PROGRESS REPORT (NYO 910-9)
TO
THE UNITED STATES ATOMIC ENERGY COMMISSION
ON
PROPOSAL #9

RADIATION STUDIES: STUDIES OF THE KINETIC STATE OF CALCIUM IN BONE LESIONS, USING CALCIUM-47 AND STRONTIUM-85

CONTRACT NO. AT(30-1)910
BIOLOGICAL EFFECTS OF RADIATION AND RELATED
BIOCHEMICAL AND PHYSICAL STUDIES

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During the period of this report - June 1, 1963 to May 30, 1964 - 34 studies of the distribution and kinetics of Calcium-47 and Strontium-85 were conducted in 33 patients who had cancer metastatic to bone or who were suspected of having such metastases. The results of this work are presently being analyzed and prepared for publication.

The studies were carried on with the following objectives:

1. to detect very early metastases which were not apparent by x-ray techniques;

2. to improve and simplify kinetic studies by (a) sampling the saliva instead of the blood, and (b) using the High Energy Gamma (HEG) scanner to measure the total body retention of the isotopes used;

3. to study the effect of radiation treatment on the retention of Ca-47 and Sr-85 by bone lesions;

4. to determine by weekly, then monthly follow-up, the retention in bone of tracer doses of Ca-47 and Sr-85 under various conditions;

5. to study the disappearance rate from blood of a single intravenous dose of tetracycline -- as a pilot study in 7 patients with various bone diseases, in order to develop a non-isotopic technique of following the course of metastatic or metabolic bone disorders by means of a "tetracycline tolerance test".

1. Detection of early metastatic lesions

a. Technique. Single doses of 100 microcuries of Sr-85 were injected intravenously into patients who were suspected of having bone metastases (on the basis of pain in a given area) but in whom x-ray studies failed to reveal any lesion. Most of the patients had breast carcinoma, and back pain was the most frequent initial symptom.

Scanning of the suspected area of disease was carried out at 24, 48 and frequently at 96 hours following the injection. Scanning of the vertebral column, pelvis, greater trochanters, patellas, tibias, and skull, as well as of any other bone which according to symptoms might have been indicated, was always performed. When lesions were detected, appropriate therapy was started, and scanning was repeated at weekly, then monthly, intervals.

Our earlier studies (described in our previous Progress Report) indicated that in the absence of x-ray evidence of metastases in the bones, patients with pain, or those with metastatic lesions at other sites, were most likely to reveal the presence of a lesion in bone as determined by the radioisotope. We have, therefore, largely confined ourselves to the study of such patients.

b. Results. During the past year, seven such patients were studied. Six had breast carcinoma, and one had carcinoma of the colon. Among the group with breast carcinoma, three had pain but no metastases were detectable by x-ray examination; three had metastases in locations other than the painful areas.

In all except the patient with carcinoma of the colon, an increase in Sr-85 deposition was detected over the suspicious area. In four patients the presence of a lesion was subsequently confirmed by x-ray examination. The other two

patients are still being followed, but no confirmation of a lesion has yet been obtained.

The presence of a metastatic bone lesion was presumed when the uptake of Sr^{85} or Ca^{47} was elevated at the given point. When this occurred in a vertebra, it appeared on the scan as a peak of radioactivity above the level of the other vertebrae. When the uptake was elevated in a long bone, it showed either a peak above the scan of the shaft or a level of radioactivity higher than the one measured over a symmetrical uninvolved side, whenever such a comparison was possible. We have found that the highest uptake by a lesion takes place 24 to 48 hours after injection of Sr^{85} but continues to rise for several days when Ca^{47} is used.

We have thus confirmed our preliminary observations, which indicated that after the administration of a tracer amount of Ca^{47} and Sr^{85} bone metastases may be detected by scanning at a time when they are not yet apparent by x-ray diagnostic studies. However, we plan to expand the number of studies in order to improve our scanning technique and to fine the optimal period of scanning.

One patient study appears to answer the question of redistribution of a previous labeling tracer dose of Sr^{85} into metastatic lesions developing in the interim. This patient had undergone bilateral radical mastectomy for breast carcinoma but had no evidence of metastases at the time a tracer dose of Sr^{85} was given. She subsequently developed multiple metastatic lesions which did not reveal any abnormally elevated radioactivity level from the original Sr^{85} tracer. We concluded, in this case, that a labeling tracer dose of Sr^{85} would not help in detecting later development of metastases and that serial tracer doses of either Sr^{85} or Ca^{47} would be more appropriate for this purpose. However, we plan to expand the number of such cases studied.

2. Improvement and simplification of kinetic studies

In recent years the study of bone physiology and calcium metabolism has been greatly stimulated by the use of calcium-47 and strontium-85. During the course of such studies the calculation of the size of the exchangeable calcium pool and the rate of accretion of calcium in bone has been based on measurements of the curve of specific activity of the isotope in blood and its excretion in the urine and stools.

The HEG scanner, which was designed and built by members of the Division of Biophysics of the Sloan-Kettering Institute for Cancer Research to measure the distribution of Ca^{47} and Sr^{85} in the body, has recently been adapted for the external measurement of the total body retention of such isotopes. Thus it is now possible to measure this retention directly in the patient, while previously this had to be based upon measurements of the isotopes in the fecal and urinary excretions of the patient.

We have endeavored to find a method of sampling the exchangeable calcium pool in order to establish the curve of specific activity of the Ca^{47} or Sr^{85} without having to resort to multiple venipunctures. It was thought that saliva might reflect the changes occurring in blood since collections of samples of this fluid can be timed more precisely than can urine samples. With urine samples there is a delay between its excretion by the kidney and its passage through the bladder. We have therefore compared, in the same patient, the data obtained by the conventional blood and excretion measurements with the data

obtained by the direct measurements of total body retention (HEG scanner) and by saliva measurements.

Eleven patients were studied. The determination of stable and isotopic calcium was carried out in samples of blood, saliva, urine, and stools. Sr-85 was likewise measured in these biological substances. The specific activity of Sr-85 or Ca-47 relative to stable calcium was calculated in both blood and saliva samples. The body retention of the isotope used was calculated by subtracting daily the cumulative amount excreted in urine and stools from the administered dose expressed as 100 per cent. The body retention was also measured directly with the HEG scanner. This scanner is composed of two scintillation detectors, heavily shielded with tungsten and lead, which move synchronously, one above and one below the lucite platform upon which the patient lies. Back and forth scanning is done across the platform (171 cm long and 72 cm wide) at a uniform speed with a 2.8 cm advance between each sweep. Total counts are recorded at the end of the scan, which takes approximately 20 minutes. The patient is counted one hour after the intravenous administration of the Ca-47 and Sr-85 tracer (before passing any urine or feces). The total counts thus accumulated represent 100 per cent of the dose distributed in the body. Subsequent measurements on the following day are done after the patient has had a bowel movement and emptied his bladder. When such measurements are obtained, they are corrected for physical decay of the isotope and compared with the 100 per cent figure in order to obtain the actual retention at any given time.

We found that the saliva gave as suitable a sampling of the exchangeable pool as blood for the measurements of the specific activity of Ca-47 or Sr-85 relative to stable calcium. The specific activity in saliva was identical to that in serum at any given time (Figs. 1 and 2), regardless of variations in the concentration of stable calcium in both these fluids. (Normocalcemic, hypercalcemic, and hypocalcemic patients were studied).

The studies of total body retention of Calcium-47 and Strontium-85 showed that direct measurements (HEG scanner) frequently revealed slightly less retention than did measurements based upon excretion of the isotopes (Figs. 3 and 4). This difference may be inherent in the technique of external counting or may represent some constant loss in the collection of excreta or possibly unmeasured losses in sweat. A comparison of both methods in the determination of the exchangeable calcium pool and the accretion rate was made in 6 studies conducted on 3 patients. The results were practically identical except in 2 studies where differences of the order of 15 per cent were noted in the values of the accretion rates. A proposed explanation was that there was, in these cases, a constant loss of calcium through perspiration which gave a lower retention measured directly than calculated from the excretion data.

3. Effects of radiation treatment on bone lesions

In order to study the effect of radiation therapy on a given bone lesion in a patient, a tracer dose of 100 microcuries of Ca-47 or Sr-85 is given intravenously, and the uptake by the lesion is measured for four days prior to starting radiation therapy and compared to measurements in uninvolved bone. After the start of radiation therapy, measurements of retained Sr-85 are continued at weekly and then monthly intervals, and repeat measurements of Ca-47 uptake are done at intervals of four to eight weeks. Eight patients were started on this study, but the results could not be fully evaluated because of the introduction of intercurrent therapy, necessary to a patient's welfare or because of rapid deterioration of a patient's clinical state. The data available in one adequately

studied patient indicate that radiation of a lesion may suppress the uptake of Ca-47 (Fig. 5) and favor its rapid release from bone (Fig. 6).

4. Follow-up of Ca-47 and Sr-85 retention

Over a period of several weeks to several months, sequential measurements of total body retention of Ca-47 and Sr-85, as well as local retention in normal and tumor-involved bone, have brought to light certain characteristics of bone with respect to these two isotopes:

a. There is greater total body retention of Ca-47 than of Sr-85 (Figs. 3 and 4).

b. Both Ca-47 and Sr-85 are retained in areas of active bone metastases of patients with breast carcinoma to a greater extent than in normal bone. However, whereas the uptake of Ca-47 continues to rise in the bone lesion over several days (Fig. 7), the Sr-85 uptake falls after the first day (Fig. 8).

c. The total body retention of Sr-85 has been followed over several months, and when plotted as a function of time, a change in slope appears on the curve after approximately 50 days. The significance of this in terms of incorporation of this isotope in the various parts of bone mineral is at present unclear.

5. Tetracycline studies

During these studies, 400 mg of tetracycline were injected intravenously over 15 minutes as a single dose. The urine output was fractionated during the first 24 hours and multiple blood samples were obtained. Tetracycline was determined fluorometrically in serum and urine, and serial determinations were carried out for 72 hours.

Twelve studies were conducted in 7 patients. In 3 patients a study was conducted before and repeated after treatment for diffuse bone disease. A response to treatment was associated with a decrease in the rate of disappearance of tetrcycline from blood in 2 patients. In the third, a lack of clinical response was associated with no significant change in the tetracycline disappearance rate. In one additional patient, two studies were conducted at an interval of 3 days without treatment; the resulting curves of disappearance of tetracycline from blood were found to be reproducible.

Kinetic studies with Ca-47 were conducted simultaneously. The results of this preliminary work indicate that the "tetracycline tolerance test" may become a useful method of studying bone disease. It is therefore planned to extend this investigation.

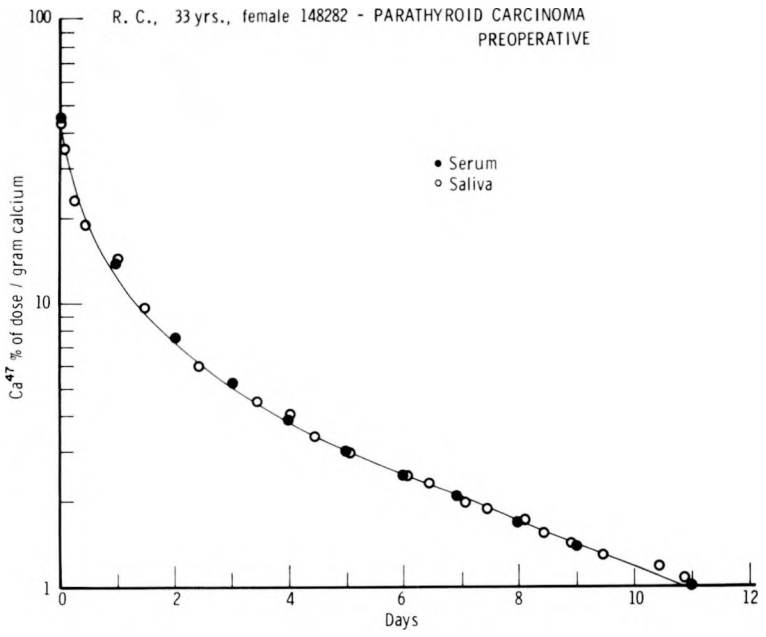
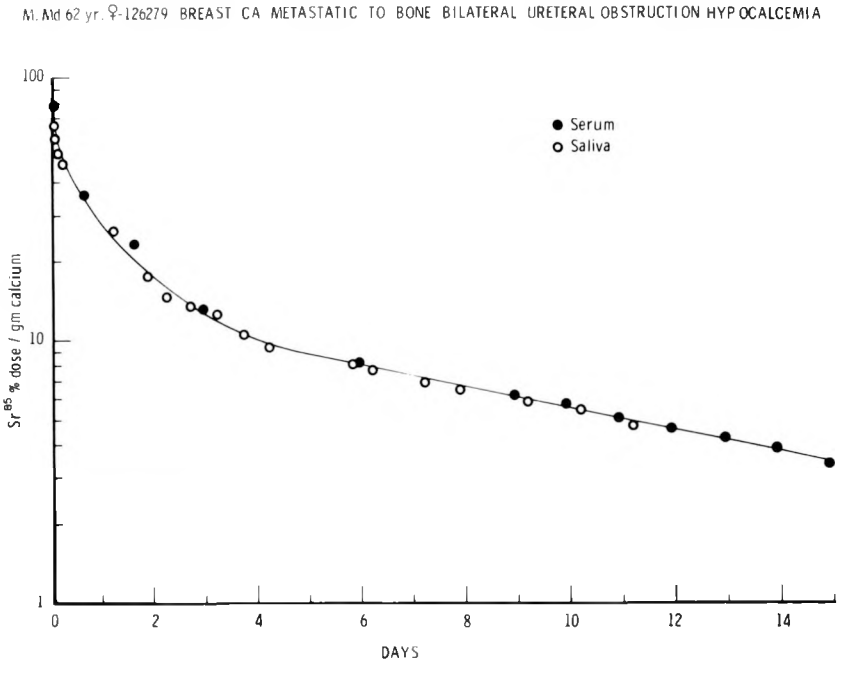


FIGURE 1



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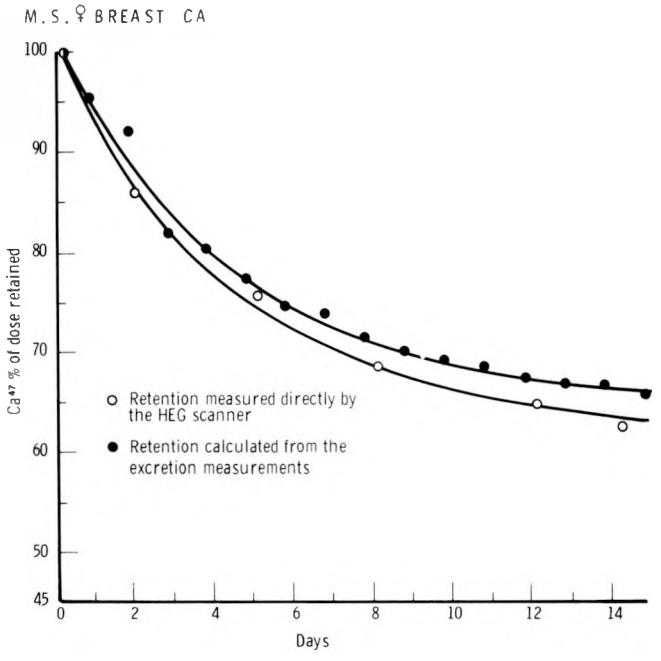

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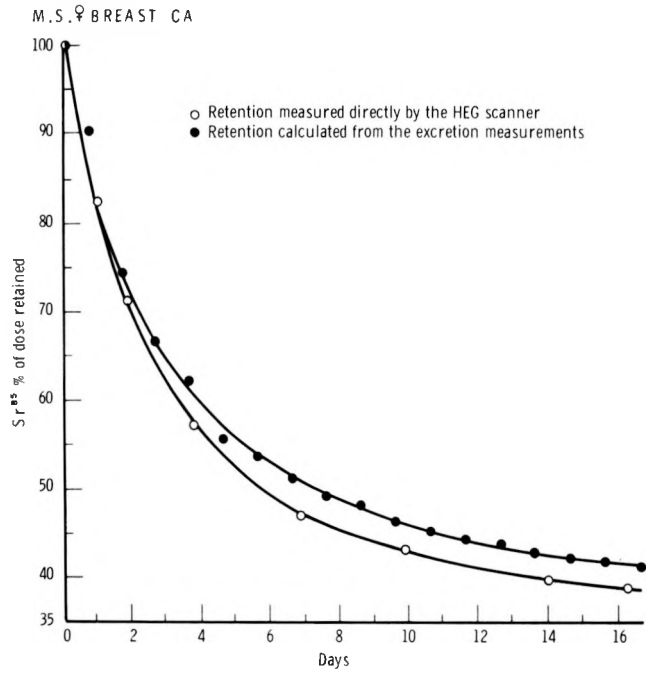

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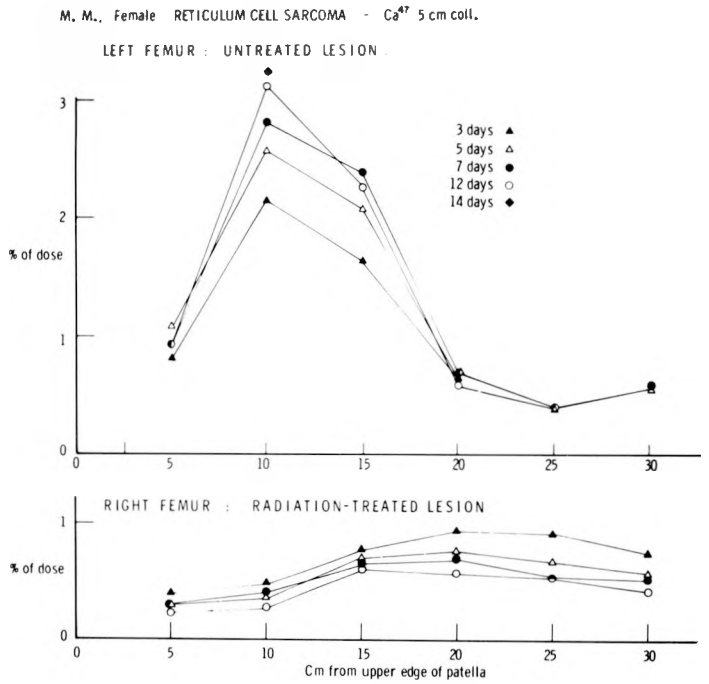

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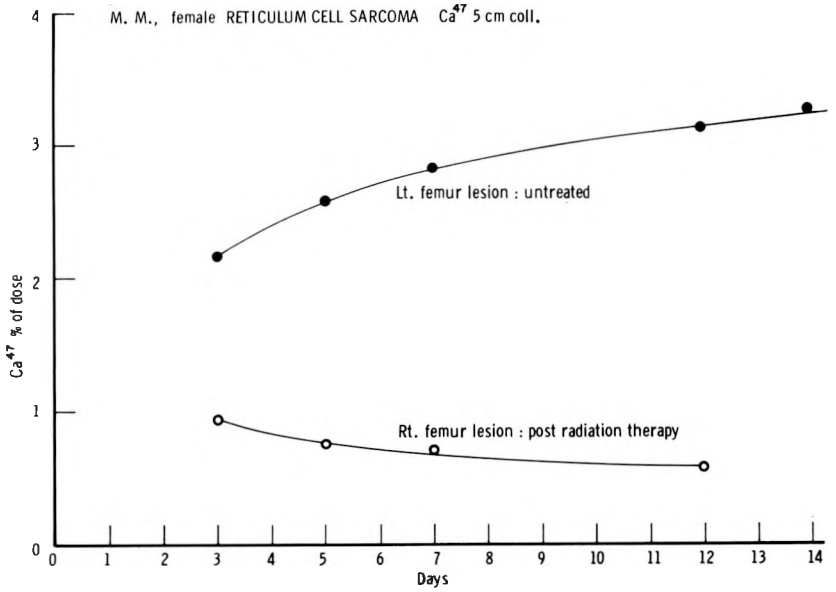

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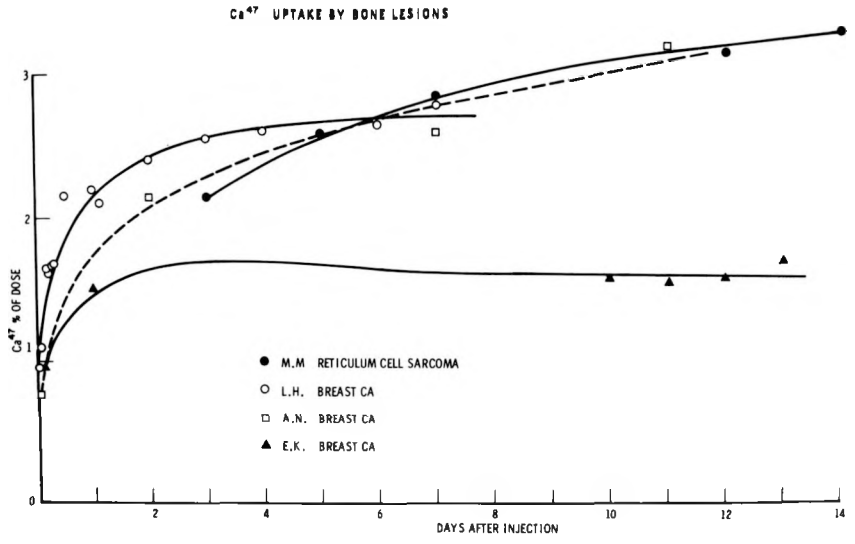

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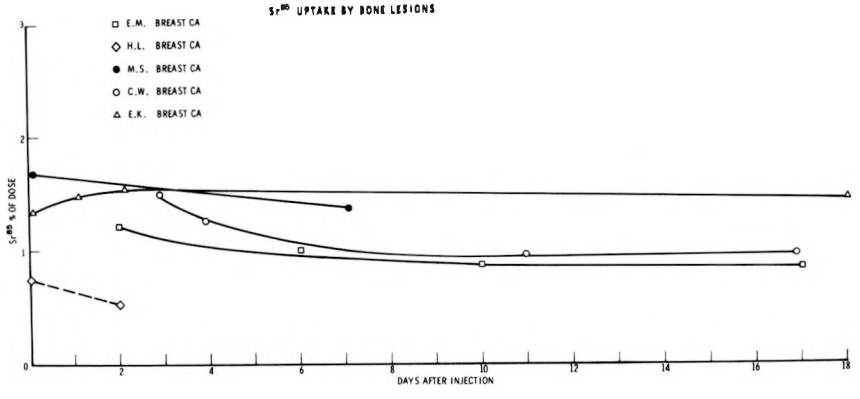

FIGURE 8