PROGRESS REPORT

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METABOLISM OF ^{90}Sr AND OF OTHER ELEMENTS IN MAN

July 1, 1974 to June 30, 1975
extended without additional funding to March 31, 1976

AND

RENEWAL PROPOSAL

April 1, 1976 to March 31, 1977

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The proposed work will be carried out in the Metabolic Research Ward of the Veterans Administration Hospital, Hines, Illinois under the administrative supervision of the Loyola University of Chicago Stritch School of Medicine, Maywood, Illinois.

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DATE: December 31, 1975

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This Progress Report covers the work conducted under Contract AT(11-1)-1231 for the Contract period July 1, 1974 to June 30, 1975, extended without additional funding to March 31, 1976. The program for the proposed studies and the budget for the Contract period April 1, 1976 to March 31, 1977 are herewith submitted.

The proposed studies will be carried out in collaboration with the Health and Safety Laboratories of the U. S. Energy Research and Development Administration, New York, New York.

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I. ABSTRACT

Trace element studies have been carried out under strictly controlled dietary conditions in adult males during different calcium intakes. These studies are listed in Table 1. Complete metabolic balances of cadmium, copper, zinc, lead, manganese, and nickel were determined in each 6-day metabolic period by analyzing the constant diet and the urinary and fecal excretions of these "naturally" occurring elements. No additional stable or radioactive trace element was given. Complete metabolic balances of calcium and phosphorus were also determined. The analyses of cadmium, copper, lead, manganese, and nickel, determined during the low calcium intake, have been completed. The trace element analyses of the remaining eight studies are in progress. This delay is due to carrying out an intercomparison study of trace element analyses in several participating laboratories because of the known difficulties involved in the reliability, accuracy, and precision of the methodology of trace element analysis. The zinc balances determined in all 10 studies have been completed. The studies carried out in the present Contract year have shown that the balances of cadmium and nickel ranged from slightly negative to slightly positive values, those of manganese ranged from slightly negative to very positive values, while the copper and lead balances were positive. In addition to the trace element studies, radiostrontium studies (^{85}Sr) have been carried out in man in order to complete previously initiated investigations. Extensive data obtained on zinc metabolism in man, on ^{210}Pb and ^{210}Po metabolism in man, and rare earth studies have been published and/or summarized for publication during the current Contract year.

II. PROGRESS REPORT

A. Trace Element Studies in Man

Table 2 shows the data of the cadmium, lead, and copper balances which were determined during a low calcium intake of about 200 mg/day.

Cadmium balances: These balance studies show that the dietary cadmium content averaged 10.3 µg/day. On this intake cadmium was predominantly excreted via the intestine and the urinary cadmium excretion represented roughly one-third of the fecal cadmium excretion. In Patient 1, the urinary and fecal cadmium excretions fluctuated little except that the fecal cadmium excretion was higher and the urinary cadmium excretion was lower in one 6-day study period than in the other five 6-day study periods. The average cadmium balance of Patient 1 was negative, -1.7 µg/day. In Patient 2, both the urinary and fecal cadmium excretions were lower than those of Patient 1 and the average cadmium balance for 30 days was slightly positive, +1.5 µg/day.

Lead balances: In Patient 1, during an average dietary lead intake of about 210 µg/day, the urinary lead excretion was about 30 µg/day in four of the five 6-day study periods, and was highest during the first 6-day study period. The lead excretion in stool fluctuated little from one study period to the next. The average lead balance of +125 µg/day for Patient 1 was unexpectedly high and reflected the low lead excretions in stool. The lead balances of Patient 2 were similar to those of Patient 1 showing both low urinary and fecal excretion values of lead, averaging 36 and 42 µg/day, respectively, and resulting in a positive lead balance of +132 µg/day.

Copper balances: The dietary copper content (Table 2) varied little from one 6-day period to the next and the copper intake of the two patients averaged 897 µg/day. The main pathway of the copper excretion was via the intestine. The copper excretions in stool of Patient 1 were greater than those of Patient 2 and the fecal copper excretion averaged 782 µg/day for Patient 1 and 718 µg/day for Patient 2. The urinary copper excretion varied little in the different 6-day study periods and the excretion values were similar for both patients averaging 90 µg/day for Patient 1 and 85 µg/day for Patient 2. The copper balances of the two patients were similar, the copper balance of Patient 1 was essentially in equilibrium, +25 µg/day, and was more positive for Patient 2, +94 µg/day.

Table 3 shows data of the manganese and nickel balances of Patients 1 and 2 during a low calcium intake of about 200 mg/day:

Manganese balances: During an average dietary manganese intake of about 2232 µg/day the urinary manganese excretion of the two patients was very low in the individual 6-day study periods ranging from 8-13 µg/day in Patient 1 and from 6-18 µg/day in Patient 2, averaging 11 µg/day and 13 µg/day for the two patients, respectively. The manganese excretions in stool differed considerably in the two patients and ranged from 2100-2400 µg/day for Patient 1 with an average of 2260 µg/day and were considerably lower for Patient 2, ranging from 928-1663 µg/day and averaging 1240 µg/day. Due to the marked difference in the stool manganese excretion, the manganese balances of the two patients differed greatly: due to the high stool manganese excretion the manganese balance of Patient 1 was slightly negative, -39 µg/day, while the low fecal manganese excretion of Patient 2 resulted in a highly positive manganese balance of an average of +979 µg/day.

Nickel balances: The nickel balances of the two patients listed in Table 3 shows that the dietary nickel intake of the same two patients averaged 269 µg/day. The excretions of nickel in urine differed somewhat in the two patients and some fluctuations of these excretions were noted in both patients from one study period to the next. The ~~average~~ urinary nickel excretion of Patient 1 averaged 164 µg/day and the excretions of Patient 2 averaged 133 µg/day. The excretions of nickel in stool of Patient 1 were greater than those of Patient 2. These excretions fluctuated in both patients from one 6-day period to the next and averaged 170 µg/day for Patient 1 and 117 µg/day for Patient 2. The average nickel balance of Patient 1 was slightly negative, -65 µg/day, and was slightly positive, +19 µg/day, for Patient 2.

Zinc balances: Table 4 shows data of the zinc balances determined in two patients during a low calcium intake. On an average dietary zinc intake of 14.8 mg/day and a low calcium intake averaging 229 mg/day, the urinary zinc excretion was low and very constant from one 6-day period to the next. Most of the ingested zinc was passed in stool and the fecal zinc excretions fluctuated somewhat from one study period to the next. The zinc balance of the two patients averaged +1.3 mg/day and +2.8 mg/day, respectively, the difference in the zinc balance being due to differences in dietary zinc intake of Patients 1 and 2.

Table 5 shows data of the zinc balances of all five patients determined during different calcium intakes. The zinc balances during the low calcium intake averaging 229 mg/day have been described in detail in Table 4 and the average values obtained in these studies

are listed in Table 5. In the zinc balance studies carried out in four patients during a normal calcium intake of an average of 791 mg/day, the dietary zinc intake was similar to that during the low calcium intake and averaged 15.6 mg/day. The average urinary zinc excretion of 1.0 mg/day was slightly greater than during the low calcium intake, the fecal zinc excretion fluctuated from patient to patient, averaging 13.6 mg/day, and the average zinc balance was positive, +1.0 mg/day. In the zinc balance studies carried out in four patients during a higher calcium intake of an average of 1289 mg/day, the dietary zinc intake was similar to that during the 800 mg calcium intake and averaged 15.3 mg/day. Again, the fecal zinc excretions reflected the zinc intake and these excretions were comparable to those during the 800 mg calcium intake. The urinary zinc excretion in this study was also in the normal range and averaged 0.8 mg/day and the average zinc balance was in equilibrium, +0.6 mg/day.

The difficulties in trace element analysis of human biological samples are well recognized. As a result of this problem widely varying results of trace element balances have been reported (1,2). In order to evaluate this aspect, samples of the diet, urine, and stool were sent to several laboratories for comparative trace element analysis of Cd, Cu, Pb, Mn, Ni, and Zn. The great variability of the results obtained are shown in Table 6. Each of these laboratories claim that their analyses determined under strictly standardized conditions are highly accurate and precise.

As the trace element studies were carried out during different calcium intakes metabolic balances of <u>calcium and phosphorus</u> were determined during these calcium intakes in order to characterize the calcium metabolism status of these patients. Table 7 shows these data. During the low calcium intake

of an average of 229 mg/day the fecal calcium approximated the calcium intake or slightly exceeded this intake. The urinary calcium of one of the two patients was in the low normal range, 68 mg/day, while the urinary calcium of the second patient was very low, 11 mg/day. The calcium balances were negative during the low calcium intake, -51 mg/day for Patient 1 and -27 mg/day for Patient 2. These negative calcium balances are normal for this low calcium intake. The phosphorus balance data show that the major pathway of phosphorus excretion is via the kidney, while the fecal phosphorus excretion was about half the urinary phosphorus excretion. The phosphorus balance was slightly positive.

During the normal calcium intake of an average 791 mg/day (Table 7), the urinary calcium of the four patients studied was higher than during the low calcium intake and averaged 240 mg/day. The fecal calcium represented 76% of the calcium intake and the calcium balances of two of the four patients were in equilibrium and those of the other two patients were negative. The average calcium balance of the four patients was -53 mg/day. On a phosphorus intake of an average of 1130 mg/day the pattern of the urinary and fecal phosphorus excretions were in the expected range and the average phosphorus balance was -62 mg/day, in agreement with the average negative calcium balance.

During an intermediate calcium intake averaging 1289 mg/day (Table 7), the urinary calcium of the four patients studied ranged from very low levels of 22 mg to moderately high levels of 250 mg/day and the average urinary calcium of 149 mg/day was 100 mg lower than during the 800 mg calcium intake. The stool calcium during the 1300 mg calcium intake was greater than during the 800 mg calcium intake and represented 83% of the calcium intake. It

appears that most of the ingested calcium passed unabsorbed in stool. The average calcium balance was slightly positive, +64 mg/day. During an average phosphorus intake of 1542 mg/day (Table 7), the urinary phosphorus excretion as well as the excretion of phosphorus in stool were 100 mg greater than during the 800 mg calcium intake, however, the increase in the phosphorus intake during the higher milk intake resulted in a positive phosphorus balance, averaging +134 mg/day. Due to the higher phosphorus intake during this calcium intake, the average urinary phosphorus was relatively high and averaged 940 mg/day, the fecal phosphorus excretion was 120 mg greater than during the 800 mg calcium intake and the phosphorus balance was positive, +134 mg/day.

B. Short Description of Recently Published or Presented Material

I. Trace Element Studies

1. Studies of zinc metabolism

Studies of zinc metabolism were carried out in man under strictly controlled metabolic and dietary conditions. Metabolic balances of zinc were determined for several weeks by analyzing the dietary intake of zinc and the excretions of zinc in urine and stool.

a. Studies of zinc metabolism in normal man and in patients with neoplasia

This study has shown that the amount of zinc necessary to attain zinc equilibrium is about 12 mg/day under normal conditions. The studies have demonstrated that the loss of zinc during weight reduction induced by a low

calorie intake is as great as the loss of zinc induced by total calorie restriction during total starvation. These studies have also shown that the plasma level of zinc does not reflect the dietary intake of zinc nor the continued loss of zinc. The marked loss of zinc during weight loss was not associated with a decrease of the plasma zinc level but rather with an increase of these levels. During repletion of individuals who are in a state of malnutrition, the zinc balance may be very positive during a normal zinc intake and this balance remains strongly positive until nutritional repletion is attained. The studies have also shown that the metabolism of zinc may differ greatly in normals and in patients with certain types of neoplasia.

This study has been published: Spencer, H., Osis, D., Kramer, L., and Wiatrowski, E. Studies of zinc metabolism in normal man and in patients with neoplasia. In: Clinical Applications of Zinc Metabolism. W. J. Pories, W. H. Strain, J. M. Hsu, and R. L. Woosley (eds.); Charles C. Thomas (publ.); Springfield 1974 pp. 101-112.

b. Certain aspects of zinc metabolism in man

Some of the results obtained in the studies of zinc metabolism in man were presented at the Fall Meeting of the American Physiological Society in Albany, New York, August 1974. These studies emphasized that the main pathway of zinc excretion in man is via the intestine, that the urinary zinc excretion is low, about 0.5 mg/day, and that increasing the dietary zinc intake from 12 mg/day to 22 mg/day or lowering it to 7 mg/day did not alter the urinary zinc excretion. The changes in urinary zinc excretion, therefore, do not

always reflect the zinc intake. The urinary zinc does, however, increase when large amounts of supplemental zinc are given.

The summary of this study was published: Spencer, H., Osis, D., Wiatrowski, E., and Coffey, J. Certain aspects of zinc metabolism in man. The Physiologist, 17:334, 1974 (ENCLOSURE 1).

c. Dietary zinc intake in man

Prior to carrying out zinc balance studies in man, it was necessary to determine the exact zinc content of the daily diet. In these studies the total dietary zinc intake, as well as the zinc content of individual meals was determined in constant metabolic diets and in regular hospital diets. The total zinc content of the hospital diets varied greatly, ranging from 7.0 to 16.3 mg per day and averaging 11.3 mg/day, due to differences in the composition of these diets, depending on the protein content of the diet. In contrast, the zinc content of the metabolic diets varied little and ranged from 11.6 to 12.7 mg per day. The study has shown that foods and diets high in protein are also high in zinc while those high in carbohydrate content have a low zinc content.

This study has been published: Osis, D., Kramer, L., Wiatrowski, E., and Spencer, H. Dietary zinc intake in man. Amer. J. Clin. Nutr., 25:582-588, 1972 (ENCLOSURE 2).

d. Intestinal absorption and secretion of ^{65}Zn in the rat

This study demonstrated that the absorption of zinc from the intestine is rapid in the intact rat as well as from isolated, ligated intestinal loops. ^{65}Zn was maximally absorbed from the duodenum, the absorption from the other portions of the small intestine being considerably less. The secretion of ^{65}Zn from the vascular space into the intestine was also rapid and most of the secreted ^{65}Zn was found in the small intestine.

This paper was presented at the meeting of the 2nd International Symposium on Trace Element Metabolism in Animals, Madison, Wisconsin, in June 1973 and was subsequently published: Methfessel, A. H. and Spencer, H. Intestinal absorption and secretion of ^{65}Zn in the rat. In: Trace Element Metabolism in Animals-2. W. G. Hoekstra, J. W. Suttie, H. E. Ganther, and W. Mertz (eds.); University Park Press (publ.); Baltimore 1974 pp. 541-543 (ENCLOSURE 3).

e. Metabolic balances of ^{210}Pb and ^{210}Po at natural levels

An extensive study has been carried out in adult man under strictly controlled dietary conditions on the dietary intake and excretions of ^{210}Po and ^{210}Pb. Also, the intake of these radioisotopes, due to inhalation from the atmosphere and to smoking of cigarettes, was estimated. The dietary intake of ^{210}Pb averaged 1.25 pCi/day and that of ^{210}Po averaged 1.62 pCi/day. Both nuclides were mainly excreted via the gastrointestinal tract and the urinary excretion was very low. The total excretions of ^{210}Pb and ^{210}Po were greater than the dietary intake and the overall balances were -0.24 pCi and -0.34 pCi/day for the two nuclides, respectively. The studies have also shown that changes of the calcium intake did not significantly affect the ^{210}Pb balances while the ^{210}Po balances were more negative during higher calcium intakes than during a low calcium intake. The intake of other compounds which affect the metabolism of calcium, such as sodium fluoride and diuretic compounds, did not affect the urinary or fecal excretions of the two radionuclides.

The data were summarized and a manuscript: Spencer, H., Holtzman, R. B., Kramer, L., and Ilcewicz, F. H. Metabolic balances of ^{210}Pb and ^{210}Po at natural levels, has been submitted for publication (ENCLOSURE 4).

f. Studies of electrophoretic binding of radioactive rare earths

An in vitro study has been carried out on the binding of the radioactive rare earths ^{140}La, ^{91}Y, ^{153}Sa, and ^{46}Sc to protein components of human serum and of mouse serum utilizing paper electrophoresis and radioassays. ^{140}La migrated with the γ-globulin, ^{153}Sa with all globulin fractions, ^{46}Sc with the β-globulin, while ^{91}Y did not migrate with any of the protein fractions.

A manuscript: Rosoff, B. and Spencer, H. Studies of electrophoretic binding of radioactive rare earths, Health Phys., 28:611-612, 1975, was published (ENCLOSURE 5).

g. Effect of carrier and of the metal/chelate molar ratio on the metabolism of radioactive yttrium

A study of the tissue distribution and excretion of yttrium chelates, labelled with radioyttrium, of varying yttrium to chelate molar ratios, and a study of the effect of the concentration of the yttrium carrier on the tissue uptake of radioyttrium was carried out in mice. Yttrium was given in chelated form as the strong chelates Y-DTPA, Y-EDTA, Y-CDTA, and as the weak chelates Y-NTA, Y-HEEDTA, and Y-HEIDA. The metal to chelate molar ratios were 1:1.2 to 1:5 and the amounts of yttrium carrier used were 0.03 mg and 0.001 mg. The tissue distribution and urinary excretion of the yttrium chelates depended on the stability constants at both metal to chelate molar ratios. However, marked differences in the excretion and tissue distribution were noted when chelates of intermediate or low stability were used at the two molar ratios.

The details of these findings were presented at the Fifteenth Annual Hanford Life Sciences Symposium, Richland, Washington, in September 1975 and a paper: Rosoff, B. and Spencer, H. Effect of carrier and of the metal/chelate molar ratio on the metabolism of radioactive yttrium, is in press and will be published in the Proceedings of this Symposium (ENCLOSURE 6).

II. ^{85}Sr Studies in Man

The results obtained in several ^{85}Sr absorption studies were summarized and were presented at national and international scientific meetings. Two aspects of radiostrontium metabolism were summarized, namely, 1) the effectiveness of various conditions and compounds in modifying or inhibiting the absorption of radioactive strontium, and 2) the changes in radiostrontium metabolism induced by the use of certain hormones and during certain aberrations of the normal hormonal status in man.

a) Factors influencing the intestinal absorption of radiostrontium in man

The effect of several compounds on decreasing the intestinal absorption of radiostrontium has been previously investigated in this Research Unit in man under strictly controlled dietary conditions, using orally administered tracer doses of ^{85}Sr. The plasma levels, urinary and fecal excretions of ^{85}Sr were assayed and the absorption of ^{85}Sr was determined from the fecal ^{85}Sr excretions. The study conditions were found to be of greatest importance in influencing the changes of the absorption of ^{85}Sr as the presence or absence of food greatly affected the absorption of the tracer from the intestine.

The absorption of ^{85}Sr was very high in the absence of food and could be decreased by a factor of 2 to 3 by the intake of either an entire breakfast meal or by the ingestion of the small amounts of calcium or of phosphorus contained in the breakfast meal. The daily intake of relatively large amounts of calcium, given in divided doses with food, had little effect on the absorption of ^{85}Sr and the daily intake of phosphate given in the same manner decreased the absorption of ^{85}Sr by 20-25%. In contrast, a single relatively large dose of 0.5 to 1 gm calcium or a single dose of 1.2 gm phosphate given either with or without food decreased distinctly the absorption of ^{85}Sr. However, this effect was greater when these amounts of calcium or phosphorus were given in the absence of food. Aluminum phosphate gel was most effective in decreasing the absorption of ^{85}Sr, by an average of 87%, while aluminum alone given as $Al(OH)_3$ was about 50% as effective as aluminum phosphate gel. Orally administered stable strontium decreased markedly the total body retention of ^{85}Sr but this decrease was not due to a decrease of the intestinal absorption of ^{85}Sr but to an increase of the urinary ^{85}Sr excretion.

The results of these studies were presented at the 5th International Congress of Radiation Research in Seattle, July 1974.

b) Effect of hormones and of the hormonal status on radiostrontium metabolism in man

As certain hormones affect the intestinal absorption and excretion of calcium in man these hormones may also affect certain parameters of radiostrontium metabolism. The effect of the functional state of the thyroid gland, of the administration of thyroid extract, parathyroid extract,

corticosteroids, and of male and female sex hormones was investigated. These studies have shown that in a state of increased thyroid function, in hyperthyroidism, the intestinal absorption of ^{85}Sr was in the low normal range and that it increased markedly after the correction of the hyperthyroid state. This was demonstrated by the marked increase of the ^{85}Sr plasma levels and by the decrease of the fecal ^{85}Sr excretions in this state of thyroid function (Figs. 1-3). Similar results were obtained following the correction of a hypermetabolic state which was due to the administration of thyroid extract. Due to the increased intestinal absorption of ^{85}Sr and also of ^{45}Ca in the state of normal thyroid function (euthyroid state) following the correction of the hyperthyroidism, the urinary excretion of both ^{85}Sr and ^{45}Ca increased markedly (Fig. 4).

In another state of endocrine dysfunction, in hypoparathyroidism, the intestinal absorption of ^{85}Sr was very low and the administration of 200 Units of parathyroid extract (PTE), given daily for 42 days, did not change the absorption of ^{85}Sr from the intestine. The ^{85}Sr absorption values were 12.2% in the control study and 11.6% during the administration of parathyroid extract (PTE) in Patient 1 and the corresponding values for Patient 2 were 10.2% and 8.5%, respectively, the normal values for the absorption of ^{85}Sr ranging from 20% to 30% of the administered dose. Fig. 5 shows that the administration of PTE did not correct this low absorption as the ^{85}Sr plasma levels did not increase and, in fact, these levels were actually lower than in the control study. This was further corroborated by the lack of change of the high fecal ^{85}Sr excretion. However, the urinary ^{85}Sr excretion increased during the administration of PTE. This increase was, therefore, not a result of increased intestinal absorption of ^{85}Sr but was due to the action of PTE on bone resulting in the release of calcium and phosphorus.

Changes in ^{85}Sr metabolism were also noted in patients with spontaneous hyperparathyroidism and following the correction of this abnormal state of parathyroid function. Fig. 6 shows that the urinary ^{85}Sr excretions differed markedly in these states of parathyroid function. The urinary ^{85}Sr excretion was high, 20% of the dose, following the intravenous administration of ^{85}Sr in the hyperparathyroid state and these excretions decreased sharply at the different time intervals in the post-operative phase. These excretions were lowest, 2%, in the first post-operative month and increased gradually in a 12-month period, however, these excretions reached only a maximum of 6% vs. 20% in pre-operative phase. The changes in ^{85}Sr excretion in the different phases of parathyroid function were associated with corresponding changes of the urinary calcium excretion (Fig. 6).

Corticosteroids are known to increase the urinary calcium excretion, however, not all corticoids result in this increase. Those compounds which increased the urinary calcium also increased the urinary ^{85}Sr excretion while corticosteroids which did not affect the urinary calcium did not change the urinary ^{85}Sr excretion. The intestinal absorption of ^{85}Sr and ^{45}Ca was somewhat lower during the administration of the corticosteroid than in the control studies.

Estrogens decreased the urinary excretion of both ^{85}Sr and of calcium (Fig. 7), while androgens had a variable effect. The decrease of the urinary calcium excretion during estrogen administration is most likely a result of decreased bone resorption. However, neither of these two hormonal compounds affected the intestinal absorption of ^{85}Sr. Fig. 8 shows data of the effect of an estrogenic compound on ^{85}Sr absorption. The ^{85}Sr plasma levels and the fecal ^{85}Sr excretions were similar during the administration of 15 mg diethyl-stilbestrol per day and in the control study.

The overall conclusion of the study of the effect of hormones and of the hormonal status on radiostrontium metabolism is that the intestinal absorption of ^{85}Sr and the excretion of ^{85}Sr in man is altered by those hormones which induce similar changes in calcium metabolism. The data obtained in this study have been presented at the Annual Meeting of the Radiation Research Society in Miami Beach, May 1975.

The summary of this study was published: Spencer, H. Effect of hormones and hormonal status on radiostrontium metabolism in man. Radiat. Res., 62:578, 1975.

III. PLANS FOR FUTURE STUDIES AND PROPOSED WORK

It is planned to continue the studies of trace element metabolism in man as previously proposed. These investigations will include studies of the metabolism of cadmium, copper, lead, and zinc, manganese and nickel. It is also planned to carry out certain ancillary radiostrontium studies in order to complete some of the previously initiated studies.

A. Trace Element Studies

Metabolic balances of cadmium, copper, zinc, lead, manganese, and nickel will be determined in man under the following study conditions:

a) in control studies during a normal calcium intake;

b) during the addition of calcium;

c) during the addition of phosphorus;

d) during the addition of both calcium and phosphorus;

e) during a low calcium intake;

f) during the intake of added zinc.

Lead balances will also be determined during the addition of calcium, given in two different forms, namely, as calcium gluconate tablets and as milk, and during the intake of different levels of dietary protein.

Methods to be used

1. Cadmium

a) Cadmium balances will be determined during a constant dietary intake of the metabolic diet having a normal calcium content of 800 mg per day and a phosphorus content of 1200 mg per day. These balances will be determined by analyzing the dietary intake of cadmium and the urinary and fecal excretions

of cadmium. Representative aliquots of the diet and aliquots of 6-day collections of urine and stool will be analyzed in each 6-day metabolic period. The cadmium balances will be determined for several weeks. Cadmium will be analyzed by atomic absorption spectroscopy.

b) The effect of a higher calcium intake on the cadmium balance will be studied by determining cadmium balances as outlined above during a high calcium intake of 2000 mg per day which will be achieved by adding calcium gluconate tablets to the constant diet.

c & d) As the metabolism of calcium may be affected by the amounts of phosphate in the diet, cadmium balance studies will be carried out during the addition of phosphate as well as during the addition of both calcium and phosphate to the constant diet. The phosphorus intake will be increased from 1200 mg/day in the control study to 2000 mg/day in the experimental study. When calcium and phosphorus will be used together the calcium intake will be increased to 2000 mg/day and the phosphorus intake will also be raised to 2000 mg/day. The high phosphorus intake will be due to the addition of glycerophosphate to the constant diet.

e) The results obtained in the cadmium balance studies carried out during the different calcium and phosphorus intakes will be compared with those obtained during a low calcium intake of 200 mg/day and a relatively low phosphorus intake of 800 mg/day.

f) The effect of zinc on the cadmium balance will be studied by determining cadmium balances during supplementation of the constant diet with added zinc given as zinc sulfate tablets. The dietary intake of zinc

in the control study will be 14-15 mg per day. During zinc supplementation the zinc intake will be raised to 100 mg per day. The cadmium balances will be continued for several weeks during zinc supplementation and the results will be compared with those obtained in the corresponding control studies.

2. Copper

Metabolic balances of copper will be determined for several weeks under strictly controlled dietary conditions during a normal calcium and phosphorus intake (control studies). The copper balances will be determined by analyzing representative aliquots of the diet in each 6-day metabolic period and by analyzing aliquots of 6-day collections of urine and stool. Copper will be analyzed by atomic absorption spectroscopy.

In studies of the effect of calcium on copper metabolism in man, copper balances will be determined during a normal calcium intake of about 800 mg/day and during a high calcium intake of 2000 mg/day. This high calcium intake will be due to the addition of calcium gluconate tablets to the normal calcium intake of 800 mg/day.

As phosphate may play a role in the copper-calcium interaction, the effect of phosphate alone and the effect of phosphate in conjunction with calcium on the copper balance will be studied. Phosphate will be added as glycerophosphate to the constant diet thereby raising the phosphorus intake from a basal intake of 1200 mg/day to an intake of 2000 mg/day. When calcium and phosphorus will be used together the calcium intake will be 2000 mg/day and the phosphorus intake will also be 2000 mg/day.

The results obtained in the studies carried out during a normal or high calcium intake will be compared with those obtained during a low calcium intake of 200 mg/day and a phosphorus intake of 800 mg/day.

In the study of the copper-zinc interaction, metabolic balances of copper will be determined during supplementation of the diet with zinc given as zinc sulfate tablets thereby raising the zinc intake from about 14 mg/day in the control study to 100 mg/day in the experimental studies.

The intestinal absorption of copper will be determined by using tracer doses of ^{64}Cu. The double tracer technique will be used, i.e., ^{64}Cu will be given orally as well as intravenously in each study phase in order to determine the intestinal absorption of copper. The plasma levels following the oral and intravenous administration of ^{64}Cu will be used to determine the absorption of ^{64}Cu based on the method for the calculation of the absorption of ^{85}Sr and of ^{47}Ca previously described in this laboratory (3).

Plasma levels of copper will be determined serially in each study phase.

3. Zinc

Metabolic balances of zinc will be determined during the intake of the constant diet having a normal calcium and phosphorus content (control studies). This diet will contain an average of 14-15 mg zinc per day. Aliquots of the diet and aliquots of urine and stool collections will be analyzed for zinc in each 6-day metabolic period by atomic absorption spectroscopy. The duration of these studies will be several weeks. Plasma levels of zinc will be determined serially.

The effect of a high calcium intake on the zinc balance will be determined. The high calcium intake fo 2000 mg/day will be attained by supplementing the diet with calcium as calcium gluconate tablets. These studies will be carried out for several weeks.

The effect of phosphate on the zinc balance will be studied by determining the zinc balances during the addition of phosphate given as glycerophosphate to the constant metabolic diet thereby increasing the phosphorus intake from an average of 1200 mg per day in the control study to 2000 mg in the high phosphate study. In another phase of the studies zinc balances will be determined during a high calcium and a high phosphorus intake, i.e., during an intake of 2000 mg calcium and of 2000 mg phosphorus per day. In all study phases the zinc balances will be determined in each 6-day metabolic study period for several weeks.

The effect of added zinc on the excretions of zinc and on the zinc balance will be studied by adding zinc as zinc sulfate tablets to the constant metabolic diet. These zinc supplements will increase the zinc intake from about 14 mg/day to 100 mg/day. The zinc balances will be determined for several weeks during supplementation of the diet with zinc.

The data obtained in the zinc balance studies during the different calcium and phosphorus intakes will be compared with those obtained during a low calcium intake of 200 mg/day and during a relatively low phosphorus intake of 800 mg/day.

Plasma levels of zinc will be determined serially in all study phases.

Tracer doses of ^{65}Zn or preferably of the short-lived radioisotopes ^{69m}Zn or ^{72}Zn, if available, will be given orally in the different study phases. Should either of these two short-lived radioisotopes become available, the intestinal absorption of zinc will be determined from the plasma levels of these radioisotopes following a single intravenous and a single oral dose, in a manner similar to that used for the calculation of the absorption of ^{85}Sr or ^{47}Ca (3). Tracer doses of ^{65}Zn will be given to patients over the age of 50 years only.

4. Lead

Balances of lead will be determined for several weeks during a constant dietary intake having a normal calcium and phosphorus content (800 mg calcium and 1200 mg phosphorus per day). The lead content of the diet and the urinary and fecal lead excretions will be analyzed by atomic absorption spectroscopy in each 6-day metabolic period throughout the studies.

In the study of the effect of calcium on lead metabolism, balances of lead will be determined during three intake levels of calcium, namely, during a low calcium intake of 200 mg/day, a normal calcium intake of 800 mg/day, and a high calcium intake of 2000 mg/day. The low calcium intake will be that contained in the constant metabolic diet. At the 800 mg intake level of calcium a comparative study of the effect of calcium, given as milk and as calcium gluconate tablets, will be carried out. The high calcium intake of 2000 mg/day will be achieved by adding calcium gluconate tablets to the constant low calcium metabolic diet.

As phosphate may affect the metabolism of lead, metabolic balances of lead will be determined during supplementation of the constant diet with phosphate given as glycerophosphate. The phosphorus intake will be increased from an intake of 1200 mg/day in the control studies to 2000 mg in the high phosphate studies. Lead balances will also be determined during supplementation of the diet with both phosphate and calcium, i.e., during a calcium intake of 2000 mg and during a phosphorus intake of 2000 mg/day.

The results obtained in the studies carried out during the different calcium and phosphorus intakes will be compared with those obtained during a low calcium intake of 200 mg/day and during a relatively low phosphorus intake of 800 mg/day.

The effect of protein on the metabolism of lead will be studied by determining lead balances during a normal, low, and high protein intake. The normal dietary protein intake will be 1 gm/kg body weight, the low protein intake 0.5 gm/kg, and the high protein intake 2 gm/kg.

5. Manganese and nickel balances will be determined along with the other trace element studies during the different study conditions outlined above.

B. Radiostrontium Studies

The following radiostrontium studies will be carried out in order to complete investigations which have been previously initiated in this Research Unit:

1) studies of the effect of aluminum phosphate gel on the intestinal absorption of radioactive strontium during a normal calcium intake;
2) continuation of the study of the effect of the combined use of orally administered ammonium chloride and of intravenously administered stable strontium on the removal of ^{90}Sr which is chronically ingested with food due to nuclear fallout.

Methods to be used

re 1: Studies of the effect of aluminum phosphate gel on the intestinal absorption of radioactive strontium during a normal and high calcium intake: The studies will be performed during two levels of a normal calcium intake. In the first study a calcium intake of 800 mg/day will be used and in the second study a higher calcium intake of 1200 mg calcium will be given. A single tracer dose of ^{85}Sr will be given orally at the midpoint of a constant

breakfast during the intake of these calcium intakes and the absorption of ^{85}Sr will be determined from the fecal ^{85}Sr excretions. Urinary excretions of ^{85}Sr and plasma levels of this radioisotope will also be determined. Following the completion of this control study the effect of aluminum phosphate gel will be tested. A single dose of 100 ml aluminum phosphate gel will be given immediately prior to the oral dose of ^{85}Sr during the same calcium intakes and the absorption of ^{85}Sr will be determined in the same manner as in the control study.

re 2: Study of the effect of the combined use of orally administered ammonium chloride and of intravenously administered stable strontium on ^{90}Sr excretions: Several of these studies have been previously reported from this Research Unit. Ammonium chloride (9 gm/day) was given orally on 6 consecutive days and stable strontium was infused intravenously on the last 3 days of the ammonium chloride administration. This study has shown that ammonium chloride increased the urinary ^{90}Sr excretion and that the combined use of both ammonium chloride and of intravenous stable strontium was more effective than the use of ammonium chloride alone. It is planned to carry out additional studies in order to determine whether higher excretions of ^{90}Sr can be achieved by a modification of the protocol used in the previous study. In the proposed study, 9 gm ammonium chloride will be given orally daily for 6 days and the intravenous infusions of 600 mg stable strontium will also be given daily on these 6 days instead of for 3 days as has been done in the previous study. The dietary ^{90}Sr intake and the excretions of ^{90}Sr in urine and stool and the ^{90}Sr balances will be determined in each 6-day period in the control and experimental phases.