I. TITLE: Mobilization of Radioactive Emitters from Bone

II. INSTITUTION:

University of Illinois Hospital
The Abraham Lincoln School of Medicine
Department of Orthopaedic Surgery
840 South Wood Street
Chicago, Illinois 60612

III. SUMMARY OF RESEARCH ACCOMPLISHMENTS:

For purposes of convenience, this summary will be divided into two sections according to the main project objectives: (A) the mechanism of uptake and mobilization of radioactive emitters by bone and (B) the factors affecting uptake and mobilization.

A. The Mechanism of Uptake and Mobilization of Radioactive Emitters by Bone.

1. Uptake by dead versus living bone:

   In using bone-seeking radioactive nuclides to study bone metabolism, it has been postulated that uptake by bone is proportional to the metabolic activity and in particular to mineralization of bone. To check this assumption, an earlier study was carried out comparing uptake by living bone to that by bone devitalized by repeated cycles of freezing and thawing. This study showed an appreciable uptake by dead bone (70 per cent as much in adult rats, 20 per cent in young growing animals). An explanation for the uptake by dead bone was sought. At first, it
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was thought to be due to exchange. However, by definition exchange is a process in which one ion replaces another. Theoretically, with the large surface area of bone in contact with the body fluids, this process should reach a steady state relatively early. In the case of dead bone, however, it was found that the curve did not follow that of the plasma but remained elevated for a prolonged period of time. Several possible explanations arose for the phenomenon; one was the possibility of "slow exchange" advanced by John Marshall; another possibility was adsorption on the surface of the dead tissue or hypermineralization. However, careful quantitative analyses of the dead bone showed no significant change in such composition. Ultimately, a method was developed for sectioning undecalcified bone to simultaneous autoradiograms, microautoradiograms, and histological sections could be performed on the bone tissue. From this study, it was found that there is slow diffusion of the nuclide through the lacunar and canalicular network of devitalized bone and that the isotope is apparently "caught" in these spaces rather than being incorporated into the crystallized mineral phase. It is our feeling that diffusion rather than exchange, secretion or adsorption accounts for the uptake of radioactive nuclides by dead bone.

One major difference between viable and non-viable bone is that the mineral phase is mobilized more rapidly
during biological remodelling of living bone than from dead bone during replacement by the classical process of resorption and "creeping substitution". One of the factors related to mobilization of bone-seeking radioactive emitters in vitro is the changing pH of the medium due to accumulation of lactic acid. As the pH decreases the rate of release of a bone-bound isotope into the culture medium increases.

Publications


2. Correlation of Balance and Kinetic Studies, Bone Density and Morphological Studies.

Two major problems confront the scientist studying bone metabolism: (1) that of measuring the total mass of the skeleton and (2) that of measuring the rate of bone turnover, i.e., the rates of formation and resorption. Some progress was made on both of these problems. As pointed
out by Robinson and by Arnold, the definition of bone density as well as the techniques for measuring it are still the subject of controversy. In a non-homogeneous material such as bone "apparent" density must be differentiated from "real" density. Apparent density can be determined in the case of a whole bone by measuring the total volume (using millet seed displacement or some modification of this technique), and the total weight. At a microscopic level, apparent density is a function of porosity. After several trials, an instrument was designed to obtain a bone biopsy of standard volume and attempts were made to correlate such biopsies with the wet weight, dry weight and ash weight of the sample (in a study previously reported volume was determined by water displacement but this gives a figure for "real" rather than "apparent" density).

Studies were undertaken to determine the porosity of bone specimens by preparing undecalcified sections and from these microradiograms which were subjected to computer analysis. The same sections were stained for calcium by the von Kossa technique and also submitted to computer analysis for porosity. In addition, an attempt was made to correlate porosity with compression strength samples of trabecular bone/(Salante). The results showed a correlation between porosity and compression strength and also a correlation between compression strength
and trabecular orientation. Thus, a biopsy taken from the same vertebral body in a transverse plane had a different compression failure than a biopsy obtained in a vertical plane.

With regard to the "real" density of bone at a microscopic level, there are variations from one area to another as noted by the classical pathologists. In some metabolic disorders, this range may be great (osteomalacia as opposed to osteoporosis). Efforts were made to measure the range of density in biopsies of human bone using microradiograms of undecalcified bone sections of measured thickness with a density standard introduced into the film (this was carried out in collaboration with Dr. Lesley).

**Metabolic Activity**

Classically, the metabolic activity of the skeleton has been assessed by balance studies, by the level of alkaline phosphatase in the serum, by the excretion of hydroxyproline in the urine (bone resorption) and by the histological pattern of bone biopsies (the number of osteoblasts, the width of the osteoid seams and by the number of osteoclasts). In 1958, Bauer and Kay postulated that the metabolic activity of the skeleton might be assessed by use of bone-seeking radioactive tracers, such as strontium 85 or calcium 47. A technique for kinetic analysis was developed based on a series of assumptions which have
continued to arouse controversy. These assumptions concern at least one of us and have been the subject of several papers. However, regardless of the assumptions, the method does provide a figure for plasma clearance by skeletal uptake that is reproducible and useful since it is significantly altered in many metabolic disorders of the skeleton. In addition and simultaneously, tracer distribution studies can be carried out for localized areas of abnormal skeletal activity. Various modifications of the method have been used by other investigators (Whedon, Neary, Rich and Lauer to mention only a few). Recently, a variety of methods for assessing the metabolic activity of bone from bone biopsies have been advocated (e.g., Jowsey and Frost).

Three clinical papers are being submitted for publication, illustrating application of techniques developed under this contract - including the correlation of kinetic studies, balance studies, and morphological studies of bone in systemic disorders of calcium metabolism.

The first paper describes a 13 year old girl with calcium deficiency rickets. It was found that the aminoaciduria disappeared and the serum calcium and phosphate levels returned to normal by simply correcting the dietary imbalance even in the absence of supplementary vitamin D.
The second paper describes a 9 year old girl with calcinosis universalis. The massive deposition of calcium in the soft tissues showed marked resolution under diphosphonate therapy.

The third paper describes use of mithromycin in the treatment of 5 patients with hypercalcemia secondary to metastatic malignancy. It was found that the drug caused accelerated removal of calcium from the blood, but this was not associated with an increase in calcium excretion in the urine or stool.

Publications:


D. The Factors Affecting Uptake and Mobilization

1. Circulation

From a variety of clinical observations, it has long been suspected that circulation and coincident alterations in oxygen tension may be one of the factors regulating bone metabolism. A study designed to determine the effects of altered circulation on bone growth using a femoral arteriovenous fistula in a young animal as a model was instituted. A technique was developed for measuring bone blood flow based on the Fick Principle. Bone circulation following creation of an arteriovenous fistula was compared with the normal side of the same animal in a series of experiments.

Publications:


2. Oxygen Tension:

Because of limitations inherent in the previous study, it was elected to study the effects of oxygen tension in vivo by maintaining rats at ambient pressure and high and low levels of oxygen in a suitably designed chamber. This study was completed as partial requirement for Master's Degree by David Barman and although the results were suggestive - high oxygen tension was accompanied by increased bone resorption (or decreased bone formation) and low oxygen tension by the reverse - the study was not statistically significant and hence, has not been published.

An in vitro study based on organ cultures of endochondral ossification was completed as partial requirement for the Ph. D. Degree by Dr. Carl Brighton.

Summary: These experiments revealed maximal metaphysical bone formation, as determined by microphotography, micro-radiography and tetracycline staining occurred in 5 per cent (thirty-eight millimeters of mercury) oxygen but the cartilaginous portion of the epiphysial plate exhibited maximum growth in 21 per cent oxygen. With higher oxygen
tensions, the cartilage portion of the plate showed narrowing, a progressive loss of acid mucopolysaccharide stainability, eventual loss of the zone of hypertrophic cells and accumulation of neutral micropolysaccharide or glycomucoprotein at its junction with metaphyseal bone.

A clinical study was inaugurated in cooperation with Dr. Guillermo Polo at the Chuluc General Hospital at La Oroya, Peru (14,000 feet altitude) and Dr. Robert Temple at the University of Peru in Lima. The tools for obtaining standard biopsies were constructed and it was hoped to carry out a series of bone biopsies on patients undergoing surgery for acute injuries and to compare these biopsies by the techniques previously mentioned. Unfortunately, because of political problems beyond the senior investigator's ability to solve, this portion of the study has not been completed. If differences are found, additional studies will be necessary including dietary controls, isotope and metabolic studies.

Publication:


3. Vitamin D

Another factor involved in bone metabolism is vitamin D
although the possibility of a direct action of the vitamin on the skeleton is still controversial. Initially, a study was carried out by Dr. Selu Sankaran repeating Carlsson, Blau and Lindquist's early studies (copies of monograph appended). This revealed that rats maintained on a low calcium diet showed increased urinary excretion of calcium following vitamin D administration along with a lowered ash content of the skeleton and a rise of the serum calcium to normal levels. The experiment was conducted under self-fed metabolic balance conditions and the results were evaluated on the basis of histology, microradiography, isotope kinetic studies and direct analyses of bone. However, it was impossible to tell from the results whether the effect of vitamin D was due to direct action on the skeleton or to an indirect mechanism mediated through the parathyroid glands since these were intact. Accordingly, an in vitro study was undertaken by Dr. John Sevestrelogion on the effects of vitamin D at physiological levels on bone maintained in organ culture. This study failed to confirm the results of Goldhaber who demonstrated resorption of bone maintained in vitro exposed to massive doses of vitamin D. However, the study did reveal an increased turnover of bone under the influence of vitamin D.

Along with these studies, an experiment on the binding of vitamin D by human serum was published.
Publications:


CONTENTS:

1. Effect of Vitamin D on Thyroparathyroidectomyed Rats.

2. Effect of Vitamin D and Calcium on Survival of the Thyroparathyroidectomyed Rat.

3. Effect of Parathyroid Hormone in the Absence of Vitamin D.

4. Effect of Vitamin D on Bone Calcium Metabolism in Vitro.

5. Chemical Studies of Cortical Diaphyseal Bone during Vitamin D Deficiency in the Rat.


Earlier studies on mobilization of Sr$^{90}$ from bone revealed that of the various possible methods, a low phosphorus
diet was the most effective and over a period of 55 days resulted in a decrease in the body burden by 90 per cent. However, since the effects of the diet were not apparent before 7 days had elapsed, it was proposed to find a method for mobilizing bone-seeking radioactive sattures during the early interval. The first method attempted was by parathormone injections. As a control, a diuretic was used and the results revealed that the diuretic was equally effective. It was felt that one possible reason for this was that the strontium had not become incorporated into the "secreted" fraction of the bone mineral. To test this hypothesis, the experiment was repeated 6 weeks after administration of the isotope. Again, it was found that the diuretic was just as effective as massive doses of parathyroid hormone.

The question arose as to whether the effect of the diuretic on the skeleton could be mediated through endogenous secretion of parathyroid hormone. To test this possibility a series of rats was given strontium 85 and after a suitable interval they were divided into two groups, one of which was parathyroidectomized and then both were given the diuretic. The parathyroidectomized group showed no significant mobilization of strontium following administration of the diuretic confirming the fact that the effect of the diuretic on mobilization of bone mineral
was due to increased endogenous secretion of parathyroid hormone.

Publication:


V. PERSONNEL:

One of the major features of this contract was that it permitted training a number of individuals in radioactive isotope techniques and in their application to problems involving the skeleton. Many of these individuals were supported by grants from other sources but contributed their efforts to completion of the contract, and thus, not only the individuals but the A.E.C. stood to profit from this cooperative effort. Among these were the following individuals:

1) Ramaz Aouad, M.D. Department of Orthopaedic Surgery Hospital St. Charles Borromeo Aithania-Fayadie Beirut, Lebanon

2) Riad Barreda, M.D. Assistant Professor of Orthopaedic Surgery, University of Illinois Hospital, The Abraham Lincoln School of Medicine, Chicago, Illinois.
   Chief of the Orthopaedic Service, West Side Veterans Administration Hospital.

3) Esther de Silva, Ph.D. Department of Biochemistry, Universidad Peruana Lima, Peru
4) Kasemsant Dhanyasobhak

Head of the Department of Orthopaedic Surgery, University of Chiangmai Medical School, Chiangmai, Thailand

5) Jorge O. Galante, M.D.

Assoc. Professor, University of Illinois Hospital, The Abraham Lincoln School of Medicine, Chicago, Illinois

6) Etienne P. Hugo, M.D.

University of Pretoria, Pretoria, South Africa

7) Richard L. Jacobs, M.D.

Assoc. Professor of Orthopaedic Surgery, University of Illinois Hospital, The Abraham Lincoln School of Medicine, Chicago, Illinois

8) Masaya Kawabata, M.D.

Department of Orthopaedic Surgery, Toranomon Hospital, Tokyo, Japan

9) Brian McKibbin, M.D.

University Department of Orthopaedics, The Royal Infirmary, Sheffield, England

10) Karl Mueller, M.D.

Marquette University, Milwaukee, Wisconsin

11) Earl Solomon, M.D.

Hebrew University Hadassah Medical School, Jerusalem, Israel

12) Olu Sankaran, M.D.

Professor and Chairman of the Department of Orthopaedic Surgery, Maulana Azad Medical College, University of Delhi, New Delhi 2, India

13) J.A. Savastikoglou, M.D.

Professor and Chairman of the Department of Orthopaedic Surgery, University of Umea, Umea 6, Sweden

14) Yutaka Shimomura, M.D.

Acting Head of the Department of Orthopaedic Surgery, Osaka University Medical School, Hekushima-Ku, Osaka, Japan

15) Antoni Trías, M.D.

Professor and Director, Dept. of Orthopaedic Surg. Université de Sherbrooke, Sherbrooke, P.Q., Canada
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<tr>
<th></th>
<th>Graduate Students Trained (1966-1970)</th>
<th>Degrees Granted</th>
<th>Post-doctoral Tenures</th>
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<tr>
<td>1</td>
<td>Daniel V. Olivas, M.D.</td>
<td>M.S. 1967</td>
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<td>2</td>
<td>Robert S. Bills, M.D.</td>
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<td>3</td>
<td>Harle J. Schradt, M.D.</td>
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<td>4</td>
<td>David Berman</td>
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<td>5</td>
<td>Carl T. Brighton</td>
<td>Ph.D. 1968</td>
<td>1968</td>
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<td>6</td>
<td>Roger A. Lueck, M.D.</td>
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<td>Clarence H. Forster, M.D.</td>
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<td>8</td>
<td>Alad Barmada, M.D.</td>
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<td>9</td>
<td>Richard L. Gram, M.D.</td>
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<td>10</td>
<td>Edward Laufer, M.D.</td>
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<td>11</td>
<td>David L. Levine, M.D.</td>
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<td>1970</td>
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VI. PUBLICATIONS SUPPORTED BY ASC GRANTS, 1966-1973

1. The Autoradiographic Distribution and Localization of Ca**<sup>45</sup> in Undecalcified Fresh and Devitalized Rat Bone Autografts.
   Solomon, C.D., Ray, R.D.
   C00-1601-44

2. An Experimental Comparison of Living and Dead Bone in Rats
   Stevens, J., Ray, R.D.
   L00-1601-45

3. Experimental Study of Peripheral Circulation and Bone Growth.
   Surface Counting of Blood Borne Radioactive Emitter. Part I.
   Ray, R.D., Aoudad, A., Kawabata, M.
   C00-1601-46

4. Experimental Study of Peripheral Circulation and Bone Growth.
   The Pattern of Venous Return in Experimental Arteriovenous Fistulae. Part II.
   C00-1601-70

5. Experimental Study of Peripheral Circulation and Bone Growth.
   An Experimental Method for the Quantitative Determination of Bone Blood Flow. Part III.
   Ray, R.D., Kawabata, M., Galante, J.O.
   C00-1601-8

6. Experimental Study of Peripheral Circulation and Bone Growth.
   Blood Flow Distal to Iliac Arteriovenous Fistulae. Part IV.
   Kawabata, M., Ray, R.D.
   C00-1601-9

7. Studies of Vitamin D Binding in Normal and Rachitic Serum.
   Jacobs, R.L., Ray, R.D.
   C00-1601-11

   Ray, R.D.
   C00-1601-50
PUBLICATIONS (Cont.)

5. In Vitro Binding of Histologic Stains by Collagen.
   Jacobs, R.L., Ray, R.D.

    Brighton, C.T., Ray, R.D., Sobin, L.H., Kuettner, K.E.

11. Vitamin D and Skeletal Metabolism (Experimental Studies in the Rat).
    Sevastikoglou, J.A., Ray, R.D., Hjertquist, S.O., Bergquist, E.

12. A Monograph on the Action of Vitamin D on Bone (A Metabolic, Isotopic and Histological Study on Rats).
    Sankaran, S., Ray, R.D.

Accepted for Publication

    Jacobs, R.L., Ray, R.D., Shapiro, P.D.

14. Mobilization of Strontium from the Rat Skeleton.

Chapter 29: Clinical Orthopaedics & Related Research, 1970
250-260
C00-1601-10

J. Bone & Joint Surg., 57
C00-1601-18

Acta Orthopaedica Scand.
C00-1601-20

Page 1-95, 1970.
C00-1601-21

Clinical Orthopaedics & Related Research, 1970
C00-1601-19

Clinical Orthopaedics & Related Research, 1970
C00-1601-22