3048

FINAL REPORT

Coo-1601-23

ATOMIC ENERGY CONTRACT AT(17-1) 1601 April 1966 to August 1970

I. **TITLE:** Mobilization of Radioactive Emitters from Bone

II. **INSTITUTION:**

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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 ash composition. Ultimately, a method was developed for sectioning undecalcified bone to simultaneous autoradiograms, microradiograms, and histological sections could be performed on the same 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

- a) Salomon, C.D., and Ray, R.D.: The Autoradiographic Distribution of Ca^{45} in Fresh and Devitalized Rat Bone Autografts, *J. Bone & J. Surg.* 48-A: 1575-1584, Dec. 1966.
- b) Stevens, J., and Ray, R.D.: An Experimental Comparison of Living and Dead Bone in Rats, *J. Bone & J. Surg.* 49-B: 154-163, Feb. 1967.
- c) Jacobs, R.L., and Ray, R.D.: In Vitro Study of Mobilization and Uptake of Bone Mineral. Accepted for Publication in *Clin. Ortho. and Rel. Res.*

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

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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 of trabecular bone/^{samples}(Galante). 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. Leelay).

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 Ray 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

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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, Neany, Rich and Bauer 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 aminocalderia 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.

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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:

- a) Galante, J.D., Ray, R.D., and Rostoker, W.:
Physical Properties of Trabecular Bone, J. of
Calcified Tiss. Res. 5: 236, 1970. (Reprints not
received as yet).
- b) Hugo, E.P., and Ray, R.D.: Bone Density Measure-
ments. (In preparation)
- c) Hugo, E.P., and Ray, R.D.: Assessment of Bone
Turnover Rates. (In preparation)
- d) Cram, R., and Ray, R.D.: Aminoaciduria of Simple
Rickets: Dissociation from Hypovitaminosis D -
Case Report. Submitted for Publication.
- e) Cram, R., and Ray, R.D.: Polyphosphates for Cal-
cinosis Universalis. Submitted for publication.
- f) Cram, R., Perile, C.P., and Ray, R.D.: Mithromycin
Therapy of Hypercalcemia. Submitted for Publication.

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:

- a) Ray, R.D., Aouad, R., Kawabata, H.: Experimental Study of Peripheral Circulation and Bone Growth. Part I. Surface Counting of a Blood-Borne Emitter, Clin. Ortho. Rel. Res. 52: 221-232, 1967.
- b) McKibbin, J., Ray, R.D.: Experimental Study of Peripheral Circulation and Bone Growth. Part II. The Pattern of Venous Return in Experimental Arterio-Fistulas, Clin. Ortho. & Rel. Res. 53: 175-183, 1967.
- c) Ray, R.D., Kawabata, H., and Galante, J.: Experimental Study of Peripheral Circulation and Bone Growth, Part III. An Experimental Method for the Quantitative Determination of Bone Flow. Clin. Ortho. & Rel. Res. 54: 175-185, 1967

-3-

- d) Kawabata, H., Ray, R.D.: Experimental Study of Peripheral Circulation and Bone Growth. Part IV. Blood Flow Distal to Iliac Arteriovenous Fistula, Clin. Ortho. & Rel. Res. 55: 177-189, 1967.
- e) Ray, R.D.: Circulation and the Skeletal System, J Bone & Joint Surg., 50-A: 764-765, 1968.

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 Barnan 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 metaphyseal bone formation, as determined by macrophotography, microradiography and tetracycline staining occurred in 5 per cent (thirty-eight millimeters of mercury) oxygen but the cartilaginous portion of the epiphyseal plate exhibited maximum growth in 21 per cent oxygen. With higher oxygen

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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 Chulec 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:

- a) Brighton, C.T., Ray, R.D., Soble, L.W., and Kuettner, K.: In Vitro Epiphyseal Plate Growth in Various Oxygen Tensions, J. Bone and Joint Surg., 51-A: No. 7, 1383, 1969.

3. Vitamin D

Another factor involved in bone metabolism is vitamin D

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although the possibility of a direct action of the vitamin on the skeleton is still controversial. Initially, a study was carried out by Dr. Balu Sankaran repeating Carlsson, Sauer and Lindquist's early studies (copies of Bonegraph 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 galy-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 Savastikoglou 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:

- a) Jacobs, R.L., and Ray, R.D.: Studies of Vitamin D Binding in Normal and Rachitic Serum. Clin. Ortho. & Rel Res. 56: 275-281, 1968.
- b) Jacobs, R.L., Ray, R.D., and Shapiro, F.D.: In Vitro Study of Mobilization and Uptake of Bone Mineral. Accepted for Publication. Clin. Ortho. & Rel. Res.
- c) Savastikoglou, J.A., Ray, R.D., Hjertquist, S.O., and Bergquist, L.: Vitamin D and Skeletal Metabolism, Experimental Studies in the Rat, Acta Orthopaedica Scandinavica Suppl. No. 136, 1970.

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CONTENTS:

1. Effect of Vitamin D on Thyroparathyroidectomized Rats.
 2. Effect of Vitamin D and Calcium on Survival of the Thyroparathyroidectomized 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.
4. Influence of Diuretics, Parathormone and a low Phosphorus Diet on Mobilization of Radioactive Emitters from the Skeleton:

Earlier studies on mobilization of Sr^{90} from bone revealed that of the various possible methods, a low phosphorus

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diet was the most effective and over a period of 55 days resulted in a decrease in the body burden by 80 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 emitters 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 "accreted" 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

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was due to increased endogenous secretion of parathyroid hormone.

Publication:

a) Barnada, R., Thomson, D.M., and Ray, R.D.:

Mobilization of Strontium from the Rat Skeleton,
Accepted for publication in Clin. Ortho. & Rel.
Research.

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) Ramez Aoun, M.D.

Department of Orthopaedic Surgery
Hospital St. Charles Borromeo
Rithanie-Fayadie
Beirut, Lebanon

2) Riad Barnada, M.D.

Asst. 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

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- 4) Kesemsant Dhanayasobhak
Head of the Department of Orthopaedic Surgery, University of Chiangmai Medical School, Chiangmai, Thailand
- 5) Jorge O. Salante, 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) Carl Salomon, M.D.
Hebrew University Hadassah Medical School Jerusalem, Israel
- 12) Balu 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. Savastikoglov, 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, MUKUSHIMA-KU, Osaka, Japan
- 15) Antoni Trias, M.D.
Professor and Director, Dept. of Orthopaedic Surg. Universite de Sherbrooke, Sherbrooke, P.Q., Canada

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IV: <u>GRADUATE STUDENTS TRAINED (1966-1970)</u>	<u>Degrees Granted</u>	<u>Post-doctoral Tenures</u>
1) Daniel V. Girsadas, M.D.	M.S. 1967	1966-67
2) Robert S. Billis, M.D.		1967
3) Harle J. Schrott, M.D.		1967
4) David Berman	M.S. 1967	
5) Carl T. Brighton	Ph.D. 1968	1968
6) Roger A. Lunck, M.D.		1968
7) Clarence H. Foster, M.D.		1968
8) Alad Barnada, M.D.		1968
9) Richard L. Cram, M.D.		1969
10) Edward Lambert, M.D.		1969
11) David L. Levine, M.D.		1970

VI. PUBLICATIONS SUPPORTED BY ABC GRANTS 1966-1970

1. The Autoradiographic Distribution and Localization of Ca^{45} in Undecalcified Fresh and Devitalized Rat Bone Autografts. Salomon, C.D., Ray, R.D. J. Bone & Joint Surg., #8 48-A: 1575-1584, December, 1966. C00-1601-4A
2. An Experimental Comparison of Living and Dead Bone in Rats 3. Uptake of Radioactive Isotopes. Stevens, J., Ray, R.D. J. Bone & Joint Surg., #1, 47-B: 154-163, February, 1967. C00-1601-4B
3. Experimental Study of Peripheral Circulation and Bone Growth. Surface Counting of a Blood Borne Radioactive Emitter. Part I. Ray, R.D., Aoud, R., Kawabata, M. Clinical Orthopaedics & Related Research, #52, 221-232, May-June, 1967. C00-1601-6B
4. Experimental Study of Peripheral Circulation and Bone Growth. The Pattern of Venous Return in Experimental Arteriovenous Fistulae. Part II. Clinical Orthopaedics & Related Research, #53, 175-183, July-August, 1967. C00-1601-7B
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., Salento, J.D. Clinical Orthopaedics & Related Research, #54, 175-185, September-October, 1967. C00-1601-8
6. Experimental Study of Peripheral Circulation and Bone Growth. Blood Flow Distal to Iliac Arteriovenous Fistula. Part IV. Kawabata, M., Ray, R.D. Clinical Orthopaedics & Related Research, #55, 177-189, November-December, 1967. C00-1601-9
7. Studies of Vitamin D Binding in Normal and Rachitic Serum. Jacobs, R.L., Ray, R.D. Clinical Orthopaedics & Related Research, #56, 275-281, January-February, 1968. C00-1601-11
8. Circulation and the Skeletal System. Ray, R.D. J. Bone & Joint Surg., #4 50-A: 764-765, June, 1968. An Instructional Course Lecture. The Am. Academy of Orthopaedic Surgeons. C00-1601-5B

PUBLICATIONS (Cont.)

9. In Vitro Binding of Histologic Stains by Collagen.
Jacobs, R.L., Ray, R.D.
10. In Vitro Epiphyseal Plate Growth in Various Oxygen Tensions.
Brighton, C.T., Ray, R.D., Sobla, L.W., Kuettnar, R.E.
11. Vitamin D and Skeletal Metabolism (Experimental Studies in the Rat).
Savastikoglou, 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.

Chapter 29: Clinical Orthopaedics & Related Research #60.
256-260
C00-1601-10

J. Bone & Joint Surg., #7
51-A: 1383-1396,
October, 1969.
C00-1601-18

Acta Orthopaedica Scand.
Supplementum #136, 1-85,
1970.
C00-1601-20

Page 1-99, 1970.
C00-1601-21

Accepted for Publication

13. In Vitro Study of Mobilization and Uptake of Bone Mineral.
Jacobs, R.L., Ray, R.D., Shapiro, F.D.
14. Mobilization of Strontium from the Rat Skeleton.
Darmada, R., Thomson, D.M., Ray, R.D.

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

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