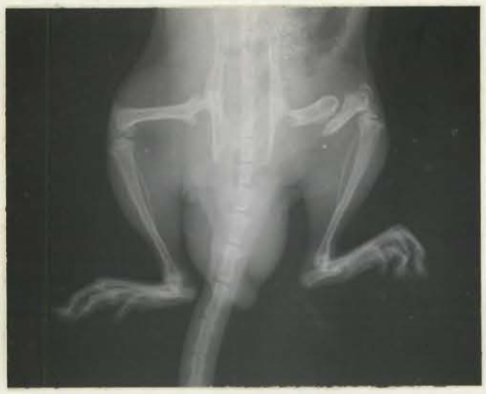


Fig. 72 B. Radiogram 56 days after fracture, showing lack of bony union.

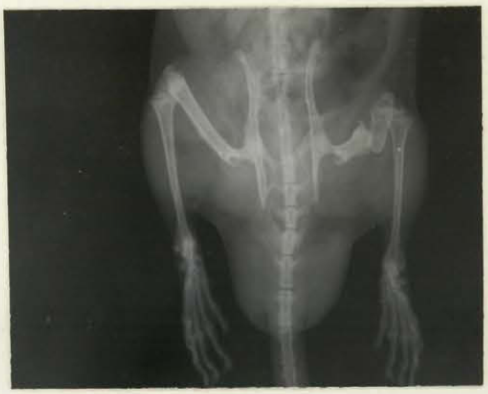


Fig. 72 C. Radiogram 76 days after fracture, showing lack of bony union.

Fig. 72.
Radiograms showing marked displacement and lack of bony union of fractures in animals on low calcium and vitamin D deficient diet.

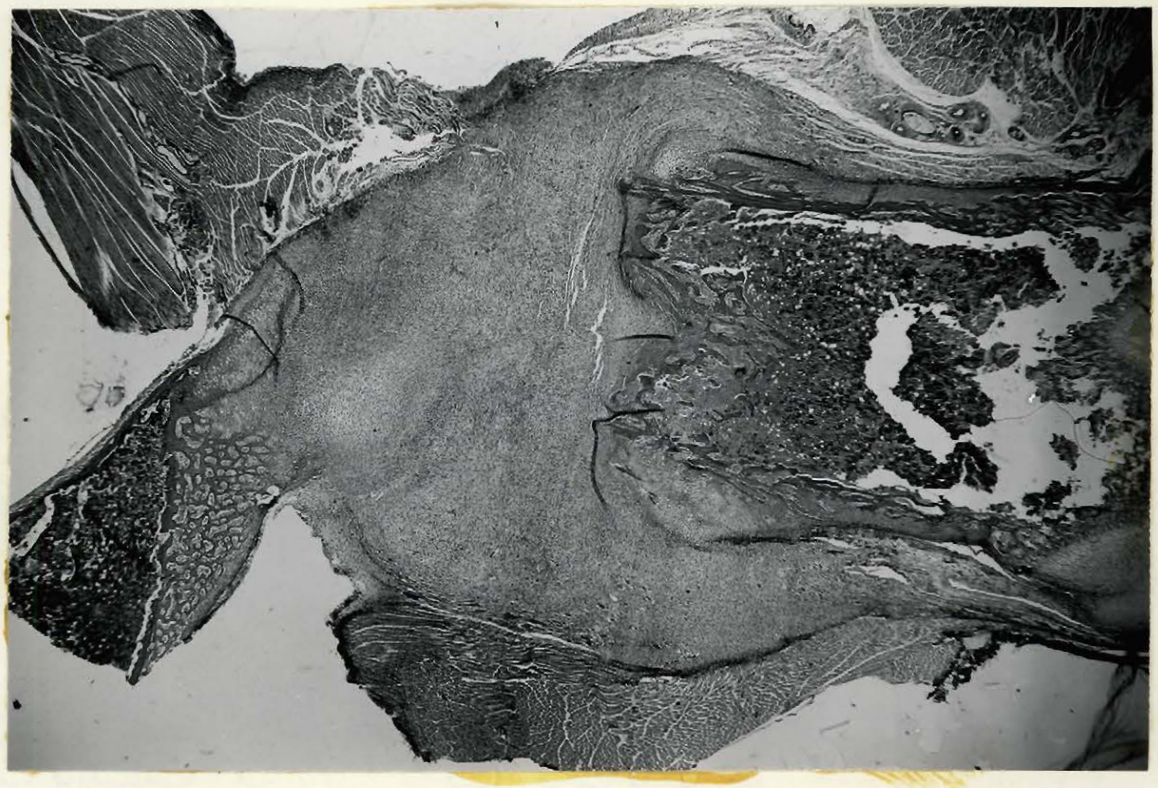


Fig. 73.

Shows histological appearance of a fracture (X 20) 8 days after its production. Note the bulk of the cartilaginous callus.

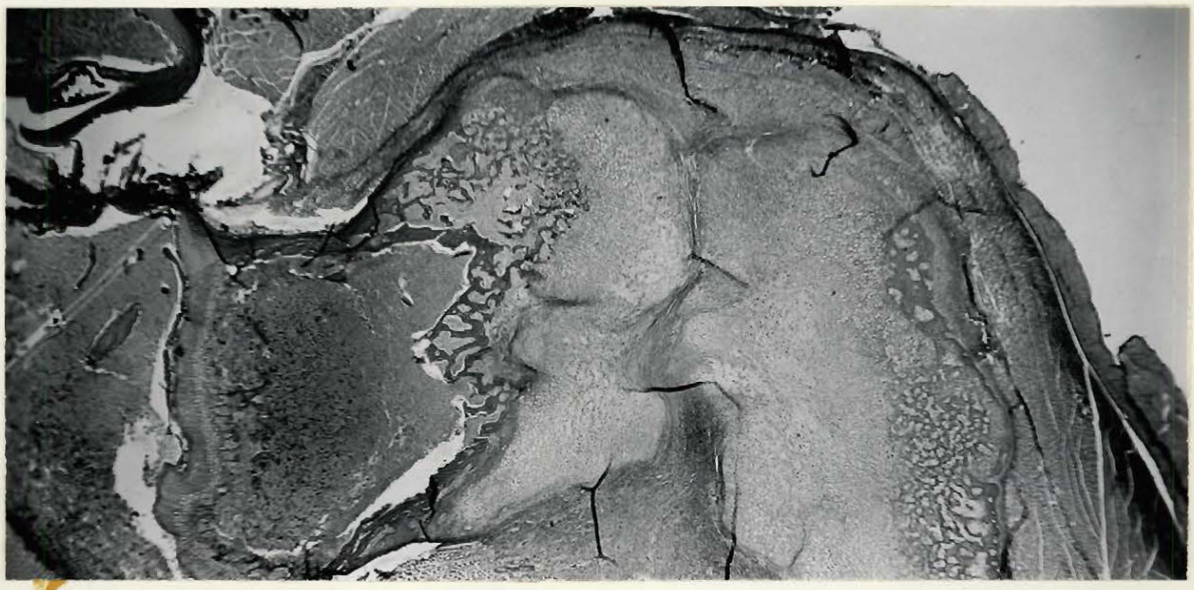


Fig. 74.

Shows histological appearance 15 days after production of fracture (X 20). There is replacement of cartilaginous callus by bone near the fracture ends. A cleft in the intermediate callus is seen.

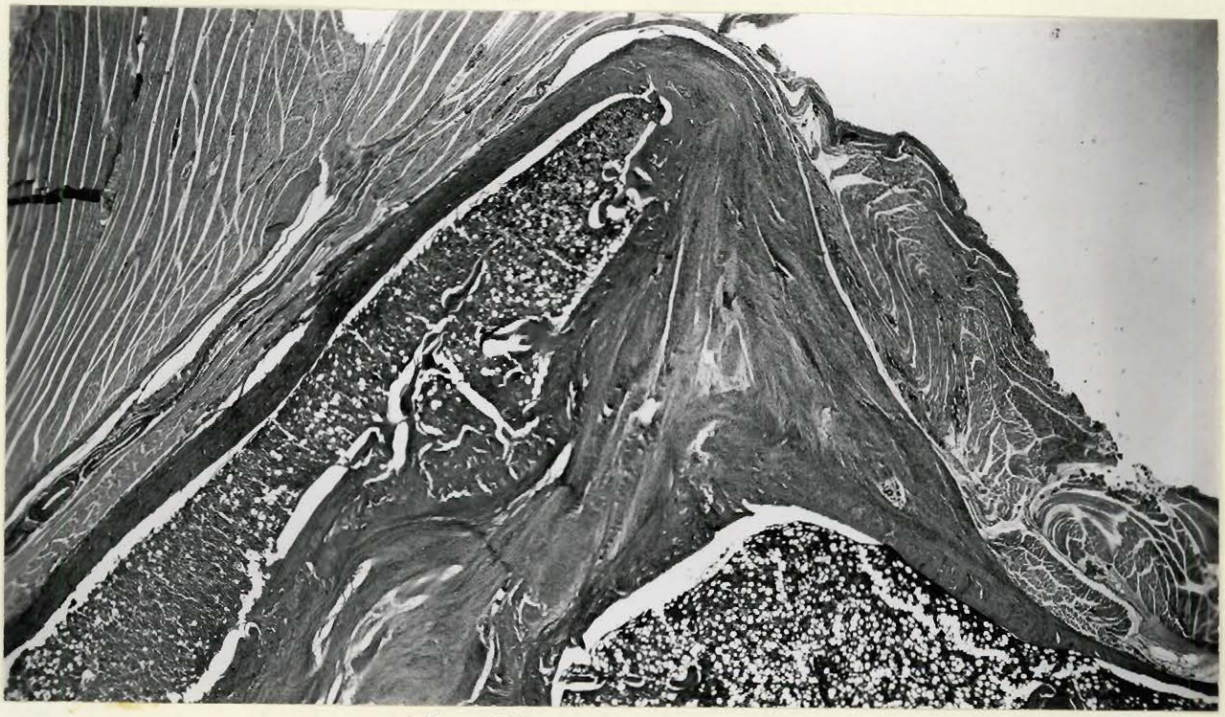


Fig. 75.
Shows histological appearance 44 days after production of fracture. There is no further advance in the healing process. There is suggestion of pseud-arthrosis (X 20).

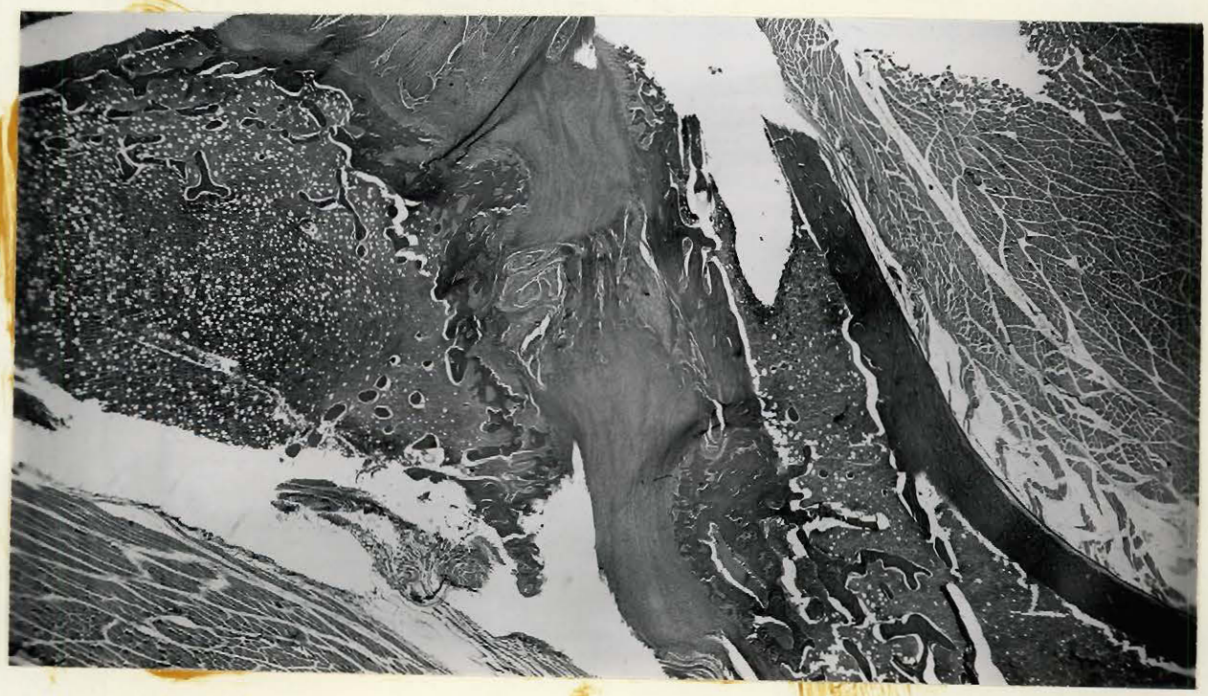


Fig. 76.
Shows histological appearance 55 days after fracture (X 20). There is lack of further advance in the healing process.

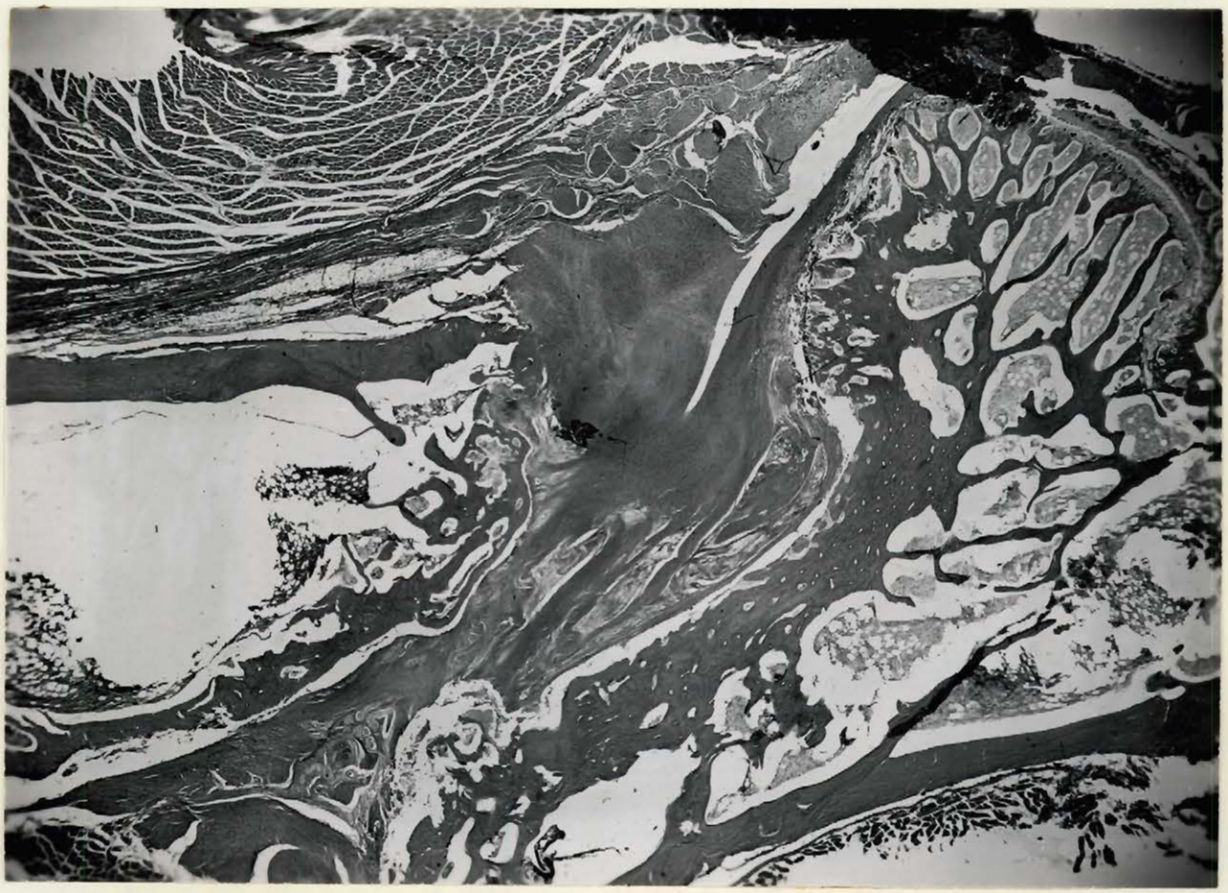


Fig. 77.

Shows a fracture 107 days after production of fracture in a rat kept on low calcium and vitamin D deficient diet. Note the persistence of cartilaginous callus. (X 20).

Experiment A.

Group X (P). Excision of Periosteal Cuff, 1 cm wide; no Fracture.

Except for a very high mortality rate both operatively and post-operatively (up to 45% of animals died in the first one-half hour of operation), no significant alterations were observed at the operated site, when re-examined one week later. There were adhesions between the denuded cortex and the surrounding muscle, but the denuded cortical bone appeared perfectly viable.

In 10 of these animals routine fractures were produced through the area of previous periosteal removal. No significant alterations in the repair process as compared to the control series were seen.

Experiment B.

Study of Osteogenesis utilizing Radioactive Strontium 89.

Group B I Study of uptake of radioactivity at the fracture site as compared to the diaphysis of the contralateral femur, from post-fracture day 1 to 289. (Uptake study).

As outlined earlier, animals in this group are injected with Sr89 (0.1 uc per gm.body wt.) at different post-fracture days and are sacrificed 24 hours after the administration of the isotope.

This study includes the results of uptake of radioactivity by the fracture site in the femur and the contralateral site from post-fracture 2 to 289.

Table I shows graphic representation of corrected counts per minute per mgm. weight of bone at the fracture site and contra-lateral diaphysis. Increase in uptake of radioactivity at the fracture site was apparent by post-fracture day 5 and was maintained throughout the study period, up to post-fracture day 289. The peak was observed from post-fracture day 15 to post-fracture day 145.

Following fracture the callus formation results in local increase in weight (up to 250 mgm.). The uptake in non-mineralized callus is very low, while the callus that is being mineralized takes up the maximum radioactivity; in the early post-fracture days this is mainly the periosteal callus.

UPTAKE STUDY

EXPERIMENT B, GROUP 1

Uptake of Sr89 at the fracture site and the contralateral diaphysis of femur, calculated per mgm. weight of bone (wet weight), in male, R.V.H. strain rats.

	FRACTURE SITE		CONTRALATERAL DIAPHYSIS			
Days after Fracture	Corr. Uptake Per Min.	Uptake Per Mgm. Wt.	Corr. Uptake Per Min.	Uptake Per Mgm. Wt.	Ratio	
2	8150(220)	185	4943(100)	247	0.74	
	5158(75)	343	2960(50)	296	1.15	*
	1284(60)	107	1176(50)	118	0.90	
3	6902(205)	168	4758(85)	279	0.60	
	6622(160)	206	4032(90)	224	0.91	*
5	16390(340)	241	5376(100)	268	0.89	
	31943(340)	469	5612(70)	400	1.17	
	31697(240)	660	5612(70)	400	1.65	*
	23890(340)	351	4682(90)	260	1.34	
7	16177(240)	337	3907(70)	279	1.20	*
	18747(440)	213	7977(130)	306	0.69	
	17611(360)	244	5497(100)	274	0.89	
9	8460(410)	103	2558(80)	161	0.63	
	15646(320)	244	4555(70)	325	0.75	
	26764(370)	361	6367(110)	289	1.24	
12	23589(370)	316	7571(100)	378	0.83	
	27155(400)	339	9422(200)	235	1.44	
	26466(320)	413	8294(120)	345	1.19	
	30814(400)	385	4822(40)	219	1.75	
15	24881(380)	327	6513(110)	287	1.13	
	29414(420)	420	8000(150)	333	1.26	
	25187(400)	314	7123(150)	273	1.15	
	36787(220)	856	6716(60)	551	1.51	*
	59391(530)	560	8191(100)	255	2.19	
16	47270(250)	1027	22299(180)	619	1.65	*
	51188(410)	624	22299(180)	619	1.00	
17	11649(405)	143	1986(105)	94	1.52	
	36266(470)	385	7079(140)	262	1.46	
	41279(520)	396	8589(160)	268	1.47	
	10140(200)	253	2406(50)	240	1.10	*
18	20418(350)	291	5096(125)	203	1.43	
	11176(558)	558	4538(100)	226	2.46	
	18113(510)	177	5472(110)	248	0.71	
21	33560(380)	441	7391(110)	335	1.31	
	15427(230)	335	5554(120)	231	1.45	
	17884(250)	357	5595(120)	233	1.53	
25	23454(350)	335	7381(150)	246	1.36	
	24674(320)	385	7376(150)	245	1.57	
28	34968(320)	546	7666(110)	348	1.56	
	21944(280)	391	7502(110)	341	1.14	
	21104(250)	422	7953(120)	331	1.27	
	20170(360)	280	10166(130)	391	0.71	
	35761(180)	993	10370(90)	576	1.73	
30	34589(350)	494	7652(140)	273	1.80	
	16117(210)	383	7528(130)	289	1.32	
	10178(200)	254	6220(100)	311	0.81	
	22794(290)	393	3779(100)	188	2.09	

Days after Fracture	Corr. Uptake Per Min.	Uptake Per Mgm. Wt.	Corr. Uptake Per Min.	Uptake Per Mgm. Wt.	Ratio	
35	29777(305)	488	11434(150)	381	1.28	
	30681(290)	528	10289(130)	395	1.33	
	29464(305)	483	14554(150)	485	1.00	
	16389(130)	630	7115(100)	355	1.77	*
45	62800(230)	1370	19648(110)	893	1.53	
	24804(230)	539	5579(90)	309	1.74	
	33655(210)	801	7052(60)	587	1.36	**
48	24984(220)	567	6036(90)	335	1.69	
53	20261(250)	405	5695(120)	239	1.70	
	16130(230)	350	5401(120)	225	1.55	
	11530(230)	250	4201(120)	175	1.42	
65	20282(160)	633	16193(100)	426	1.48	
	26703(210)	635	11948(190)	314	2.01	
70	20624(280)	361	10304(210)	215	1.47	
	19624(350)	280	11304(210)	269	1.04	
	24326(250)	486	10636(150)	354	1.37	
75	7771(350)	111	4531(150)	151	0.73	
	21705(240)	452	7080(200)	177	2.55	**
	24339(220)	553	10938(210)	260	2.12	
80	17903(210)	426	6607(145)	227	1.87	
	22442(320)	350	9262(200)	231	1.51	
87	28920(260)	556	15556(200)	388	1.43	
	23492(140)	839	11946(100)	597	1.40	
101	13015(160)	406	7296(200)	182	2.23	
108	9990(240)	208	5214(190)	137	1.51	
	18691(260)	364	9696(200)	242	1.50	
113	32882(400)	411	5277(210)	125	3.28	
130	9571(280)	170	4917(200)	122	1.38	**
	212936(450)	2365	56608(170)	1664	1.42	**
135	13709(420)	163	7732(180)	214	0.76	
139	5902(270)	109	4414(120)	183	0.59	
145	32369(340)	476	9885(150)	329	1.44	
	31190(380)	410	9608(170)	282	1.45	
153	6113(175)	174	5115(165)	155	1.12	***
	17228(350)	246	6789(240)	141	1.74	
158	10188(300)	169	7480(220)	170	1.00	***
166	19932(300)	332	9129(170)	268	1.23	
	151994(380)	1999	61790(180)	1716	1.16	
181	6093(190)	160	7104(180)	197	0.81	***
218	58288(210)	1387	46174(190)	1215	1.14	***
232	1376(320)	21	1376(140)	49	0.42	
238	12153(470)	129	3904(180)	108	1.19	***
242	3268(210)	77	4608(220)	104	0.74	
	6214(360)	86	3376(250)	67	1.28	
256	6136(210)	146	5244(220)	119	1.23	***
260	8429(260)	162	4958(200)	123	1.31	***
262	13648(320)	213	4257(180)	118	1.80	**
283	100648(210)	2369	54730(180)	1520	1.57	**
289	14416	167	7529(235)	160	1.04	***

* Fracture end.

** Bony union.

*** Bony union with reconstruction.

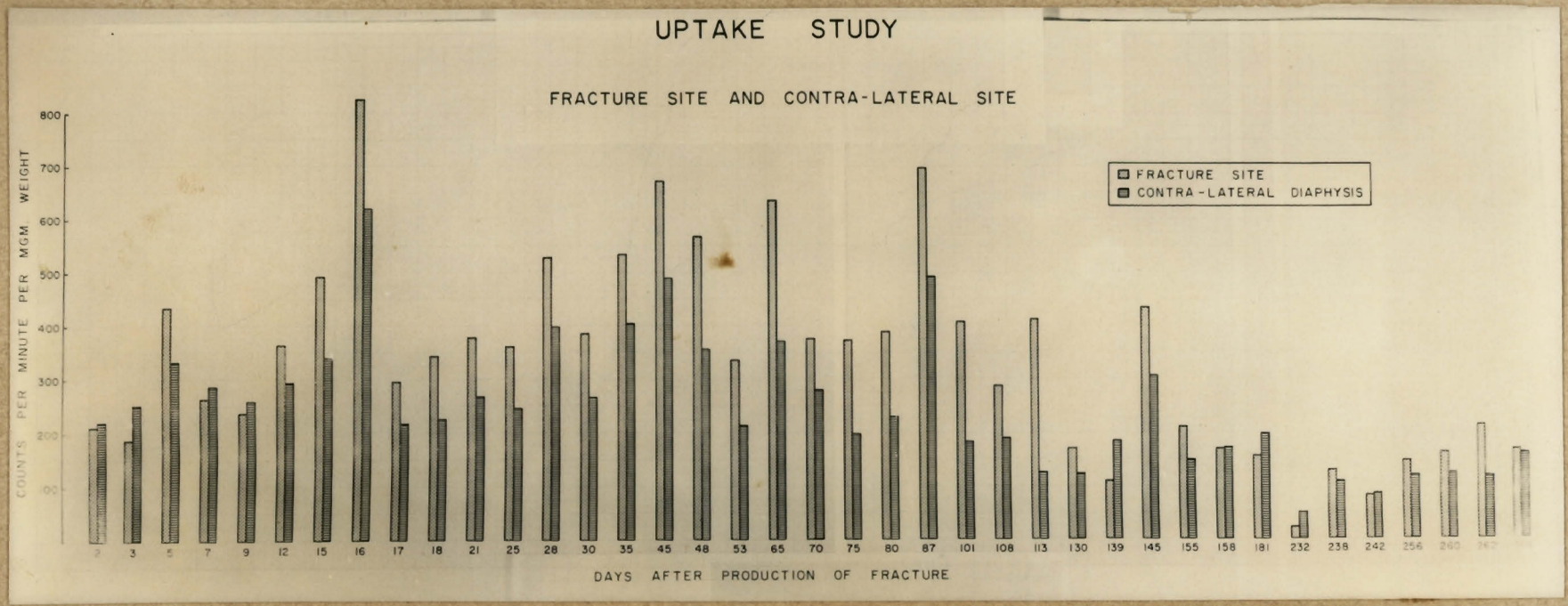


Fig. 78.

Shows radioactivity at the fracture site in comparison to uptake at the contralateral site from post-fracture day 2 to 289. The increase at the fracture site is apparent from post-fracture day 5 and is maintained throughout the study period.

By using the formula

$$\text{Increased Percent} = \frac{R_F - R_{F_{cl}}}{R_{F_{cl}}} \times 100$$

(R_F means radioactivity at the fracture site and

$R_{F_{cl}}$ means radioactivity at the contralateral diaphysis)

The radioactivity at the fracture site as compared to contralateral diaphysis is increased at the fracture site 4 - 5 fold.

There was also a close relation between the stage of healing and the uptake of radioactivity.

a) In those fractures where bony union was observed but fracture site was still recognizable (the reconstruction was not yet completed), the fracture site showed a ratio of 1.68 when compared to contralateral diaphysis.

b) In those fractures where bony union was observed and fracture site could no longer be recognized because of adequate reconstruction, the fracture diaphysis showed a ratio of 1.10 when compared to contralateral diaphysis.

CORRELATIVE STUDY

EXPERIMENT B, GROUP II

Uptake of Sr89 at ipsilateral proximal, distal metaphyses and fracture site, and contralateral proximal, distal metaphyses and diaphysis of femur, calculated per min. per mgm. wt. (wet weight), in male, R.V.H. strain rats.

	PROXIMAL METAPHYSIS		DISTAL METAPHYSIS		FRACTURE & DIAPHYSIS	
Days after Fracture	Corr. Uptake Per Min.	Uptake Per Mgm. Wt.	Corr. Uptake Per Min.	Uptake Per Mgm. Wt.	Corr. Uptake Per Min.	Uptake Per Mgm. Wt.
2	(IL) 1269(40)	158	1448(60)	120	1284(60)	107
	(CL)1257(60)	104	1681(80)	105	1176(50)	118
	(IL) 3881(70)	277	12316(150)	410	5158(75)	343
	(CL)3838(60)	319	13635(135)	505	2960(50)	296
5	(IL) 2264(50)	226	4563(60)	380	31697(240)	660
	(CL)2530(55)	230	3972(65)	305	5612(70)	400
9	(IL) 3973(60)	331	8402(80)	525	15646(320)	244
	(CL)3702(50)	370	13468(100)	673	4553(70)	325
15	(IL) 6710(70)	479	25557(220)	580	59391(530)	560
	(CL)8930(115)	388	25780(250)	515	8191(160)	255
17	(IL) 2140(40)	267	5391(80)	336	10140(200)	253
	(CL)2004(30)	334	9131(100)	456	2406(50)	240
21	(IL) 153(50)	215	2372(190)	162	3977(400)	249
	(CL) 422(60)	235	1330(130)	255	407(100)	220
25	(IL) 4656(50)	465	13738(100)	686	23454(350)	335
	(CL)7327(366)	366	17980(200)	449	7381(150)	246
	(IL) 3255(80)	203	13615(125)	544	24674(320)	385
	(CL)6816(110)	309	16341(180)	453	7367(150)	245
28	(IL)26213(110)	1191	17444(140)	623	35761(180)	993
	(CL)5112(90)	284	20400(180)	566	10370(90)	576
30	(IL) 4947(50)	494	16303(110)	741	16117(210)	383
	(CL)6819(80)	426	17061(160)	533	7528(130)	289
35	(IL) 7443(60)	620	12209(95)	642	16389(130)	630
	(CL)6703(80)	418	17973(130)	691	7115(100)	355
45	(IL)12386(130)	476	17309(110)	786	24804(230)	539
	(CL)14387(180)	399	18824(130)	632	5579(90)	309
	(IL) 6756(60)	563	16920(130)	650	33655(210)	801
	(CL)7158(60)	596	22239(140)	794	7052(60)	587
53	(IL) 822(110)	37	1509(130)	58	20261(250)	405
	(CL)2569(100)	128	2934(150)	97	5695(120)	239
	(IL) 1136(60)	94	1595(155)	51	11530(230)	250
	(CL)1176(95)	61	1606(180)	44	4201(120)	175
65	(IL) 5730(90)	318	20899(160)	653	20282(160)	633
	(CL)8469(130)	325	23147(220)	526	16193(100)	426
70	(IL) 9048(80)	565	20254(140)	733	20624(280)	361
	(CL)7643(100)	382	21549(220)	489	10304(210)	215
75	(IL) 2086(70)	149	5055(170)	148	7771(350)	111
	(CL)3429(110)	155	7471(240)	155	4531(150)	151
	(IL) 4587(90)	254	12786(170)	376	21705(240)	452
	(CL)5693(100)	284	16310(200)	407	7080(200)	177
	(IL) 4980(70)	356	22084(160)	690	24339(220)	553
	(CL)10281(160)	320	26171(190)	688	10938(210)	260
80	(IL) 2639(50)	263	10298(130)	396	17903(210)	426
	(CL)4668(100)	233	11553(120)	481	6607(145)	227

Days after Fracture	PROXIMAL METAPHYSIS Corr. Uptake Per Min.	PROXIMAL METAPHYSIS Uptake Per Mgm. Wt.	DISTAL METAPHYSIS Corr. Uptake Per Min.	DISTAL METAPHYSIS Uptake Per Mgm. Wt.	FRACTURE & DIAPHYSIS Corr. Uptake Per Min.	FRACTURE & DIAPHYSIS Uptake Per Mgm. Wt.
108	(IL) 3732(110)	169	8993(170)	264	9990(240)	208
	(CL)4597(155)	148	13350(210)	317	5214(190)	137
113	(IL) 3990(85)	234	8346(85)	490	32882(400)	411
	(CL)7439(120)	309	13114(890)	345	5277(210)	125
127	(IL) 6148(110)	279	13068(185)	353	Absorption of fracture site	
	(CL)7305(145)	251	16434(190)	434	5768(120)	237
130	(IL) 2804(110)	127	12422(230)	270	9571(280)	170
	(CL)4153(130)	159	8112(170)	238	4917(200)	122
(	(IL)60887(110)	2767	10285(180)	285	212936(450)	2365
	(CL)71294(150)	2376	10661(190)	280	56608(170)	1664
135	(IL) 4995(90)	277	12981(150)	432	13709(420)	163
	(CL)4827(100)	241	19098(180)	530	7732(180)	214
139	(IL) 4608(85)	271	18476(180)	513	5902(270)	109
	(CL)6116(90)	339	20406(170)	600	4414(120)	183
145	(IL) 8736(80)	546	22481(130)	864	32369(340)	476
	(CL)8799(90)	488	31086(170)	914	9885(150)	329
	(IL) 5519(100)	275	21284(200)	532	31190(380)	410
	(CL)5912(110)	268	21901(230)	476	9608(170)	282
153	(IL) 4680(120)	195	15626(240)	325	6113(175)	174
	(CL)4456(120)	185	17091(270)	316	5115(165)	155
	(IL) 5795(70)	413	17971(215)	417	17228(350)	246
	(CL)9259(150)	308	17327(220)	393	6789(240)	141
158	(IL) 3466(70)	247	14266(200)	356	10188(300)	169
	(CL)7221(150)	240	16710(240)	348	7480(220)	170
166	(IL)10073(110)	457	15998(160)	499	19932(300)	332
	(CL)8268(100)	413	23063(200)	576	9129(170)	268
	(IL)42309(100)	2115	117555(170)	3457	151994(380)	1999
	(CL) 0016(180)	1944	116592(170)	3429	61790(180)	1716
181	(IL) 2918(80)	182	12045(150)	401	6093(190)	160
	(CL)3395(80)	212	14538(190)	382	7104(180)	197
218	(IL)40010(130)	1538	62204(110)	2827	58288(210)	1387
	(CL)34063(120)	1419	24332(105)	1158	46174(190)	1215
232	(IL) 4204(140)	150	5392(170)	158	9376(280)	167
	(CL)4189(145)	144	5415(180)	150	3376(140)	120
238	(IL) 4415(180)	122	6632(170)	195	12153(470)	129
	(CL)2617(100)	130	7960(180)	221	3904(180)	108
242	(IL) 2464(120)	102	6182(150)	206	3268(210)	77
	(CL)4958(170)	151	9185(170)	270	4608(220)	104
	(IL) 5169(150)	172	9489(180)	263	6136(210)	146
	(CL)3853(150)	128	14820(210)	352	5244(220)	119
260	(IL) 2831(110)	128	6627(200)	165	8429(260)	162
	(CL)4031(130)	155	1583(80)	98	4958(200)	123
262	(IL) 3126(120)	130	7973(200)	199	13648(320)	213
	(CL)5961(140)	212	10836(246)	220	4257(180)	118
283	(IL)55013(120)	2292	42939(80)	2683	100648(210)	2396
	(CL)53598(130)	2061	78324(110)	3560	54730(180)	1520
289	(IL) 4449(110)	202	12912(190)	339	14416(430)	167
	(CL)6466(150)	215	12926(180)	359	7529(235)	160

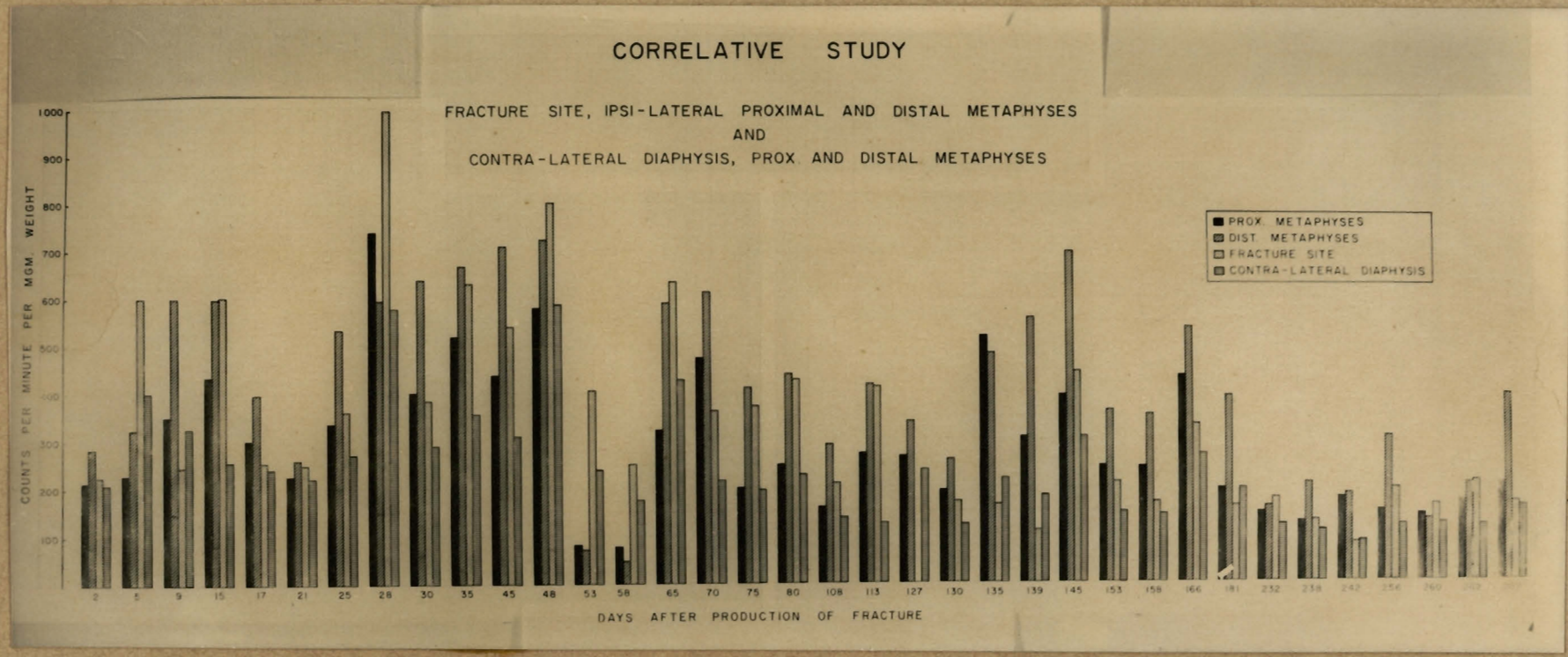


Fig. 79.

Shows correlative study of radioactivity at the fracture site, proximal and distal metaphyses from post-fracture day 2 to 289. The maximum uptake of radioactivity at the fracture site has its peak from post-fracture day 15 to 145.

From the accumulated data it would appear that the uptake-capacity of bone per mgm. wt. at the fracture site varies with the stage of healing of the fracture. A rise in uptake occured at post-fracture day 5 and increased uptake was maintained with a peak from post-fracture day 28 to post-fracture day 155. Towards the end of this study period, the uptake-capacity at the fracture site was at low levels. This coincides fairly well with the mineralization phase of the fracture callus. As the mineralization follows a non-homogenous pattern, the variations can be explained. It is the callus undergoing mineralization at the time of Sr89 injection which picks up the greater amount of radioactivity. Also, the recently laid down mineral takes up more radioactivity than the older well mineralized bone (Neuman & Neuman, 1953; Nilsonne, 1959).

In the group of animals maintained on low calcium and vitamin D deficient diet, a very slight increase in the uptake of radioactivity was observed at the fracture site, per minute per mgm. weight (ratio 1.2), but by post-fracture day 9 an increased ratio 1.67 and 1.45 were observed when compared to contralateral diaphysis. This increase was apparent till post-fracture day 45. There seemed to be a more diffuse distribution of radioactivity in the latter half of the study period, from post-fracture day 60 to post-fracture day 213. The peak of this increased uptake followed from post-fracture day 9 till post-fracture day 181 when the uptake of radioactivity was of lower magnitude. Uptake on post-fracture day 213 was of the same magnitude as of post-fracture day 3.

UPTAKE STUDY

EXPERIMENT B. GROUP V

(Low Calcium and Vitamin D Deficient Diet)

Uptake of Sr89 at the fracture site and the contralateral diaphysis of femur, calculated per mgm. weight of bone (wet weight), in male, R.V.H. strain rats.

	FRACTURE SITE		CONTRALATERAL DIAPHYSIS		
Days after Fracture	Corr. Uptake Per Min.	Uptake Per Mgm. Wt.	Corr. Uptake Per Min.	Uptake Per Mgm. Wt.	Ratio
3	1783(90)	99	1251(40)	156	0.6
	1155(60)	96	2409(50)	240	0.4
6	5555(140)	198	1596(40)	199	1.00
	17587(360)	244	10592(260)	203	1.20
9	6788(70)	484	2892(50)	289	1.67
	6182(150)	206	1141(40)	142	1.45
15	36787(220)	836	6616(60)	551	1.51
	23487(190)	618	4141(50)	414	1.49
30	3138(30)	513	2588(30)	431	1.21
	12932(90)	718	1518(30)	253	2.83
35	17557(200)	438	2300(65)	176	2.48
	14954(150)	498	9115(80)	569	0.87
40	6817(110)	309	2106(70)	150	2.06
45	22140(170)	651	6603(60)	550	1.18
	4827(130)	185	1649(80)	103	1.79
60	24093(270)	446	7438(100)	371	1.20
	12812(160)	400	9526(95)	501	0.79
65	22618(330)	324	7797(105)	380	0.90
70	29578(240)	616	9776(90)	543	1.13
	18045(350)	257	9154(110)	416	0.61
85	21967(340)	323	10065(140)	359	0.89
	23492(140)	839	11946(100)	597	1.40
94	11816(220)	268	7175(120)	298	0.89
124	29386(450)	315	8206(170)	241	1.30
138	22531(375)	300	7874(140)	281	1.06
156	15709(250)	314	6422(110)	292	1.07
181	9352(250)	187	3635(90)	201	0.93
	8113(250)	162	3393(95)	178	0.91
213	8749(380)	115	3609(150)	120	0.95

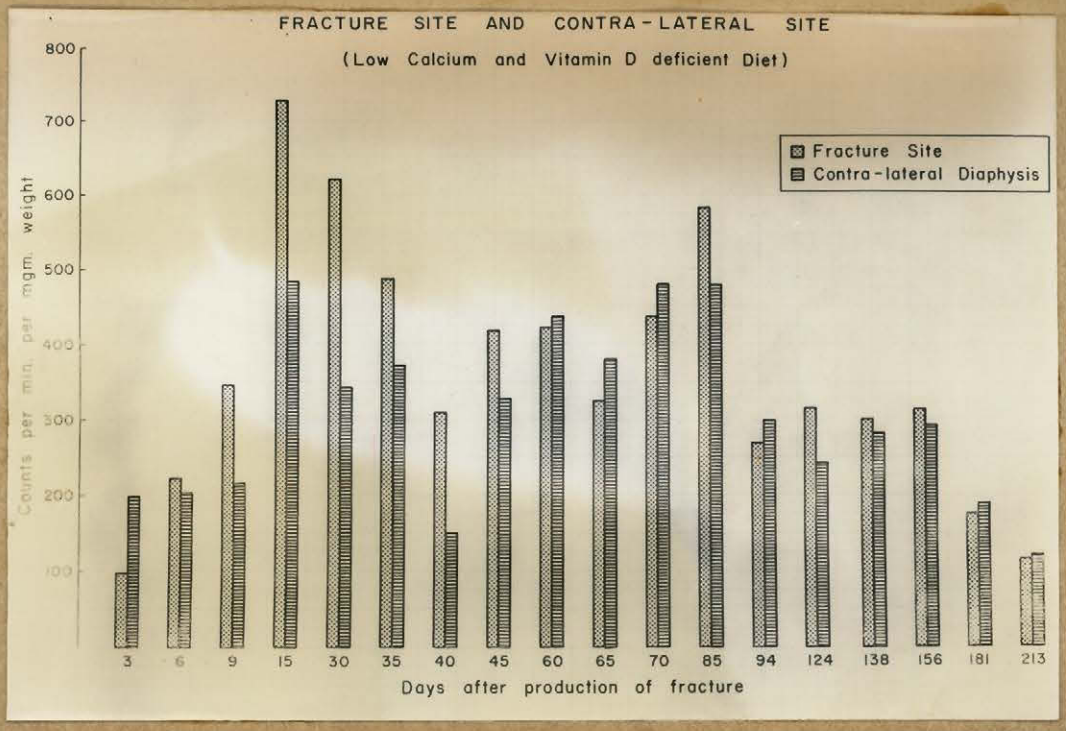


Fig. 80. A.

Shows uptake of radioactivity at the fracture site and the contra-lateral diaphysis in animals maintained on low calcium and vitamin D deficient diet. The peak of uptake in this study extends from day 15 to day 45 after the production of fracture.

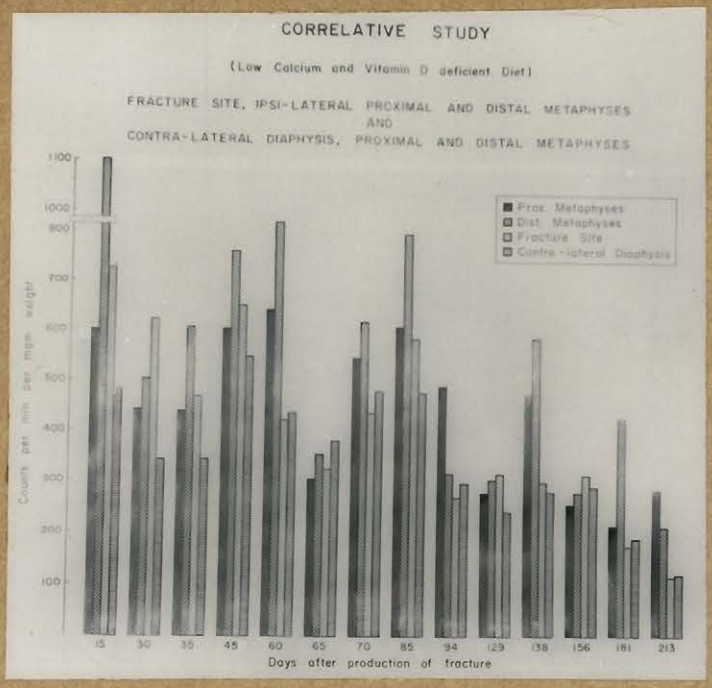


Fig. 80. B.

Shows correlative study of radioactivity at the fracture site, proximal and distal metaphysis in animals maintained on deficient diet.

Group B II — Study of uptake of radioactivity at the fracture site in comparison to uptake at proximal and distal metaphyses on the fractured femur and diaphysis and the two metaphyses in the contralateral femur (Correlative Study).

Animals were sacrificed 24 hours after administration of Sr^{89} and radioactivity was recorded at fracture site, ipsilateral proximal and distal metaphyses, and contralateral diaphysis, proximal and distal metaphyses. This study extends over a period of post-fracture day 2 to post-fracture day 289.

There is an increased uptake of radioactivity at the fracture site from post-fracture day 5 (vide supra). This was maintained throughout the study period with peak from post-fracture day 25 to post-fracture day 145. Highest uptake was recorded from post-fracture day 28 to post-fracture day 80.

The uptake of radioactivity at the fracture site was higher than that of the ipsilateral proximal metaphysis with the exception of the findings on post-fracture days 9, 17, 30, 70, 130, 135, 139, 153, 158, 166, 181, 242, and 289.

The uptake of radioactivity at the fracture site was higher than the ipsilateral distal metaphysis on post-fracture days 5, 15, 28, 48, 53, 58, 65, 232, 260, and 262.

CORRELATIVE STUDY

EXPERIMENT B, GROUP V

(Low Calcium and Vitamin D Deficient Diet)

Uptake of Sr89 at ipsilateral proximal, distal metaphyses and fracture site, and contralateral proximal, distal metaphyses and diaphysis of femur, calculated per min. per mgm. wt. (wet weight), in male, R.V.H. strain rats.

	PROXIMAL METAPHYSIS		DISTAL METAPHYSIS		FRACTURE & DIAPHYSIS	
Days after Fracture	Corr. Uptake Per Min.	Uptake Per Mgm. Wt.	Corr. Uptake Per Min.	Uptake Per Mgm. Wt.	Corr. Uptake Per Min.	Uptake Per Mgm. Wt.
15	(IL) 5061(40)	633	15367(70)	1097	36787(220)	836
	(CL)8482(60)	706	22353(100)	1117	6616(60)	551
	(IL) 4179(40)	522	15099(60)	1257	23487(190)	618
	(CL)3293(30)	548	15022(80)	938	4141(50)	414
30	(IL) 2462(30)	410	3803(30)	633	12932(90)	718
	(CL)1417(20)	367	3988(50)	398	1518(30)	253
	(IL) 2671(30)	445	11567(115)	502	3148(30)	524
	(CL)5352(50)	535	10647(110)	483	2588(30)	431
35	(IL) 1838(50)	183	7412(130)	285	17557(200)	438
	(CL)2610(60)	217	6409(110)	291	2300(65)	176
	(IL) 8400(60)	700	13289(90)	738	14954(150)	498
	(CL)10529(80)	658	31478(140)	1124	9115(80)	569
45	(IL) 4403(35)	629	9614(55)	874	22149(170)	651
	(CL)4068(35)	581	9096(70)	649	6603(60)	550
60	(IL) 6241(50)	624	18099(120)	754	24093(270)	446
	(CL)8434(70)	602	16829(90)	934	7438(100)	371
	(IL) 6604(45)	733	14802(95)	779	12812(160)	400
	(CL)7350(60)	612	16243(100)	812	9526(95)	501
65	(IL) 4695(80)	293	11691(180)	324	22618(330)	324
	(CL)6252(100)	312	12982(170)	381	7997(105)	380
70	(IL) 6883(50)	688	9924(70)	708	29578(240)	616
	(CL)4757(60)	396	9344(100)	467	9776(90)	543
	(IL) 5225(50)	522	11464(100)	573	18045(350)	257
	(CL)8266(70)	590	15381(105)	732	9154(110)	416
85	(IL) 5036(50)	503	12208(110)	554	21967(340)	323
	(CL)7335(75)	489	24365(160)	761	10065(140)	359
	(IL) 9617(70)	686	22326(120)	930	23492(140)	839
	(CL)13029(80)	814	26642(140)	951	11946(100)	597
94	(IL) 5842(60)	486	11797(110)	536	11816(220)	268
	(CL)7946(80)	496	18825(130)	724	7175(120)	298
124	(IL) 6069(110)	275	19241(170)	565	29386(450)	315
	(CL)6766(120)	281	12550(180)	348	8206(170)	241
138	(IL) 7828(85)	460	18410(160)	575	22531(375)	300
	(CL)8866(90)	492	7299(260)	600	7874(140)	281
156	(IL) 3500(60)	291	7707(150)	256	15709(250)	314
	(CL)5256(120)	219	14722(120)	304	6422(110)	292
181	(IL) 1316(40)	164	8543(90)	474	8113(250)	162
	(CL)4687(90)	260	8286(95)	436	3393(95)	178
	(IL) 2658(60)	171	10610(140)	378	9352(250)	187
	(CL)5743(110)	261	11972(140)	427	3635(90)	201
	(IL) 6209(110)	282	10546(200)	263	8749(380)	115
	(CL)7006(120)	291	3567(110)	162	3603(150)	120

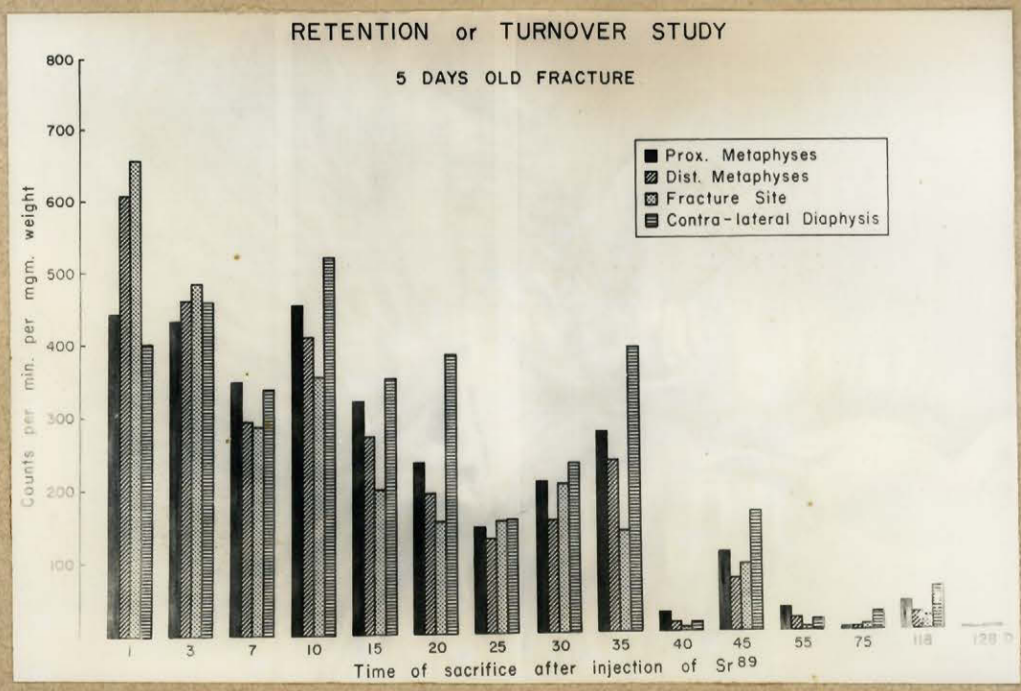


Fig. 81. A.

Shows retention of radioactivity in a group of animals where strontium was administered as a single dose, 5 days after production of fracture, and animals were sacrificed at varying intervals.

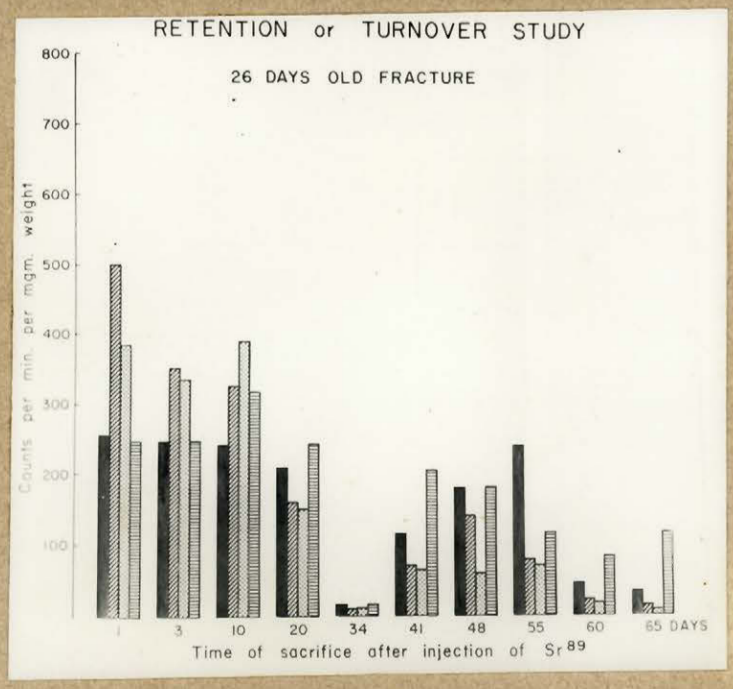


Fig. 81 B.

Shows retention of radioactivity in animals where strontium was administered 26 days after production of fracture. Animals were sacrificed at varying intervals.

RETENTION SERIES

EXPERIMENT B, GROUP III

Sr89 (0.1 uc/gm. body wt.) was administered in animals 5 days after production of fracture. Animals were sacrificed at variable intervals after the isotope administration.

Turnover of Sr89 at ipsilateral proximal, distal metaphyses and fracture site, and contralateral proximal, distal metaphyses and diaphysis of femur, calculated per min. per mgm. wt. (wet weight), in male, R.V.H. strain rats.

	PROXIMAL METAPHYSIS		DISTAL METAPHYSIS		FRACTURE & DIAPHYSIS	
Days after Admin. of Sr89	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight
1	(IL) 3666(40)	458	7828(65)	602	31697(240)	660
	(CL)2985(35)	426	8542(70)	610	5612(70)	400
3	(IL) 3633(40)	454	7028(75)	468	19387(200)	484
	(CL)3266(40)	408	7239(80)	452	11898(130)	457
7	(IL) 3350(45)	372	5579(85)	328	11404(200)	285
	(CL)3254(50)	325	4144(80)	259	7426(110)	337
10	(IL) 4750(50)	475	6926(80)	432	21825(310)	352
	(CL)4697(55)	427	6160(80)	385	9345(90)	519
15	(IL) 3587(55)	326	5017(85)	295	8792(230)	191
	(CL)3721(60)	310	3675(75)	245	7721(110)	350
20	(IL) 2939(65)	226	3151(75)	210	6082(200)	152
	(CL)2677(55)	243	2626(75)	175	6901(90)	383
25	(IL) 1896(60)	158	2335(80)	146	6497(210)	154
	(CL)1455(55)	132	1873(85)	110	3738(120)	155
30	(IL) 2888(65)	222	3188(90)	177	7296(180)	202
	(CL)2282(60)	190	2510(95)	132	7871(170)	231
35	(IL) 4414(70)	315	4944(95)	260	9490(340)	139
	(CL)3159(65)	238	3990(95)	210	6277(80)	392
40	(IL) 452(75)	30	377(125)	15	250(225)	5
	(CL) 332(75)	22	205(85)	12	280(100)	14
45	(IL) 1625(65)	125	1520(95)	80	2730(330)	41
	(CL)1350(75)	90	1302(100)	65	5545(170)	163
55	(IL) 561(80)	35	330(110)	15	287(270)	5
	(CL)365(65)	28	481(120)	20	282(90)	15
75	(IL) 48(75)	3	121(110)	5	282(175)	8
	(CL) 56(60)	4	150(125)	6	602(120)	25
118	(IL) 555(65)	42	700(130)	26	889(250)	17
	(CL)605(80)	37	582(145)	20	1766(150)	58
128	(IL) 20(80)	1	48(220)	1	34 (155)	1
	(CL) 23(100)	1	19(155)	0	68 (120)	2

The uptake of radioactivity at the ipsilateral distal metaphysis was higher on post-fracture days 2, 9, 17, 21, 25, 30, 35, 45, 70, 75, 80, 108, 113, 130, 139, 145, 153, 158, 166, 181, 238, 242, 256 and 289.

The uptake of radioactivity at the ipsilateral distal metaphysis was higher as compared to contralateral distal metaphysis by a ratio of 1.36.

The uptake of radioactivity at the ipsilateral proximal metaphysis was higher as compared to contralateral proximal metaphysis by a ratio of 1.25.

Group B III — Study of 'turn-over' of radioactivity at the fracture site and its relation to 'turn-over' at other sites (contralateral diaphysis and the metaphyses), at various intervals after injection of Sr89. This study included groups of fractures at different post-fracture intervals. (Retention or "Turn-over" study).

Fractures were produced. Sr89 was injected intraperitoneally and the animals were sacrificed from post-injection day 1 to the termination of the study period.

a) Sr89 was injected 5 days post-fracture and the animals were sacrificed from post-injection day 1 to post-injection day 128.

RETENTION SERIES

EXPERIMENT B, GROUP III

Sr89 (0.1 uc/gm. body wt.) was administered in animals 26 days after production of fracture. Animals were sacrificed at variable intervals after the isotope administration.

Turnover of Sr89 at ipsilateral proximal, distal metaphyses and fracture site, and contralateral proximal, distal metaphyses and diaphysis of femur, calculated per min. per mgm. wt. (wet weight), in male, R.V.H. strain rats.

	PROXIMAL METAPHYSIS		DISTAL METAPHYSIS		FRACTURE & DIAPHYSIS	
Days after Admin. of Sr89	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight
1	(IL) 3255(80)	203	13615(125)	544	24674(385)	385
	(CL)6816(110)	309	16347(180)	454	7084(150)	245
3	(IL) 3500(70)	250	7370(110)	335	23454(350)	335
	(CL)3600(75)	240	7602(105)	362	7097(150)	246
10	(IL) 2860(65)	220	9390(150)	313	17100(220)	388
	(CL)3668(70)	262	9686(145)	334	7587(120)	316
20	(IL) 3400(85)	250	4760(140)	170	8989(300)	149
	(CL)3888(90)	216	18850(130)	145	7275(150)	242
34	(IL) 182(55)	16	330(180)	9	945(400)	11
	(CL) 308(100)	15	319(150)	10	502(150)	16
41	(IL)1133(50)	113	2668(200)	66	3867(300)	64
	(CL)2300(100)	115	3068(210)	73	4072(100)	203
48	(IL)3596(90)	199	3670(140)	131	5099(300)	84
	(CL)2598(80)	162	5600(185)	151	3616(100)	180
55	(IL)4357(60)	363	2500(130)	96	3412(230)	71
	(CL)2227(95)	117	1198(100)	59	2764(120)	115
60	(IL) 540(100)	27	477(150)	15	1745(460)	18
	(CL)1146(90)	63	834(140)	29	2655(160)	82
65	(IL) 420(70)	30	510(150)	17	400(250)	8
	(CL) 420(65)	35	324(135)	12	3450(150)	115
75	(IL) 720(100)	36	513(160)	16	1311(350)	18
	(CL) 474(70)	33	524(130)	24	1485(100)	74

RETENTION SERIES

EXPERIMENT B, GROUP III

Sr89 (0.1 uc/gm. body wt.) was administered in animals 36 days after production of fracture. Animals were sacrificed at variable intervals after the isotope administration.

Turnover of Sr89 at ipsilateral proximal, distal metaphyses and fracture site, and contralateral proximal, distal metaphyses and diaphysis of femur, calculated per min. per mgm. wt. (wet weight), in male, R.V.H. strain rats.

	PROXIMAL METAPHYSIS		DISTAL METAPHYSIS		FRACTURE & DIAPHYSIS	
Days after Admin. of Sr89	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight
1	(IL) 7443(60)	620	12209(95)	642	16389(130)	630
	(CL)6702(80)	418	17973(130)	691	7115(100)	355
7	(IL) 3770(65)	290	9909(110)	450	17538(220)	398
	(CL)3364(60)	280	7878(125)	315	7530(130)	298
11	(IL) 2829(60)	235	5520(115)	240	19377(360)	269
	(CL)2542(60)	211	5073(125)	203	5622(130)	216
25	(IL) 1910(45)	212	7407(120)	308	9573(250)	191
	(CL)3834(65)	294	6956(150)	231	5490(130)	211
32	(IL) 1330(80)	83	2854(160)	89	9826(370)	132
	(CL)2658(120)	110	3279(200)	81	3216(120)	134
39	(IL) 1986(55)	180	2337(150)	77	5406(310)	87
	(CL)2274(90)	126	2711(130)	104	3788(150)	126
45	(IL) 1208(60)	100	2006(160)	62	2540(150)	84
	(CL)1449(65)	111	2418(180)	67	3272(120)	136
52	(IL) 888(70)	63	524(110)	23	600(125)	24
	(CL) 904(75)	60	506(100)	25	2516(120)	104
60	(IL) 245(50)	24	160(80)	10	228(130)	8
	(CL) 275(45)	25	175(80)	10	608(120)	25
75	(IL) 546(65)	42	270(75)	18	222(140)	7
	(CL) 420(60)	35	280(70)	20	2254(115)	98

Turn-over was studied at the fracture site, ipsilateral proximal and distal metaphyses, contralateral diaphysis and proximal and distal metaphyses.

The uptake of radioactivity at the fracture site was in accordance to the uptake study (vide supra). By the 3rd post-injection day the turn-over was approximately equal at the various sites counted. On the 7th post-injection day the fracture site showed a lesser degree of radioactivity in comparison to other sites. From post-injection day 7 to post-injection day 128 (termination of study), the proximal metaphyses and the contralateral diaphysis showed a higher count of radioactivity than the other sites at each day counted. The initially high radioactivity declined progressively in all sites during the follow-up period.

In those specimens where bony union and reconstruction had occured by the time of sacrificing the animal, loss of radioactivity (turn-over) was relatively faster. On post-injection days 75 and 128 in animals with completely healed fractures, no radioactivity was discernible. At post-injection day 118, the fracture studied was not firmly united and radioactivity was still present. The proximal metaphyses and the contralateral diaphysis were relatively hot.

b) Sr89 was injected 26 days post-fracture and animals were sacrificed from post-injection day 1 to post-injection day 65. Turn-over was studied at the various sites in the usual manner.

RETENTION SERIES

EXPERIMENT B, GROUP III

Sr89 (0.1 uc/gm. body wt.) was administered in animals 47 days after production of fracture. Animals were sacrificed at variable intervals after the isotope administration.

Turnover of Sr89 at ipsilateral proximal, distal metaphyses and fracture site, and contralateral proximal, distal metaphyses and diaphysis of femur, calculated per min. per mgm. wt. (wet weight), in male, R.V.H. strain rats.

	PROXIMAL METAPHYSIS		DISTAL METAPHYSIS		FRACTURE & DIAPHYSIS	
Days after Admin. of Sr89	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight.
1	(IL) 6756(60)	563	16920(130)	650	33655(210)	801
	(CL)7158(60)	596	22239(140)	794	7052(60)	587
7	(IL) 5144(60)	428	8112(140)	289	21237(280)	379
	(CL)7700(70)	550	12495(130)	480	8363(80)	522
10	(IL) 2150(50)	215	5436(120)	226	4586(200)	114
	(CL)3473(90)	92	6339(160)	198	4138(100)	206
15	(IL) 3407(40)	425	4056(100)	202	9404(230)	204
	(CL)3429(40)	428	7281(120)	303	7270(105)	346
21	(IL) 4058(55)	368	3804(130)	146	5884(230)	127
	(CL)3927(50)	392	4761(100)	238	9753(85)	573
35	(IL) 1252(60)	104	1280(110)	58	5235(350)	74
	(CL) 872(40)	109	1943(130)	74	4057(110)	184
45	(IL) 263(85)	15	282(140)	10	389(300)	6
	(CL) 699(80)	43	240(180)	6	223(130)	8
55	(IL) 1616(65)	124	1144(110)	52	4084(240)	85
	(CL)1247(70)	89	1515(115)	66	3857(95)	203
65	(IL) 631(90)	35	444(130)	17	999(275)	18
	(CL)1046(80)	65	628(125)	25	2418(155)	78
80	(IL) 1458(100)	72	4068(200)	101	8325(430)	96
	(CL)2478(150)	82	2757(230)	59	3137(180)	87
85	(IL) 223(75)	15	110(110)	5	78(130)	3
	(CL) 361(75)	24	217(120)	9	2156(125)	86
95	(IL) 518(90)	28	310(125)	12	1789(280)	31
	(CL)1118(110)	50	407(150)	13	1618(100)	80
100	(IL) 85(70)	6	166(130)	6	272(410)	3
	(CL) 106(90)	7	135(100)	7	35501(140)	1268

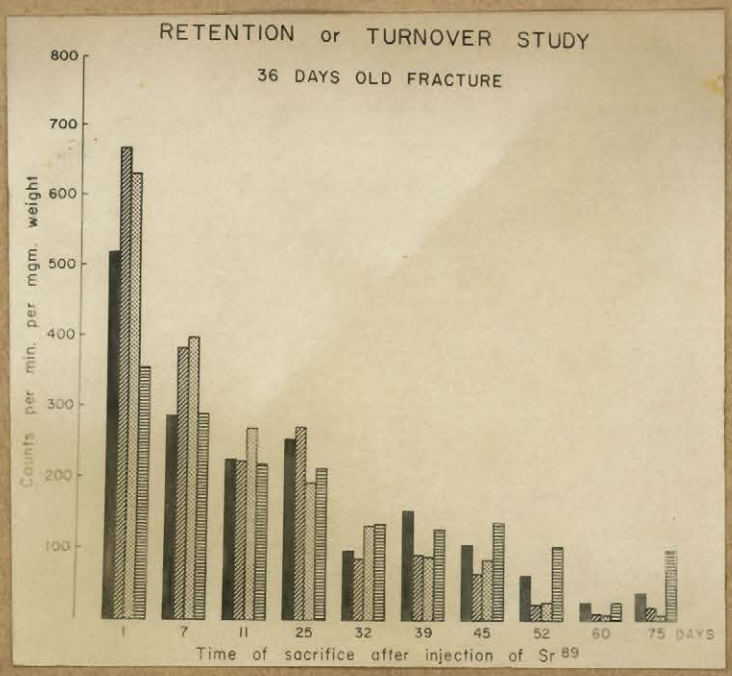


Fig. 82 A.

Shows retention of radioactivity in animals on standard diet. Sr89 was administered 36 days after fracture. Note the reversal phenomenon by post-injection day 7. Contralateral diaphysis retained radioactivity while the fracture site has extremely low reactivity 75 days after the injection.

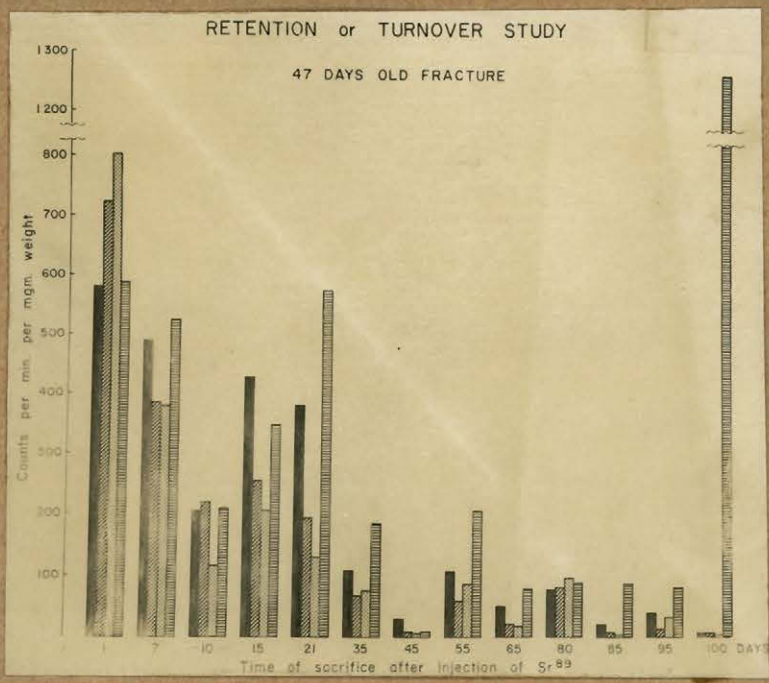


Fig. 82 B.

Shows retention of radioactivity after injection of Sr89 47 days after the production of fracture. Note the reversal phenomenon on post-injection day 10. 100 days after injection the contralateral diaphysis showed extremely high radioactivity while the other sites had lost reactivity.

An initially higher uptake (ratio 1.57) at the fracture site when compared to contralateral diaphysis was observed in accordance with uptake studies. The reversal of the ratio of turn-over at the fracture site to contralateral diaphysis was observed on post-injection day 20. This seemed to be aslow phenomenon in this group as compared to the turn-over in 5 day old fractures. Proximal metaphyses and contralateral diaphysis followed the same pattern of slow loss of radioactivity as compared to the relatively faster turn-over at the fracture site and distal metaphyses of the femur.

In a fracture with bony consilidation and undergoing reconstruction, 34 days after injection the counts were very low at the fracture site, though proximal metaphyses (a site of slower osteogenesis) still had relatively higher radioactivity left over. In other fractures where a firm union had not been achieved, contralateral diaphysis retained higher radioactivity as seen 60 and 65 days after the injection of the isotope.

c) Sr89 was injected 36 days post-fracture and the animals were sacrificed from day 1 to day 75 after injection.

An initially higher uptake (ratio 1.77) at the fracture site when compared to contralateral diaphysis was observed. Reversal of turn-over between fracture site and contralateral diaphysis was apparent on post-injection day 25. This phenomenon seemed further delayed in comparison to previous groups. Proximal metaphyses and contralateral

RETENTION SERIES

EXPERIMENT B, GROUP III

Sr89(0.1 uc/gm. body wt.) was administered in animals 61 days after production of fracture. Animals were sacrificed at variable intervals after the isotope administration.

Turnover of Sr89 at ipsilateral proximal, distal metaphyses and fracture site, and contralateral proximal, distal metaphyses and diaphysis of femur, calculated per min. per mgm. wt. (wet weight), in male, R.V.H. strain rats.

	PROXIMAL METAPHYSIS		DISTAL METAPHYSIS		FRACTURE & DIAPHYSIS	
Days after Admin. of Sr89	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight
1	(IL) 5730(90)	318	20899(160)	653	20282(160)	633
	(CL)8469(130)	325	23147(220)	526	16193(190)	426
3	(IL) 9048(80)	565	20254(140)	733	19624(300)	327
	(CL)7643(100)	382	21549(220)	489	11304(210)	269
5	(IL) 4355(85)	256	5750(125)	230	13565(230)	294
	(CL)1729(80)	108	4080(120)	170	3790(140)	135
8	(IL) 3288(60)	274	5590(130)	215	11406(330)	172
	(CL)2133(65)	164	4682(130)	180	6217(110)	282
10	(IL) 2566(70)	183	3751(125)	150	12782(380)	168
	(CL)1717(75)	132	3073(120)	128	3312(170)	97
15	(IL) 53(65)	4	45(110)	2	213(310)	3
	(CL) 39(60)	3	25(115)	1	281(210)	6
22	(IL) 130(80)	8	(125)	3	301(400)	3
	(CL) 145(80)	9	(130)	2	101(210)	2
31	(IL) 172(85)	10	147(145)	5	248(150)	8
	(CL) 237(75)	15	227(140)	8	368(120)	15
35	(IL) 1295(60)	107	5002(150)	166	8944(270)	165
	(CL)4386(100)	219	4677(160)	146	6493(130)	249
40	(IL) 261(65)	20	205(145)	7	535(300)	8
	(CL) 450(80)	28	272(135)	10	193(200)	2
50	(IL) 675(75)	45	152(125)	6	346(140)	12
	(CL) 815(70)	58	170(120)	7	418(125)	16
60	(IL) 322(80)	20	140(115)	6	297(135)	11
	(CL) 422(85)	25	201(125)	8	1395(120)	58

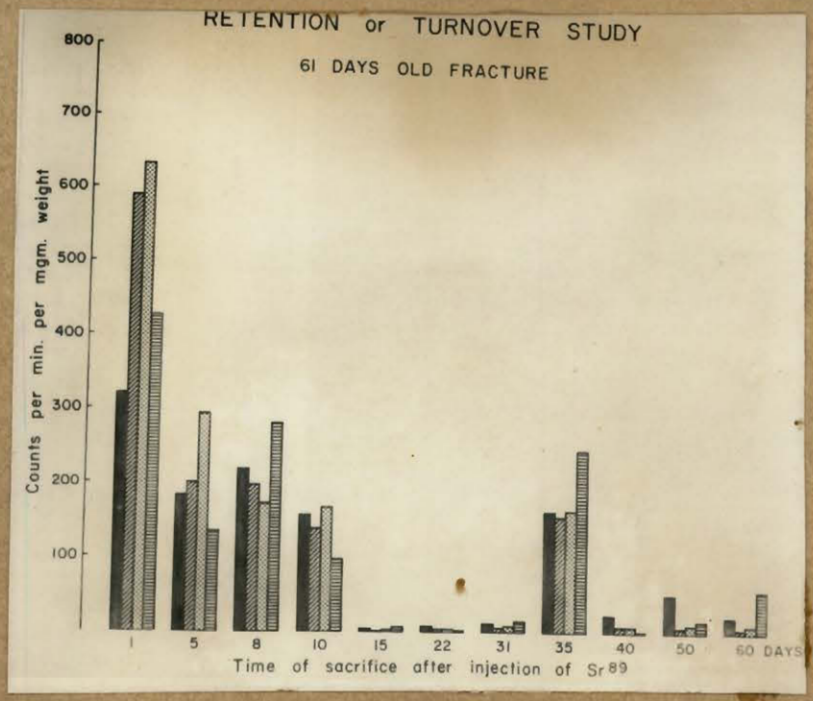


Fig. 83.

Shows retention of radioactivity in animals maintained on standard diet. Strontium 89 was administered 61 days after production of fracture. Note the loss of radioactivity on post-injection day 15.

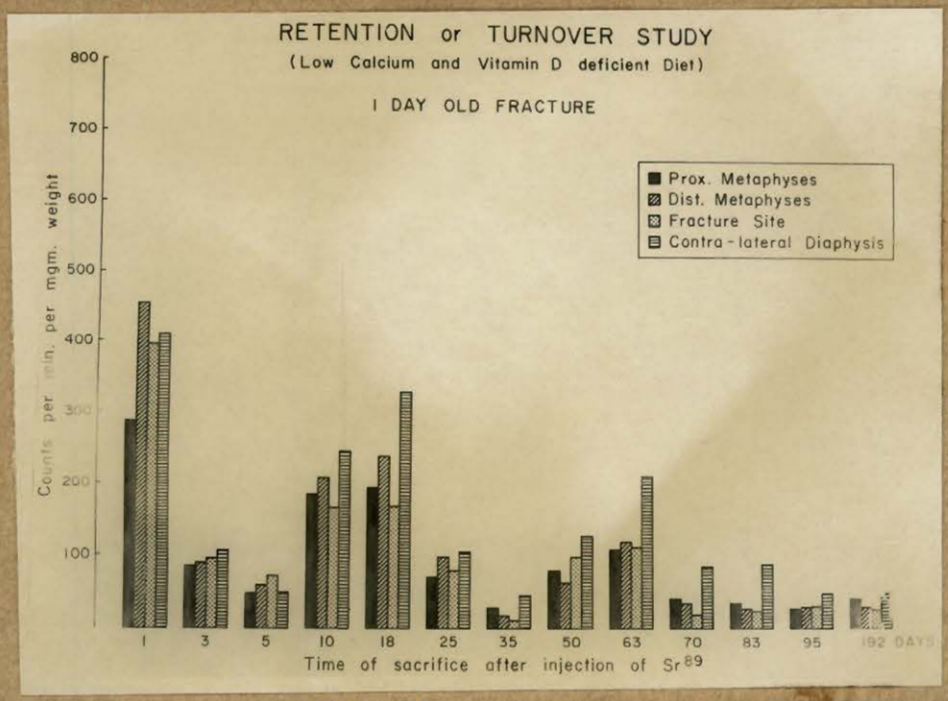


Fig. 84.

Shows retention of radioactivity in animals maintained on low calcium and vitamin D deficient diet. Note the retention of low grades of activity till post-injection day 192.

followed the same pattern of slower turn-over.

In a fracture with bony consilidation and reconstruction 75 days after injection, the fracture site had relatively no radioactivity; while proximal metaphyses and contralateral diaphysis showed higher values. Radioactivity was retained for long period of time in this group as compared to previous groups.

d) Sr89 was injected 47 days post-fracture and animals were sacrificed from post-injection day 1 to post-injection day 100. Turn-over was studied at the various sites in the usual manner.

Very high initial uptake was observed at all the sites counted in accordance with uptake studies. Fracture site showed an increased ratio of 1.36 as compared to contralateral diaphysis. Reversal of the turn-over was observed in this group by post-injection day 7. A rapid loss of radioactivity was observed at the fracture site. On the other hand, contralateral diaphysis showed a very slow turn-over.

A fracture which was completely healed showed extremely low radioactivity on post-injection day 45 while another fracture with bony union still exhibited radioactivity of larger magnitude at the contralateral diaphysis. A fracture with firm union on post-injection day 100 showed extremely high level of radioactivity at the contra-lateral diaphysis (slow osteogenesis) while other sites (active osteogenesis) showed no radioactivity. Those fractures which had not undergone complete reconstruction were still radioactive.

RETENTION SERIES

EXPERIMENT B, GROUP V

(Low Calcium and Vitamin D Deficient Diet)

Sr89 (0.1 uc/gm. body wt.) was administered in animals 1 day after production of fracture. Animals were sacrificed at variable intervals after the isotope administration.

Turnover of Sr89 at ipsilateral proximal, distal metaphyses and fracture site, and contralateral proximal, distal metaphyses and diaphysis of femur, calculated per min. per mgm. wt. (wet weight), in male, R.V.H. strain rats.

	PROXIMAL METAPHYSIS		DISTAL METAPHYSIS		FRACTURE & DIAPHYSIS	
Days after Admin. of Sr89	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight
1	(IL) 2392(40)	299	4320(50)	432	3166(40)	395
	(CL)1920(35)	274	7633(80)	477	4088(50)	408
3	(IL) 729(45)	80	1266(60)	105	1525(80)	95
	(CL) 820(45)	91	953(65)	73	1056(50)	105
5	(IL) 641(50)	64	1476(110)	58	2242(160)	70
	(CL) 332(50)	33	1479(120)	61	444(50)	44
10	(IL) 1840(45)	199	4244(100)	212	4955(150)	165
	(CL)1352(40)	168	3828(95)	201	2930(60)	244
18	(IL) 520(30)	86	1030(80)	64	3331(100)	166
	(CL)2392(40)	299	8204(100)	410	2611(40)	326
25	(IL) 435(30)	72	1999(90)	111	1888(120)	78
	(CL) 460(35)	65	1499(90)	83	1444(70)	103
35	(IL) 276(35)	38	277(85)	16	300(125)	12
	(CL) 121(35)	17	381(105)	18	588(65)	45
50	(IL) 753(40)	92	688(80)	43	2232(115)	97
	(CL) 592(45)	65	1644(100)	82	1877(75)	125
63	(IL) 969(60)	80	1042(100)	52	2865(130)	110
	(CL)2401(90)	133	5215(150)	183	4579(110)	208
70	(IL) 435(65)	33	539(95)	28	555(145)	19
	(CL) 799(85)	47	868(110)	39	1828(110)	83
83	(IL) 292(70)	20	582(100)	29	1322(270)	24
	(CL) 564(60)	47	622(130)	23	1897(110)	86
95	(IL) 499(70)	35	569(105)	27	1515(250)	30
	(CL) 222(65)	17	727(125)	29	1088(115)	47
192	(IL) 576(70)	41	1038(200)	25	2125(420)	25
	(CL) 549(70)	39	649(100)	32	1697(190)	44

RETENTION SERIES

EXPERIMENT B, GROUP V

(Low Calcium and Vitamin D Deficient Diet)

Sr89 (0.1 uc/gm. body wt.) was administered in animals 30 days after production of fracture. Animals were sacrificed at variable intervals after the isotope administration.

Turnover of Sr89 at ipsilateral proximal, distal metaphyses and fracture site, and contralateral proximal, distal metaphyses and diaphysis of femur, calculated per min. per mgm. wt. (wet weight), in male, R.V.H. strain rats.

	PROXIMAL METAPHYSIS		DISTAL METAPHYSIS		FRACTURE & DIAPHYSIS	
Days after Admin. of Sr89	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight
1	(IL) 2671(30)	445	11567(115)	502	3148(30)	524
	(CL)5352(50)	535	10647(110)	483	2588(30)	431
4	(IL) 5162(35)	737	9159(80)	572	14170(160)	442
	(CL)7564(50)	756	11948(100)	597	5870(55)	533
7	(IL) 2566(40)	320	4865(85)	285	8348(200)	208
	(CL)2777(45)	306	5588(90)	310	3042(70)	217
10	(IL) 1550(45)	170	2555(90)	142	4554(185)	122
	(CL)1355(50)	135	2444(95)	128	2166(75)	144
15	(IL) 2322(55)	210	1749(100)	85	5254(190)	138
	(CL)2277(65)	175	2536(110)	110	3333(85)	196
19	(IL) 2453(70)	175	3157(130)	121	6872(290)	118
	(CL)4598(100)	229	5327(170)	156	7565(130)	290
25	(IL) 1806(70)	129	4060(160)	126	7836(200)	195
	(CL)3487(70)	203	3483(105)	165	1962(120)	331
35	(IL) 1244(75)	83	1279(110)	58	2122(200)	53
	(CL) 888(70)	63	1155(125)	46	2636(125)	104
45	(IL) 632(75)	42	606(105)	29	1555(250)	31
	(CL) 333(60)	28	235(115)	10	1717(125)	68
53	(IL) 68(60)	5	119(150)	3	338(370)	4
	(CL) 72(50)	7	145(150)	4	330(130)	12
60	(IL) 927(70)	66	1321(320)	20	1539(390)	19
	(CL)1129(90)	62	1861(200)	46	3707(160)	115
65	(IL) 932(70)	66	1663(140)	59	2801(310)	45
	(CL)1305(70)	93	841(110)	38	1835(100)	91
70	(IL) 390(70)	27	469(180)	13	1682(380)	22
	(CL) 573(65)	44	587(170)	17	2814(110)	127

e) Sr^{89} was injected 61 days post-fracture and animals were sacrificed from post-injection day 1 to post-injection day 60. Turn-over was studied in the usual manner.

An initial higher uptake at the fracture site was noted as compared to contralateral diaphysis. Reversal of the turn-over was observed by post-injection day 8. Since most of the fractures in this group had bony union, the radioactivity measured at the various sites was of low magnitude; the contralateral diaphysis and proximal metaphyses still maintained their slow turn-over as compared to fracture site and distal metaphyses (post-injection days 15, 22, 31 and 40). A fracture which still had not achieved union showed higher radioactivity, maximum being at the contralateral diaphysis.

Retention Study in Animals Maintained on Low Calcium and Vitamin D Deficient Diet:-

a) Sr^{89} was injected in animals 1 day post-fracture and were sacrificed from post-injection day 1 to post-injection day 192.

There was a generalized increased uptake of radioactivity at the various sites, measured post-injection day 1; the fracture site did not show any localized increased radioactivity when compared to other sites. There is evidence of relatively early loss by post-injection day 3, 5, 25 and 35. Some radioactivity was still retained by post-injection days 70, 83, 95 and 192. All these fractures showed cartilagenous union.

The turnover by the contralateral diaphysis showed the same pattern of loss of radioactivity as seen in animals maintained on standard diet.

In this group low grade radioactivity seemed to be retained for a longer period of time than the period of study (192 days after injection) in this group.

b) Strontium 89 was injected 30 days post-fracture and animals were sacrificed from post-injection day 1 to post-injection day 70. Turnover was measured at the various sites in the usual manner.

There was generalized uptake of radioactivity at the various sites; fracture site showed relatively high uptake when compared to contralateral diaphysis. This initially higher uptake at the fracture site showed reversal phenomenon by post-injection day 4.

All fractures in this group had varying degrees of cartilaginous union. The radioactivity was retained for a period longer than the study period (70 post-injection days). One of the fractures showed firm union by bone and had very low radioactivity 53 days after the injection.

The contralateral diaphysis retained higher radioactivity as compared to other sites of active osteogenesis.

c) Strontium 89 was administered 71 days post-fracture and animals were sacrificed from post-injection day 1 to post-injection day 80.

There was evidence of higher uptake at the different sites. The reversal of turnover at the fracture site, as compared to contralateral diaphysis; and equilibrium between the proximal and distal metaphysis was established by post-injection day 7. A relatively rapid loss of radioactivity occurred by post-injection day 15. Further turnover seemed to follow a similar pattern as seen in other groups of animals, maintained on deficient diet. Only the fractures with firm union showed minimal radioactivity on post-injection days 55 and 70. The fracture without bony union still showed retention of radioactivity on post-injection day 80 (this fracture was 151 days since its production).

Cumulative Study.

Repeated injection of (0.1 uc per gm body weight) strontium 89 were given twice a week to groups of animals with fractures, starting on post-fracture day 2. Groups of animals were sacrificed after variable number of injections and radioactivity was measured at the various sites (fracture sites, metaphysis and contralateral diaphysis).

The data to date is not far advanced, but the following observations have so far been made:

a) The radioactivity at the fracture site, ipsilateral proximal and distal metaphysis, contralateral diaphysis and proximal and distal metaphysis did not seem to follow a rising pattern with successive injections of the isotope.

b) There was some evidence that the radioactivity is retained for a considerable length of time as evidenced by loss of only 1/4 - 1/6 of the initial radioactivity, measured 125 days after the last injection in animals, given 20 injections (total dose 2.0 uc per gm body weight).

c) It was interesting to observe that the animals lost some weight after 20 injections (total dose 2.0 uc/gm body weight). This loss in weight became marked with successive injections. After 36 injections (total dose 3.6 uc/gm body weight), a syndrome like 'radiation sickness' developed. It was marked by loss of weight, nose bleed, loss of hair and atrophy of muscle. The mortality rate increased appreciably after 40 injections (total dose 4.0 uc/gm body weight) and by the time of the 50th injection (total dose 5.0 uc/gm body weight), 80% mortality was observed. With 53 injections (total dose 5.3 uc/gm body weight), 40 days after the last injection (total dose 5.3 uc/gm body weight),

and 260 days after the first injection (0.1 uc per gm body weight) tumours were detected at the fracture sites and other sites.

By the time of preparation of this report, several tumours have appeared. After a total dose of 5.0 uc per gm body weight, bone tumours developed in the metaphyseal regions of tibia, humerus and only in few cases, at the fracture site.

Cumulative Study.

(Low Calcium and Vitamin D Deficient Diet).

Repeated injections (0.1 uc per gm body weight) of strontium 89 were given to animals with fractures maintained on low calcium and vitamin D deficient diet in the same way as in the previous group. Animals were investigated in the same manner as in the group on standard diet.

Interesting features of this study include: -

1) There was diffuse measure in the radioactivity of various parts investigated. This had no relation to the total number of injections (total dose) given. There was no evidence of increased uptake of radioactivity at the fracture.

2) As shown in the growth graph, the animals showed a definite increase in their weight. The mortality rate was nil. By 20 injections (total dose 2.0 uc/gm body weight) there was

RETENTION SERIES

EXPERIMENT B, GROUP V

(Low Calcium and Vitamin D Deficient Diet)

Sr89 (0.1 uc/gm. body wt.) was administered in animals 71 days after production of fracture. Animals were sacrificed at variable intervals after the isotope administration.

Turnover of Sr89 at ipsilateral proximal, distal metaphyses and fracture site, and contralateral proximal, distal metaphyses and diaphysis of femur, calculated per min. per mgm. wt. (wet weight), in male, R.V.H. strain rats.

	PROXIMAL METAPHYSIS		DISTAL METAPHYSIS		FRACTURE & DIAPHYSIS	
Days after Admin. of Sr89	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight	Corrected Turnover Per Min.	Turnover Per Mgm. Weight
1	(IL) 5721(60)	476	16132(130)	620	17840(270)	330
	(CL)7725(70)	551	22331(140)	797	5771(105)	274
7	(IL) 5465(v60)	455	6801(90)	377	12252(220)	278
	(CL)5581(65)	429	10924(110)	496	6761(80)	422
15	(IL) 1377(55)	125	1540(100)	77	1321(200)	33
	(CL)1543(55)	140	2020(100)	92	3656(210)	87
21	(IL) 1015(60)	84	2408(140)	86	5173(270)	95
	(CL)1726(80)	107	2851(150)	95	2401(110)	109
28	(IL) 677(65)	52	793(120)	33	210(130)	8
	(CL) 492(65)	38	568(105)	27	403(80)	25
35	(IL) 535(70)	38	586(150)	19	1010(150)	33
	(CL) 261(50)	26	622(120)	25	241(70)	17
45	(IL) 737(45)	82	1323(110)	60	4084(210)	97
	(CL) 893(50)	89	969(105)	46	1788(100)	89
55	(IL) 75(60)	6	119(135)	9	335(320)	5
	(CL) 72(50)	7	148(130)	5	330(110)	15
70	(IL) 100(50)	10	210(130)	8	200(200)	5
	(CL) 132(60)	11	155(110)	7	233(65)	18
80	(IL) 1727(100)	86	1673(150)	55	2214(100)	110
	(CL)1773(110)	80	1563(150)	52	1589(80)	99

CUMMULATIVE STUDY

EXPERIMENT B, GROUP IV

Animals were injected with Sr89 (0.1 uc/gm. body wt.) twice a week. Animals were sacrificed after variable number of injections and radioactivity was measured at various sites of osteogenesis and the fracture site.

		PROXIMAL METAPHYSIS		DISTAL METAPHYSIS		FRACTURE & DIAPHYSIS	
	Total No. of Inject.	Uptake Per Min.	Uptake Per Mgm. Wt.	Uptake Per Min.	Uptake Per Mgm. Wt.	Uptake Per Min.	Uptake Per Mgm. Wt.
	5	(IL) 14422(50)	1442	51051(130)	1963	53966(110)	2453
		(CL)23663(70)	1690	34587(70)	2470	42696(100)	2134
	20	(IL) 34333(100)	1716	78954(220)	1794	47114(140)	1682
		(CL)50608(135)	1874	97907(220)	2225	38164(120)	1590
*	36	(IL) 42354(105)	2016	81565(240)	1699	77464(270)	1434
		(CL)46562(170)	1369	88313(270)	1635	44886(150)	1496
		(IL) 34659(120)	1444	84561(250)	1691	23724(100)	1186
		(CL)37922(130)	1458	78686(280)	1405	28077(100)	1403
		(IL) 30628(120)	1276	95549(280)	1706	27093(100)	1354
	42	(IL) 19404(85)	1141	54795(185)	1480	26853(115)	1167
		(CL)19634(60)	1636	58429(175)	1669	25856(110)	1166
		(IL) 26597(100)	1329	71531(180)	1992	29381(140)	1049
		(CL)21967(100)	1098	87291(280)	1558	38715(140)	1382
	46	(IL) 20015(70)	1429	55005(200)	1375	41253(200)	793
		(CL)14927(70)	1066	68931(200)	1723	18033(80)	1127
	48	(IL) 26914(50)	2691	61659(150)	2055	44735(175)	7278
		(CL)16185(50)	1618	87774(250)	1755	33148(120)	1381
**	50	(IL) Histology					
		(CL)31878(150)	1062	63636(210)	1515	39286(170)	1155

evidence of re-mineralization of the demineralized bone.

3) There was no development of the radiation-like sickness.

4) Malignant bone tumours developed in animals with 50 or more injections (total dose 5.0 uc or more) within 36 days of the last injection and 260 days since the time of the first injection (total dose 0.1 uc/gm body weight). The incidence of such tumours studied to date is much higher in the animals on deficient diet. Several animals have developed tumours at the fracture site: the incidence of multiple tumours in the same animal was higher in these animals than the group on standard diet.

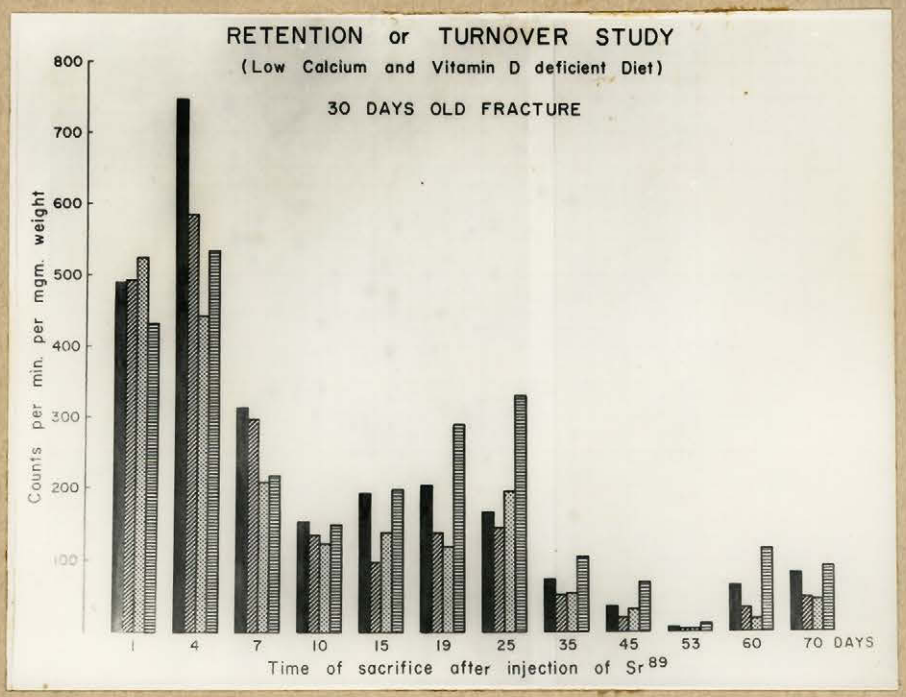


Fig. 85.
Shows retention of radioactivity in animals maintained on low calcium and vitamin D deficient diet. Sr89 was administered 30 days after production of fracture. Animals were sacrificed at varying intervals.

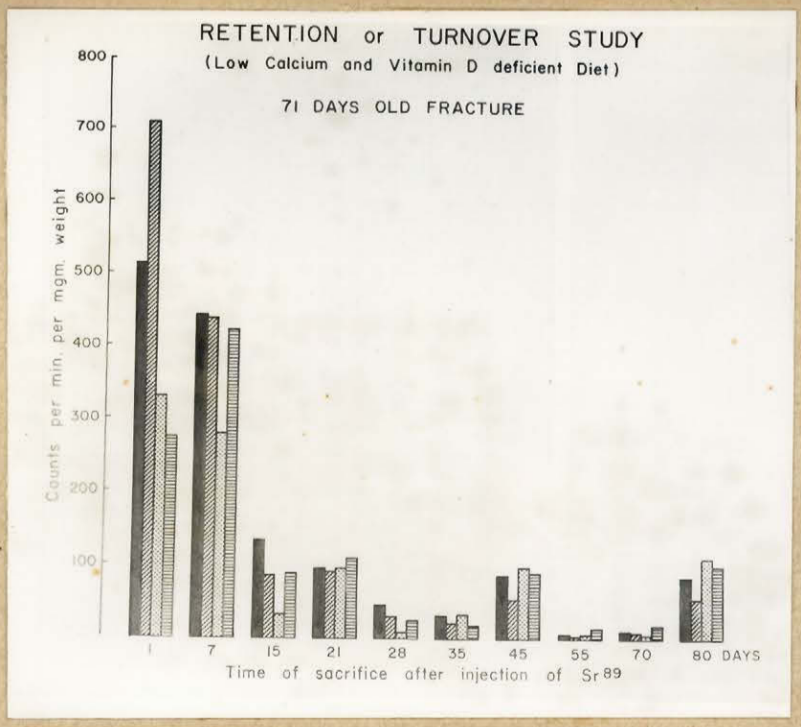


Fig. 86.

Shows retention of radioactivity in animals maintained on low calcium and vitamin D deficient diet. Sr89 was administered 71 days after production of fracture. Animals were sacrificed at varying intervals.

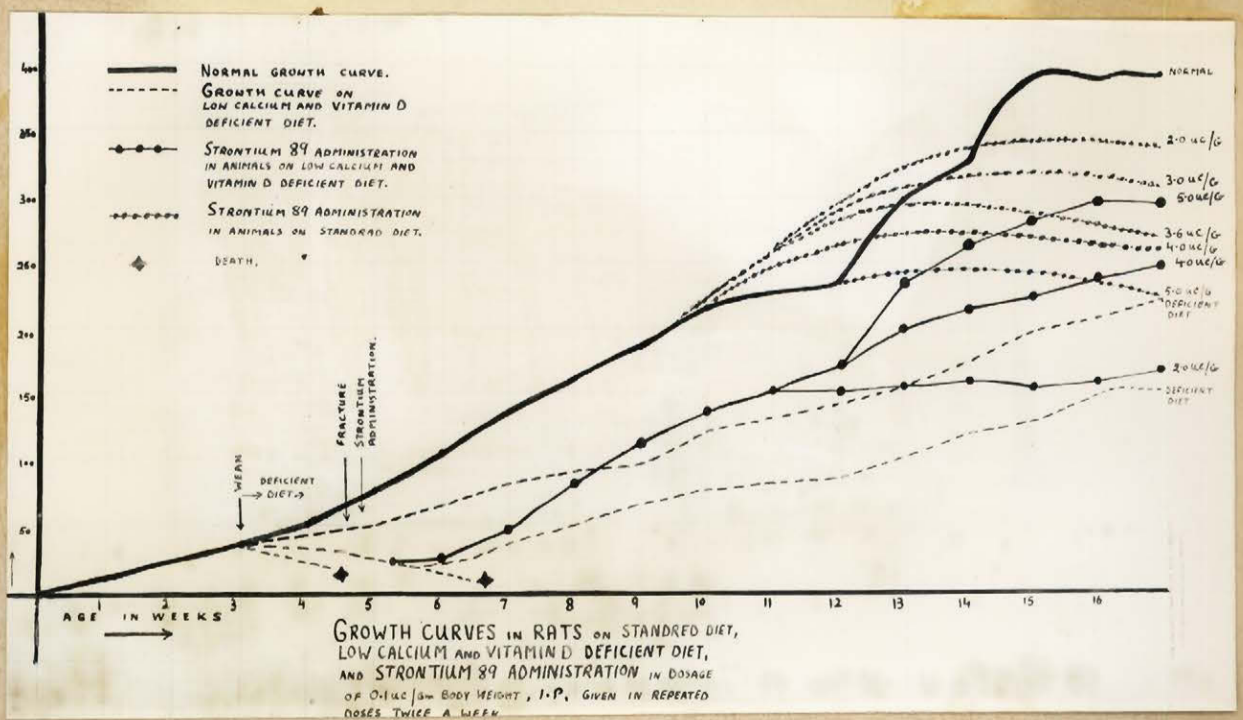


Fig. 87.

COMMULATIVE STUDY

EXPERIMENT B, GROUP IV

(Low Calcium and Vitamin D Deficient Diet)

Animals were injected with Sr89 (0.1 uc/gm. body wt.) twice a week. Animals were sacrificed after variable number of injections and radioactivity was measured at various sites of osteogenesis and the fracture site.

		PROXIMAL METAPHYSIS		DISTAL METAPHYSIS		FRACTURE & DIAPHYSIS	
	Total No. of Inject.	Uptake Per Min.	Uptake Per Mgm. Wt.	Uptake Per Min.	Uptake Per Mgm. Wt.	Uptake Per Min.	Uptake Per Mgm. Wt.
	4	(IL) Histology					
		(CL) 8375(50)	837	14756(80)	922	41370(190)	1088
		(IL) 10453(50)	1045	22065(100)	1103	17286(60)	1440
		(CL)12180(50)	1218	21014(100)	1050	14999(50)	1499
	7					10703(100)	535
						9934(40)	1241
	10					9400(60)	783
						14596(50)	1459
	17					31568(150)	1052
						23658(100)	1182
	20					10297(130)	396
						18409(130)	708
						18674(130)	718
						18906(140)	675
						12267(130)	471
						12081(80)	755
						31440(110)	1429
						36625(90)	2034
*	50	Tumor Formation					
	53	(IL) 6249(50)	624	21841(70)	1560	19600(150)	653
		(CL) 8173(60)	681	32687(140)	1167	10253(120)	427
*	54	Tumor Formation					

CHAPTER IX.

DISCUSSION.

I. General Comments;-

Radioactive strontium constitutes the most dangerous nuclear fission product from a public health point of view. Its long half life, bone seeking property and very prolonged incorporation make it the "dangerous isotope". Its chemical similarity to calcium introduces a nutritional aspect of the fall-out problem (Lisco et al, 1947, Wasserman et al, 1957). Other fission products appear to be less hazardous because of their lower fission yield, shorter half-life and lesser incorporation and more rapid turn-over in the biological systems (Schulert et al, 1959).

It is now generally accepted that strontium is rapidly accumulated in the bone. This has been shown by numerous investigators (Norris et al, Engstrom et al, Hamilton, McLean et al, Neuman et al, Jowesy et al, Leblond et al, Bauer et al, Bohr and others). Radioactive strontium represents a potent carcinogenic agent. Bone tumours have been induced by strontium 89 and 90 in rats after a latent period of 6 - 8 months. Litvinov, Kuzma, Finkel, Brues, Owen, Sissons, Vaughan, Yokoro, Skoryna, Kahn and others have succeeded in inducing such malignant tumours with strontium 89 and 90.

Late effects of strontium 89 administration in rats were investigated by Skoryna and Kahn in this laboratory. Strontium 89 was administered intraperitoneally in doses of 0.9 uc per gm body weight as the initial dose, followed by 5 injections of 0.7 uc per gm body weight at monthly intervals. 63% of the animals survived the minimum latent period of 188 days. Neoplastic changes on microscopic examination were found in all animals. 89 rats out of 100 had gross palpable tumours.

Since strontium is deposited at the site of active osteogenesis (Hamilton, Engstrom, Bohr, Baur, Neuman, McLean, Urist and many others), it was feasible to use the fracture site as a model of active osteogenesis. This model provided an excellent opportunity to study the up-take, 'turn-over' and the cumulative effects of the isotope.

The metabolism of radioactive strontium in man was found to be similar to that in the experimental animals (Comar et al, 1957). The effects of internally deposited radioactive materials in man have been reported by Aub et al, Looney, Gartland, Martland, Hoffmann, Copeland, Vaughan, Hempelmann and others.

II. Osteogenesis; -

The first part of our study consists of the investigation of normal osteogenesis. Fracture healing has been the subject of many previous investigations. (Bohr, Copp, Greenberg, Bennett, Pollock, Urist, McLean, Koskinen, Trueta). By 1943, as mentioned earlier, more than 4,000 articles on this subject had been published. Eggers has said for the regeneration of bone we are dependent on the "blue eyed" osteoblasts (Quoted by Boyd, 1960). Most surgical interventions slow down the repair process. In order to study osteogenesis it was felt necessary to learn the pattern of normal bone healing in the strain of the rat used in these experiments.

Open transverse fractures were produced in the right femur of four week old male rats and no immobilization was provided. The degree of displacement was variable and was further aggravated by partial weight bearing. The knee joint of a rat functions in a position of flexion; therefore with muscular contraction and weight bearing the fragments become further displaced. In the present experiment, the average period of time required for bony consolidation of non-immobilized transverse femoral fractures was 100 days. The earliest bony union

was obtained by the 28th day, while one specimen at 289 days showed only cartilaginous union. During the study period 85 - 90 % of fractures healed by bone.

There was a definite relation between the degree of displacement and the period necessary for complete healing of the fracture. The greater the displacement, the longer the time for healing. This had a definite relation to the uptake of radioactivity which will be discussed in the later part of this discussion.

Wray, Trueta, Urist, McLean and others agree that repair begins with a reaction of blood vessels and surrounding connective tissue. Wray et al (1959) showed that the vascular response reached a peak at 9 days after fracture and then gradually subsided to levels of the controls when union of the fracture took place. Cellular proliferation in the periosteum reached a peak at 10 to 12 days. The two responses closely approximate each other and it has been suggested that a co-ordinated effort exists between vascular response and osteo-blastic proliferation. Cohen and Lacroix (1955) have shown that the regenerating power of periosteum is confined to the cambrium layer. The time of organisation of the haematoma and its replacement by the granulation may vary from 7 - 30 to 60

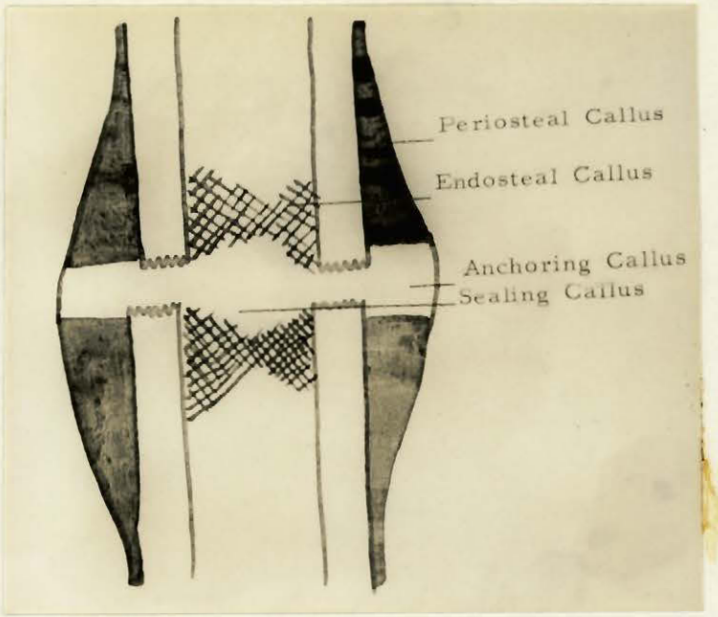


Fig. 88.
Shows various parts of the callus at the fracture site.

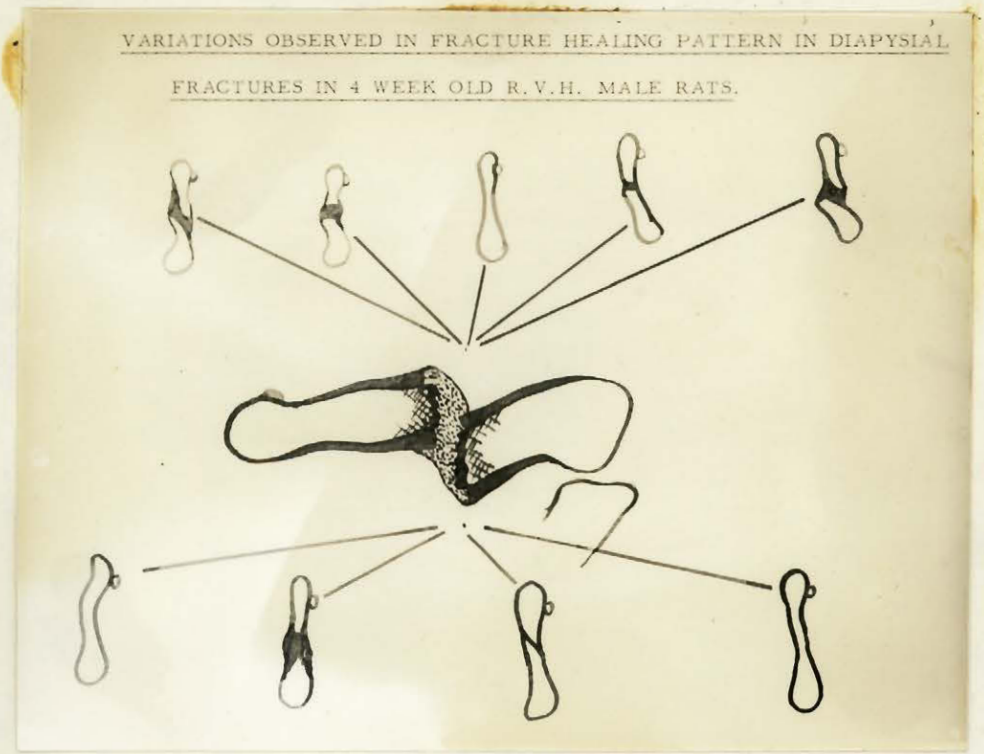


Fig. 89.

days (Wray).

In the group in which the animals after production of the fracture were immobilized with plaster spica showed that in the rat it is impossible to achieve perfect immobilization with plaster. The growth at this age is rapid and complications due to tight plaster were commonly seen; these consisted of swelling of the foot, ulcerations and gangrene. The average time required to achieve bony consolidation was 73 days, as compared to the 100 in the control group. All fractures united by bone in this study. The repair process was essentially the same although the stages were shortened. The bulk of the callus was visibly increased; this could be considered to be due to loss of muscle mass surrounding the fracture and the femur. Two very important complications were seen in this group: a) Stiffness of the knee was observed as early as the 10th day after immobilization and was progressive in nature during the 92 days of study period. Histological examination showed only fibrotic proliferation in the capsular tissue of the knee joint while the articular surfaces were normal. It was further noted that if the spica was removed and the animals allowed to resume activity, the stiffness disappeared and the mobility was recovered.

b) As early as 3 days after immobilization atrophy of the thigh musculature was observed, the muscles lost their normal colour and texture. There was also evidence that these changes were reversible. According to Iwase (1955) excitability of the muscles is lowered by immobilization in general. Production of fracture causes further reduction of the excitability of the muscles. The elements most affected in the early stages are the receptors and the neuromuscular junctions, while the changes in the muscle follow. A classical report upon affects of immobilization upon the metabolic and physiologic functions in man has been reported by Dietrick et al (1948). He reported general loss of muscle mass, greater in the immobilized thigh as compared to the non-immobilized parts.

In the group where a gap had been produced by excision of approximately 3 mm of the shaft at the fracture site, the animals limped for a period of 10 days, as compared to 3 to 5 days in the control group. The angulation at the fracture site was more prominent as seen by the 'broad base' position of the hind limbs; however this did not seem to influence the average time for bony consolidation. Although there was an initial lag, the bony consolidation was obtained,on the average,by 90 days. There

was a shortening of the healed femur by 3 - 5 mm: 95% of the fractures healed by bone during the study period. There was no increase in the incidence of non-union. In man, as stated by Urist, Mazet, McLean, a gap of 1/2 cm between the bone ends will postpone union by 12 - 18 months. Our results in this study do not suggest any adverse effect on ultimate bone healing, except for an initial lag in repair and the resultant shortening.

The group of animals in which a cuff of periosteum was excised at the time of producing fracture, yielded very interesting results. The mortality rate in this group was very high, 10 - 15 percent. Most of the animals died within one hour of the operation, an overdose of Nembutal is a possibility, but in this series it was not unusual to see the rats gasp and die when the periosteum was excised. The fracture ends from which the periosteum had been excised underwent necrosis, probably due to lack of blood supply. The repair was delayed for a considerable length of time; necrotic ends were seen up to 88 days after the fracture in one animal. While earliest bony union was obtained by the 90th post-fracture day, the average period for complete healing in this group was 126 days. Marked shortening of the

femur was probably due to absorption of the necrotic ends. This was a constant finding. The transverse width of the femoral shaft on the fractured side was found to be increased in comparison to the shaft on the non-fractured side. This probably was due to abundant periosteal new bone formation, as a result of lifting of the periosteum.

The periosteal blood supply constitutes one of the most important source of blood supply to bone; this has been confirmed by the works of Professor Trueta. The importance of the connections between the muscle, periosteum and the bone were recently reported by Zuckman, 1960. Importance of periosteal blood supply to fracture healing has been established since Kolodny (1923). Macnab (1958) reported a study of the significance of periosteal circulation. He found that the only source of blood supply to the cortex was derived from the nutrient artery. There was free anastamosis between the metaphyseal vessels and the nutrient system, but practically none between the nutrient and the periosteal systems. Death of bone does not result from interruption of the circulation of the nutrient artery; circulation is rapidly re-established after destruction of the endosteal circulation by free anastomosis

with the metaphyseal vessels. Göthman (1961) states that greatest part of the arterial reaction originates in soft tissues outside the periosteum and consists of branches radiating either directly or indirectly towards the fracture site. Göthman (1960) stated that periosteal vessels take slight part in originating arterial reaction of the callus.

According to Wray (1959) the peak of the vascular response was reached by the 9th day after fracture, while the studies of Göthman (1961) showed that the vascular response appeared to be most lively between 2 to 4 weeks after fracture. During this period, calcium deposition started at the fracture site.

In the group of animals in which fracture was accompanied by section of the thigh musculature, retardation of the fracture healing was observed; the animals were not able to use their limb for periods as long as 21 days after the operation; their activity was therefore restriced; only partial weight bearing was possible, the foot appeared clawed and edema of the leg and foot were present. The incidence of non-union was high; there was no sign of consolidation by the 40th day. The first evidence of cartilaginous union was observed 53 days after the fracture. In most of the fractures of this group, the fracture ends were markedly displaced and were covered with fibrous caps; delayed and non-union were very common,

(25 - 30%). Average time of union by bone was 176 days. This suggests that the traction exerted by muscle pull is an important factor in promoting bony healing of the fractures. Wray (1960) stressed the importance of muscular activity as a mechanical influence upon the growth of bone. Under normal circumstances, skeletal growth is largely independent of external factors. Periosteal new bone formation is destroyed by loss of muscular pull and thus periosteum is bound to the bone and therefore the osteogenic tissue formation is scanty.

In the group of animals in which the endosteum was reamed from the fracture ends of the diaphysis, the repair process was not altered to any significant degree. Average time for bony union was 105 days. All fractures healed by bone. It was, however, noted that the power to remodel the fracture was somewhat poor in this series. The only pertinent observation in this respect appears to be that of Enneking (1948) who stated that endosteal reaction is of equal duration and intensity to the periosteal reaction, in the normal repair of fractures. On the other hand, Kuntscher (1960) noted that extensive reaming did not produce any ill effects in animal experiments nor in clinical cases of intra-medullary fixation method. This applied

both to local healing and systemic reactions (fat emboli). Although Kuntscher noted that endosteal callus does not develop following the reaming (the endosteum is completely destroyed together into endosteal blood supply) this seems to be well compensated for by an increased development of intra-medullary and periosteal callus. Our experimental findings similarly indicate that the endosteum itself is not a very important structure in regard to fracture healing.

The group of animals in which the periosteal cuff was excised and the thigh musculature was sectioned showed a combination of the results present in groups separately; the fracture ends underwent necrosis to a greater extent than in the group with periosteal cuff resection only. A variable degree of absorption of the diaphysis in all the fracture femurs was observed. The incidence of delayed union was high, but if sufficient time had elapsed, the fracture did achieve bony consolidation, althoughalways with resultant shortening. The time of bony union was variable, average being 188 days after the fracture. The results in this group suggest therefore that the periosteum and the muscle pull are two extremely important factors in fracture healing. In one 127 day old femur only proximal and

distal ends were left while the whole shaft had undergone absorption. The incidence of non-union was high (40%) when compared to control series (10 - 15%).

Göthman (1961) postulated that the blood vessels from the muscles radiate directly or indirectly to the callus. According to Göthman (1960), periosteal vessels play a slight part in originating arterial reaction of the callus.

In our series of animals with excision of periosteum and section of thigh musculature, increased incidence of non-union and absorption of diaphysis can be explained as a combined effect of deficient blood supply and the elimination of the mechanical effect of muscle action.

In the group of animals in which four repeated fractures were induced at weekly intervals, previous fractures did not influence healing of succesive fractures except in a few instances of non-union. When compared to the control group, the average time of bony consolidation was 120 days after the last fracture. There was no increase (15%) in the incidence of non-union when compared to the control group (10 - 15%). However, the displacement of the fragments seemed to persist for a longer period than in the control group, which had a tendency for spontaneous

re-alignment of the shaft of the fractured femur. This would appear to agree with the findings in wound healing: a wound that has re-opened will heal in approximately the same time as the initial wound.

In studies reported by Wray (1961) extremely rapid union seemed to follow the third re-fracture. Wray studied the vascular response to repeated fractures. Both hypertrophy of fracture vascular bed and that of entire limb were involved, with a peak around the 9th day after the fracture. It receded with healing of the fracture. This is probably an example of delayed compliance on the part of the vascular bed of the entire limb. Local pH changes resulting from tissue damage probably contribute to dilatation in the region of the fracture. There was progressive enlargement of the vascular bed after re-fracture which suggested that it should be considered as a passive phenomenon. Judet (1961) suggested that hypervascularity may cause non-union of fractures, but according to Wray hypervascularity is the result and not the cause of non-union. The impaired venous outflow in the fractured limb appears to play a role and it may be even of greater significance. The impairment of venous outflow explains high incidence of delayed union and non-union (25 - 30%)

observed in the group of animals where thigh muscles were sectioned at the time of production of the fracture.

In the group of animals which were maintained on low calcium and vitamin D deficient diet, some very interesting observations were made. The animals were weaned at three weeks of age and were maintained on this deficient diet for 10 to 15 days. An x-ray at this stage revealed generalized demineralization, thinning of the cortices and widening of the epiphyseal plates. The animals showed lack of gain in weight and the mortality rate was high. There was marked restriction of activity. Rats kept on a standard diet during a similar period gained an average of 50 gms per week.

In accordance with the Erdheim's calcio-protective law, certain tissues are less susceptible to defects in calcium metabolism than others. Enamel and dentin are not as readily disturbed in their calcification as is bone, nor are they subject to calcium withdrawal; thus the bone releases its calcium readily in case of need, while the enamel and dentin do not. In fact the calcium and phosphorus made available by the bone are evidently taken up by the calcifying enamel and dentin. Growing dentin may even receive an excess supply from the skeletal source and become

hypercalcified (Clark and Smith, 1935). It is interesting to note that the rats kept on low calcium diet have a cannibalistic tendency and sometimes as much as half of an animal would be eaten.

After the production of the fracture the animals were maintained on the deficient diet throughout the experiment. Some of them died 15 days after being placed on the deficient diet, while the survivors showed very slow gain in weight; however, 16 weeks later they weighed 200 gms while animals on standard diets and of the same age would weigh 350 gms. The bones of animals kept on the deficient diet were markedly soft, the incidence of non-union was high, averaging 80 percent as compared to 10 - 15 percent of the control group. Only occasionally (15 - 20%) bony union of fracture was observed. The majority of the fractures were united either by fibrous or cartilaginous callus.

The mortality rate of the group in which only a periosteal cuff was excised (no fracture was produced), was extremely high, up to 45% in any one series. One week later, in 10 animals the femur was fractured by the open method. Adhesions were detected between the bone and the surrounding muscle at the site of previous procedure. These fractures healed in the same manner as on

the control group. The evidence suggests that the regeneration of periosteum follows at a rapid rate. The fractures healed normally in spite of the previous defect. Trueta (1958) stated that periosteal system of vessels played the main part in restoring bone continuity by callus formation and in keeping the cortex alive, provided it had not lost its connections with the tissues surrounding the periosteum, particularly the muscles. It seems that with formation of adhesions between denuded bone and surrounding muscles, blood supply to the cortex was re-established and thus when these were fractured, normal union was observed. Zuckman (1960) reported the importance of the vascular connections between periosteum, muscle and bone. The osteoblastic power of periosteum was observed to be confined to the cambrium layer of periosteum (Cohen, Lacroix, 1955).

Experiment A.

Time Intervals of Fracture Healing.

Groups	Average Period of time for bony union.	Bony union in animals during the study period.
Group I (T) (Control)	100 days	85 - 90%
II (TP) (Plaster imm. of fracture)	73 days	100%
III (TG) (Gap at fracture site)	90 days	95%
IV (PRT) (Fracture and excision of Per. cuff)	126 days	90%
V (TM) (Fracture and section of thigh musculature)	176 days	70 - 75%
VI (TE) (Fracture and reaming of endosteum)	105 days	100%
VII (PRTM) (Fracture, excision of periosteal cuff & muscle section)	188 days	60%
VIII (MT) (Repeated fracture)	120 days	85%
IX (LCT) (Fract. in animals on low Ca & Vit. D deficient diet)	85 days	15 - 20%
X (P) (Excision of periosteum; 1 week later open transverse fracture.	90 days	100%

III. Experiments Employing Radioactive Strontium.

Since 1937, when Chievitz and Hevesy first used radio-active phosphate in an investigation of rat skeleton, radio-active isotopes have been extensively employed in studying various aspects of bone metabolism.

The majority of the current studies have employed strontium 85, 89 and 90 and calcium 45 (Comar, Palmer, Bauer, Neuman, Wasserman, Jowsey, Arnold, Engstrom, Jones, Copp). From these investigations it is evident that the distribution of the bone seeking isotopes is not uniform throughout the skeleton, but varies in different bones and in different parts of the same bone. Greatest concentration was found in areas of active growth and recently deposited mineral (Neuman et al, 1953). Although in matured bone the isotope deposition is considerable, it is less than in areas of active growth (Nilsonne, 1959); the age of the animal appears to determine the pattern of distribution (Bauer and Bohr).

The uptake of strontium in cortical bone appears to follow the same biological pattern as the uptake in bone beneath the epiphyseal cartilage which is an area of active bone growth (Jowsey et al, 1953). Immediately following administration of

strontium 90 a localised concentration in areas of active bone growth can be observed. Radio-autographic studies demonstrate a diffuse general transient distribution in the young animal and a spotty distribution of discrete loci of intense radioactivity in the adult (Neuman, Leblond, Bauer, Bohr, Engstrom). In younger age groups such areas of concentration are almost entirely lost by the normal physiological processes of resorption and apposition. In the older age group, areas of concentration persist. The total amount of the radioactive strontium retained in the skeleton at any time corresponds largely to that incorporated at the time of injection, the so-called primary deposition. To a lesser degree secondary depostion derived from the blood stream after release from the bone by resorption, plays a part. It has been shown that a high uptake of strontium 90 occurs in vivo and vitro in low mineralised areas of bone (Engfeldt et al, 1954), and therefore follows the same general pattern as that of P32 and Ca45 (Amprino, 1952, Engfeldt, 1952 and Spencer et al, 1957).

In fracture healing, experiments with various radioactive isotopes have revealed that they are quickly absorbed into the newly formed bone tissue in the callus area (Marshak et al, 1945, Copp et al, 1945; Bohr et al, 1950; Karecher, 1953; Cartier et al, 1956).

Quantitative bone salt accretion in callus area has been studied by Bauer, 1954, Bauer et al, 1955. Microradiographic techniques were used by Amprino et al, 1952, Vincent, 1955, Owen, 1956, Wallgren, 1957, Engstrom et al, 1958; Bergman et al, 1958. Nilsonne (1959) reported the quantitative study of the course of mineralization during fracture healing, using microradiographic and x-ray diffraction methods. Mineralization in the callus was observed as early as the 4th day after the fracture, and it started in the periosteal cuff. In rats, the whole shaft of the bone participates in the callus formation. At the end of the 3rd week the mineral has greatly accumulated near the fracture ends. In the intermediate callus, the mineral salt has a patchy distribution and the areas in-between are completely unmineralized. The fracture ends, as well as the whole fractured bone, exhibit a more homogenous distribution of the mineral. Low-mineralized areas of the callus reveal highly intensive uptake of radioactive isotope. The isotope is particularly strongly concentrated in the periosteal callus cuff which is seen to enclose practically the whole of the fractured femur. The higher the degree of mineralization in the callus area at the time of isotope administration, the lower the

uptake of the isotope; after approximately 10 weeks the higher concentrations of the isotope are still present to a considerable extent.

The re-distribution phases of the isotopes have been reported in excellent reviews by Bauer (1954). The unequal distribution of mineralization continues in principle for a very long time even after a mineralized continuity between the fracture ends has been achieved. Neuman et al (1953) reported the mineral phase of bone and stressed that bone crystals have a hydration layer which provides the crystals an intimate contact with the organic phase, without exposure of the crystallite surface. It is the surface of the crystal that equilibrates with its surrounding fluid.

Study of the osteogenesis in irradiation disease (produced by roentgen rays in fractures treated by intra-medullary fixation (Kashkarov, 1956), showed no change in the morphology of repair of fracture. Periosteal osteogenesis slowed down during the acute stage of irradiation disease, but it caught up when the acute phase of the disease was over. Similar findings were reported by Thachenko (1958). Total retention in bones from animals of different age groups may be the same, but the risk of tumour development may differ, since in one age group, strontium 90

seems largely in localized concentrations while in another group, its deposition is diffuse. As previously mentioned, the objective of this experiment was to study the correlation of uptake of radioactivity at the various sites of active osteogenesis in the femur as well as the experimentally produced fractures. Small doses of strontium 89 (0.1 uc/gm body weight) were used throughout the experiment. Fracture was used as a model of active osteogenesis. After establishment of the pattern of normal bone healing in the strain of rats used in this experiment, the radioactive studies were carried out. The data using strontium 89 as a marker showed that the fracture site had an increased uptake of radioactivity from day 5 after fracture. This was maintained throughout the 289 days after fracture. This observation is consistent with similar findings reported by several investigators (Bohr, Bauer, Nilsonne, Engstrom, Carlsson, Leblond).

Bohr (1955) reported an increased uptake of phosphorus not only at the fracture site, but also in the epiphysis of the femur and proximal epiphysis of tibia. The increase in radioactivity at the fracture site was distinct up to 6 months after

fracture; the increase in the epiphysis was practically limited to the period between the first and the 8th week. There was no difference as regard to the uptake of radioactivity between animals with good healing and animals with poor healing.

Nilsonne (1959) made a quantitative study of mineralization of the fracture callus and found that intermediate callus was the last part to become calcified; while periosteal callus constituted the main zone of mineralization. In our studies it was observed that callus mass (intermediate callus) when separated from the fracture ends (and periosteal callus) did not show any significant radioactivity.

At this stage the callus consisted primarily of fibrous tissue; it was only after replacement by a calcified bone material, that this area showed evidence of uptake. These findings are consistent with the observations of Engfeldt (1954), who reported high uptake of strontium in low mineralized areas of bone.

The pattern of bone healing influences the determination and calculation of uptake of radioactivity at the fracture site. Since the fractures were not immobilized, variable degrees of displacement of the fractures in the animals were present. The greater the degree of displacement the more extensive callus

was found and hence longer time was required for bony consolidation. The weight of the fracture site showed progressive increase up to 250mgms due to local callus formation. The amount of callus was proportionate to the degree of displacement of the fracture. According to Nilsonne (1959) the parts of the callus showed variable and non-homogenous pattern of mineralization, starting in the periosteal callus by the fourth day and was progressive towards the fracture ends. It was also shown that in rats, unlike dogs, the whole shaft of the femur contributes to periosteal cuff of callus. The intermediate callus is the last and the least of the mineralized areas. The parts of the callus which become mineralized showed greater increase in radioactivity, while callus at a pre-mineralization stage does not have any affinity for the isotope. If the radioactivity is recorded at the fracture site, it would represent the radioactivity as a combined value for the fracture end and the intermediate callus. If calculations for radioactivity are made per mgm weight, the calculated values will be less when the callus mass is included than when the counts are made after dissecting the fracture ends free of fibrocartilaginous mass and counting the radioactivity at the fracture ends alone.

It was found that when radioactivity was measured at the fracture site alone and the increased percentage calculated by using the formula:

$$\frac{R_{F_f} - R_{F_{cl}}}{R_{F_{cl}}} \times 100$$

(where R_{F_f} means radioactivity per minute at the fracture site and $R_{F_{cl}}$ means radioactivity per minute at the contralateral diaphysis) that there was an increase of 4 to 5 fold at the fracture site as compared to the contralateral diaphysis. Also, when radioactivity is calculated as uptake per mgm weight of the bone (wet weight) the increase after day 5 was maintained throughout the study period of 289 days after the fracture. The peak of highest uptake in the series studied ranged from post-fracture day 15 to 145. From our data it appears that the uptake capacity at the fracture site (per mgm weight) varies with the stage of healing of the fracture. The rise in uptake was noted after day 5 and it was maintained with a peak around the 47th day; it declined then by the 153rd day and towards the end of the 289th day period the capacity showed lower values. This coincides fairly well with the study of mineralization reported by Nilsonne (1959). The callus undergoing mineralization at the time of strontium 89

administration picks up the greatest amount of radioactivity. The radioautographic data by Nilsonne shows that the recently laid down bone (not completely mineralized) takes up more radioactivity than the older well mineralized bone. The chief mechanism of deposition of radioactive strontium reported by MacDonald; and Neuman (1953) consists of absorption process on the surface of the crystalite and later incorporated into the interior of the lattice by preciptation phenomenon. Neuman and Neuman (1953) have discussed the ionic exchange through the hydration layer of the apatite crystal.

An interesting observation of the present experiment was that fractures which at the time of injection of Sr89 had not yet undergone full reconstruction (the fracture callus could be seen and recognized) showed a high ratio of radioactivity per mgm. weight (ratio of fracture site to contralateral diaphysis). The average value was 1.77 as compared to a value of 1.10 in those specimens in which the fracture site had undergone complete reconstruction and where it was difficult to recognize grossly the site of fracture in the diaphysis. It can, therefore, be concluded that an increased uptake of radioactivity at the fracture ends occurs, both in itself and perngm. weight.

The results of the correlative study showed higher radioactivity at the fracture site as compared to the contralateral site from day 5 and it was maintained throughout the study period up to 289 days after the fracture. The comparison of the uptake at the fracture site

with the ipsilateral distal metaphyses demonstrate variations up to day 45. The distal metaphyses had a higher uptake of radioactivity as compared to the fracture site. Fracture site showed higher radioactivity than the distal metaphyses on days 5, 15, 28, 48, 53, 58, 65, 232, 262 after fracture. This suggests that in all cases except those where the fracture was produced close to the distal metaphyseal region, the radioactivity was higher in that metaphysis as compared to the fracture site. Comparison of the uptake of radioactivity at the distal metaphysis of the fractured femur with the contralateral distal metaphysis showed some increase (ratio 1.36) on the ipsilateral metaphysis.

Bohr, (1955) reported an increase in the radioactivity (P32) at the fracture site persisting up to 6 months after fracture, while the increase in the epiphysis was practically limited to the period between the first and the 8th week, there was no difference as regard the uptake of radioactivity between animals with good and those with bad healing. Our results suggest, on the other hand, that a definite relation exists between stage of healing and uptake of radioactivity. Comparison of uptake of radioactive material in the fracture and contralateral site of femoral shaft in the group of animals which were maintained on low calcium and Vitamin D deficient diet showed an initial period of diffuse uptake with no increase on the fractured side. From post-fracture day 15, a definite rise at the fracture site was observed as compared to the contralateral diaphysis; this was maintained

for the 124 post-fracture days that the animals were studied.

As noted earlier only 15 - 20% of the fractures produced in animals on the deficient diet showed bony consolidation. The majority had cartilaginous and fibrous union. The specimens which showed bony consolidation (calcifiable callus) had higher uptake of radioactivity, similar to that of fractures in animals kept on a standard diet.

The correlative study in this deficient diet group showed a more diffuse pattern of distribution of radioactivity in the various sites which were counted as compared to the animals on a standard diet. Jones and Copp (1951) and Bohr(1961) reported on the distribution of Sr in animals on low calcium diets. In rachitic rats, the rapid initial uptake was similar to that in the normal young animals but was followed by active loss from the skeleton, thus only one third was left at the end of 124 hours. This suggests that the Sr was in a labile state in the bone. A large part of the dose of strontium was excreated in the urine by the rachitic rats within the first 24 hours. Normal crystalline matrix is not formed in the rachitic animals, therefore radiostrontium cannot be incorporated in to this, although ion exchange with existing bone salt may take place as in the adult. Rapid initial intake is a labile combination from which Sr is readily released in rachitic animals.

In the rachitic rats, the plasma clearance was 10 - 15 times greater than in the normals indicating a direct effect of this condition on the excretion of Sr by the kidneys (Jones, Copp: 1951). Similar investigations by Bohr (1961), who studied the uptake of Sr and Ca in normal and

rachitic rats were confirmed by using external counting method in the living rats. The results show that beside the more diffuse uptake in the bone tissue itself in both normal and rachitic bone, a heavy accumulation takes place in the metaphysial side of the epiphysial line in the normal bone which has almost no corresponding phenomenon in the rachitic bone.

An interesting observation was encountered when the animals with fracture received one injection of Sr89 (0.1 uc per mgm. body weight) and were sacrificed from 1 to 119 days after the injection.

Groups of animals were injected 5, 26, 36, 47 and 61 days after the production of the fractures; there was always an initial high uptake at the fracture site; this is in accordance with the findings in the uptake series described. At variable intervals a reversal phenomenon was observed and the radioactivity at the fracture site became lower when compared to the contralateral diaphysis. The turnover study showed that the fracture site loses radioactivity at a faster rate than the contralateral diaphysis. This would appear to result from the fact that the fracture site undergoes reconstruction which sets in early and hence the rapid turnover.

The second important observation was that the proximal metaphysis, in which growth takes place at a slower rate than the distal metaphysis (which is the growing end of the femur), retained radioactivity for a longer period than the distal metaphysis. In those specimens in which firm union was not obtained, the proximal

metaphyses and the contralateral diaphysis retained radioactivity for still longer period of time than the fracture site and the distal metaphyses. On the contrary, fractures with bony consolidation and when reconstruction had progressed further, very low or no radioactivity was retained. The only sites which still retained radioactivity were the contralateral diaphysis and next in order was proximal metaphysis. The rate of loss by either metaphysis in fractured and non fractured femurs was more or less of same magnitude.

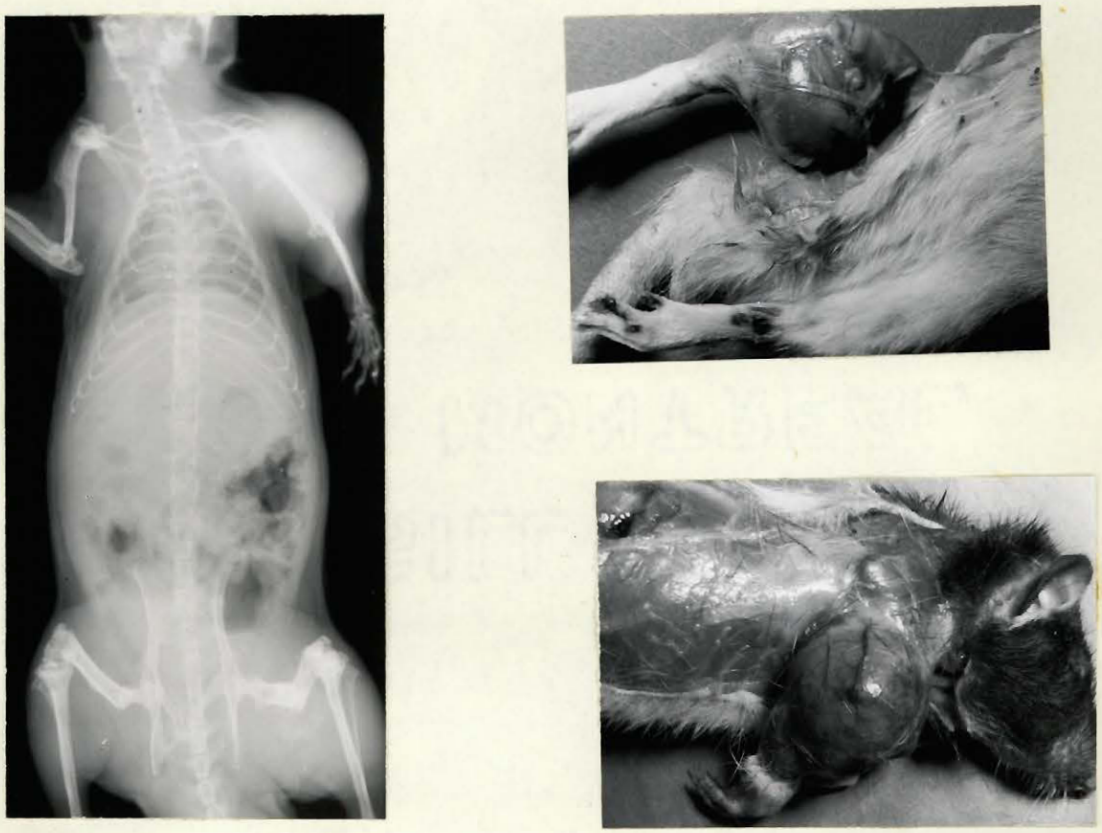
The turnover study in the animals on low calcium and Vitamin D deficient diet were carried out in groups of animals with 1, 30 and 71 days old fractures. The data obtained demonstrate that in those animals in which the fracture was mineralized in a normal control pattern, the uptake and reversal phenomena were similar to that of the animals on a standard diet. In the majority of the animals the union was mostly cartilaginous or fibrous and radioactivity, although of low magnitude was maintained for longer periods of time and no reversal of turnover was observed as in series on standard diet.

The experiments using cumulative doses require long follow up and to date only preliminary results can be reported from the experiments which are being continued. In cumulative series animals with fractures were injected twice a week for variable number of injections in doses of 0.1 uc per gm. body weight; the radioactivity of the bones was measured at different sites of active osteogenesis including

the fracture. The findings to date show that the radioactivity does not follow a cumulative pattern with successive injections. The loss of radioactivity was at a slower rate when compared to turn-over in groups where only one dose was administered.

After 36 injections (total dose 3.6 uc per gm. body weight) rats on a standard diet developed a syndrome similar to the radiation sickness produced in animals by total body radiation. The animals showed great loss of weight, nose bleed, loss of hair and muscle atrophy. The mortality rate was high and increased proportionally to the number of injections. After 50 injections (total dose - 5.0 uc per gm. body weight), 80% mortality was observed. A few animals survived 53 injections and were followed for tumor formation and histological changes. By the time of writing of this report (40 days after the last injection and 260 days from the first injection) several animals developed bone tumors. The animals maintained on low calcium and Vitamin D deficient diet did not behave in the same way as those on standard diet. When on deficient diet, after 20 injections (total dose 2.0 uc per gm. body weight), there was a definite increase in their weight, the mortality rate was nil. X-Ray of the bones showed no more evidence of generalized osteoporosis which was present in animals maintained on the deficient diet for the same length of time. Several animals in this group, within 40 days after the last injection developed osteogenic sarcomas at the fracture site. To date more tumors have

developed at the fracture site in animals on low calcium diet than animals on standard diet. No final remarks and conclusions can be made because more prolonged follow up is necessary. The radiation sickness did not develop in these animals on a deficient diet.



A B

Fig. 90.
Induction of bone tumours following repeated injections of 0.1 uc per gm body weight of Sr 89. (A) Shows a radiogram of a rat with bone tumours at the fracture site (right femur) and right humerus. (B) Shows gross appearance of the tumours at the fracture site and right fore-limb.

CHAPTER X.

CONCLUSIONS.

A. Study of Normal Bone Healing in Displaced Diaphyseal Fractures of 4 Week Old Rats, and the Influence of Various Factors on the Repair of Bone.

1. In the control group of animals, in which open transverse fractures of the right femur were produced without immobilization, the degree of displacement appeared to be an important factor in determining the ultimate time of bony consolidation. Average time for bone healing was 100 days. 85 - 90% of fractures healed by bone during this study period.

2. When the fractures were immobilized, the pattern of healing was essentially similar to that of the control series, though the stages of repair were somewhat shorter. Excessive bulk of callus formation was present. The important observations included the following:

a) Atrophy of the musculature of the thigh which occurred as early as the third day after immobilization; it was progressive in nature and some evidence of its reversibility was present.

b) Stiffness of the knee on the immobilized limb was noticed as early as the 10th day following immobilization; this

was also progressive, but reversible in the study period of 92 days. The main changes were noticed in the peri-capsular tissues rather than in the articular surfaces of the knee joint. The period of bony consolidation in this group averaged 73 days.

3. In the group of animals where a gap of approximately 3 mm was produced by excising a fragment of the diaphysis at the fracture site, there was an initial lag in the repair process; ultimate bone healing was observed in an average period of 90 days. 95% of the fractures healed by bone during the study period. A shortening of approximately 5 mm was observed in all fractured femurs in this group.

4. In the group of animals where a cuff of periosteum was excised at the time of producing the fracture, the denuded bone ends underwent necrosis. A marked initial lag in the repair process was observed, but the ultimate bony union was not adversely affected; 90% of the fractures healed by bone in this study period. Necrotic ends were visualised as late as 88 days and the average time of bone healing was 126 days. Marked shortening of the fractured femur developed as a result of necrosis and absorption of the fracture ends. There was evidence of increase in the transverse width of the shaft, probably due to a hyperplastic periosteal new-bone formation.

5. In the group of animals in which the thigh muscles were sectioned at the time of fracture production, the incidence of non-union increased to 30% as compared to 10% in the control; there was absorption of the fracture ends; the displacement of the fragments persisted for a longer period of time in these animals, hence delayed union developed. The average period of time for bone healing in this series was 176 days.

6. In the group of animals where endosteum was reamed at the time of fracture production, no adverse effects in the repair process were observed. There was some suggestion that re-modelling was poor. Average time for bony union was 105 days in this study.

7. In the group of animals in which the periosteum was excised and the muscle sectioned at the time of fracture production, the results represent a combination of those found in the two series separately. The incidence of non-union was high (45%); average time of bony consolidation was 188 days. The absorption of the diaphysis to a variable extent was noticed in all specimens. Ultimate bony union took place with marked shortening of the fractured femur.

8. When the fracture was re-manipulated, the average time required for ultimate bony union was practically the same as in

control series (120 days). It was noticed, however, that the displacement of the fragments persisted for a longer time in this series. The incidence of non-union was not increased when compared to the control group (15%).

9. The pattern of bone healing in animals on low calcium and vitamin D deficient diet appears to be arrested at the cartilaginous stage, due to calcium deficiency. A high incidence of non-union (80%) as compared to the control group was observed. In some cases (15%) bony union did occur, in an average period similar to the control group.

10. The pattern of bone healing in fractures which were produced 1 week after excision of a cuff of periosteum, was essentially the same as in the control series. The blood supply to the cortex seemed to be re-established through adhesions between the surrounding muscle and bone.

B. Study of Osteogenesis Using Radioactive Strontium.

1. This study shows that there is an increase in the uptake of radioactivity at the fracture site from post-fracture day 5 to post-fracture day 289, during the period of this study. The peak was found from post-fracture day 15 to post-fracture day 145.

The data reveals that there is a definite relationship

between the stage of healing and the uptake of radioactivity at the fracture site. Fractures without firm bony union showed a higher ratio of 1.77 as compared to a ratio of 1.10 in those fractures which had healed by bone and reconstruction was far advanced.

There was a more diffuse distribution of radioactivity in animals maintained on low calcium and vitamin D deficient diet. After post-fracture day 15 there was a definite increase in uptake of radioactivity at the fracture site. Those fractures which developed firm union showed increase in uptake of radioactivity similar to fractures in animals maintained on standard diet.

2. Correlative investigations revealed that there was higher uptake of radioactivity on the ipsilateral proximal and distal metaphysis as compared to contralateral proximal and distal metaphysis respectively, but this was not a constant finding. When the fracture happened to be closer to the distal metaphysis, the uptake of radioactivity was higher in that metaphysis when compared to the uptake at the distal metaphysis when the fracture appeared to be farther away. The uptake in the distal metaphysis of the fractured femur was higher than the uptake at the proximal metaphysis in the same femur.

3. Retention study showed that the initially high uptake of radioactivity at the fracture site followed a reversal phenomenon; the fracture site lost its radioactivity at a relatively faster rate when compared to the retention at the contralateral diaphysis. The sites of slow osteogenesis (contralateral diaphysis and proximal metaphysis) retained radioactivity for longer period of time as compared to the fracture site. Thus a fracture site which had an initially high uptake lost its radioactivity at a relatively faster rate. A fracture which showed bony union and reconstruction, lost radioactivity at a faster rate as compared to those fractures which were not firmly united at the time of sacrificing the animal. The distal metaphysis, like the fracture site, lost radioactivity at a faster rate when compared to the proximal metaphysis. The ipsilateral and the contralateral metaphysis showed the same rate of loss of radioactivity.

The loss of radioactivity in animals maintained on low calcium and vitamin D deficient diet, showed a rapid loss in the early post-injection days, however, low grades of radioactivity were retained for a considerably longer period than the period of study. Those fractures which showed bony union followed a similar pattern of loss of radioactivity as in animals on standard diet.

4. Our 'cumulative study' so far shows: -

a) A radiation-sickness like syndromewas produced after a total dose of 3.6 uc per gm body weight (in divided doses, twice a week) in animals on standard diet. This was marked by loss of weight, nose bleed and loss of hair, followed by death.

b) After a total dose of 5.0 uc per gm body weight, bone tumours developed in 40% of the animals at the fracture site and other sites of active osteogenesis.

c) No radiation-sickness syndrome developed in animals maintained on low calcium and vitamin D deficient diet. On the contrary, there was evidence of mineralization of the skeleton after a total dose of 2.0 uc per gm body weight. There was appreciable gain in weight of the animals and mortality rate was markedly reduced. The incidence of tumours at the fracture site was higher in this group.

Thus, malignant tumours were induced by repeated administration of 0.1 uc per gm body weight of Sr89, at the fracture site and other sites of active osteogenesis.

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