JOURNAL OF NUCLEAR MEDICINE 5:407-416, 1964

Use of a Whole Body Counter in Turnover Studies with Ca^{47}.[1,2]

Thornton Sargent, Ph.D., John A. Linfoot, M.D.,
Henry Stauffer, M.D., and John H. Lawrence, M.D., D.Sc.

Berkeley, California

INTRODUCTION

A number of investigations of calcium metabolism have been reported by workers using Ca^{45}, Ca^{47}, Sr^{85}, and stable strontium (1,2,3,4). Models have been proposed and mathematical treatments developed to describe the dynamics of calcium by Bauer *et al* (5), Heaney and Whedon (6) and others. Aubert and Milhaud (7) have maintained that the simplified models of these workers were incomplete chiefly because they failed to take into account the component of serum calcium disappearance which does not appear until the seventh to the eighteenth day after injection. They demonstrated that this simplification can lead to errors of 35 to 76 per cent in measurement of the size of the calcium pool, and 18 to 38 per cent in the rate of disappearance from the pool.

In any system described by a sum of exponential functions, if one cannot measure the slowest rate constant of the system, an error will be introduced into the determination of all the others. The magnitude of the error will depend on the coefficient and the rate constant of the unknown component. One cannot be certain in fact that there is not still another component with a longer turnover time. Cohn *et al* (8) in fact have measured the turnover of Sr^{85} in a series of subjects for more than a year with a whole body counter, and found very long-lived components; the retention curve was best fitted by a power function. Considering the complex nature of recycling of bone mineral discussed by Heaney and Whedon (6), it seems possible that a power function may best describe the behavior of calcium in compartments with such complex recycling; however, a power function yields little information about rates and compartment sizes. Exponentials may be more useful in describing turnover in the miscible calcium pool and the initial incorporation into bone. Although a complete and satisfactory mathematical description has not yet been evolved, studies with calcium isotopes will continue to be valuable both in developing clinically useful measurements and in evolving a better model of calcium metabolism.

A whole body counter can be used to advantage because it can measure the calcium deposited in bone without relying on calculation from serum measurements. The results of such measurements on a small group of patients will be presented here, and the advantages of the method discussed.

---

[1]Donner Laboratory of Medical Physics and Biophysics, University of California.
[2]Work done under the auspices of the U.S. Atomic Energy Commission.

METHODS

All of the patients were ambulatory and generally were studied on an outpatient basis. Patients for whom blood, urine and stool collections were made were admitted to the Donner Pavilion for periods up to the first seven days of the study. Metabolic balance technique was not employed, but diets were controlled so that calcium intake was approximately 900 mg per day during the first week of study.

Ca^{47} from Oak Ridge, with Ca^{47}/Ca^{45} approximately 10/1, was given intravenously, buffered to pH 4-5, at doses between 2 and 25 microcuries. In some patients serial blood samples were drawn, and in some complete urine and stool collections were made for periods up to two weeks.

The whole body counter is of the Argonne type with 6 inch steel walls, a single 9⅝ inch by 4 inch NaI(Tl) crystal and 100 channel pulse height analyzer. Patients were counted both in the one meter arc position and the titlting chair position. In the one meter arc the patient lies on a couch with a radius of curvature of one meter with the crystal at the center of curvature. This geometry is usually considered to yield a count with minimum dependence upon localization of the isotope. In the tilting chair the sensitivity is five times that of the one meter arc, but with Ca^{47} changes in the location of the isotope in the body yield a retention curve different from that of the one meter arc until 12 to 18 days after

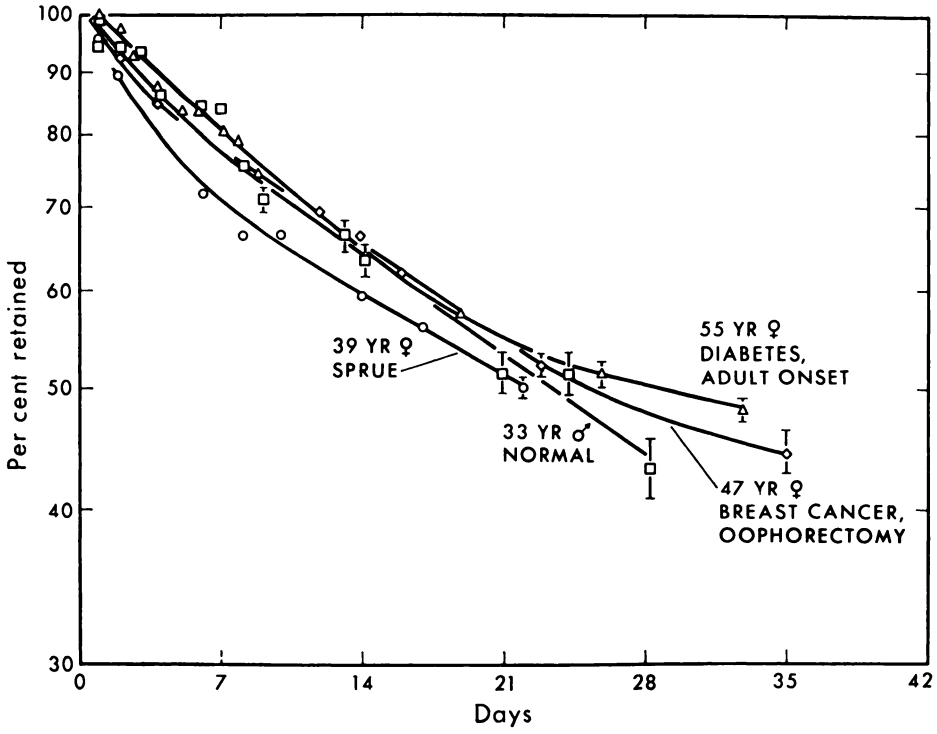

Fig. 1. Retention of whole-body radiocalcium in patients with no demonstrable bone disease, and in one completely healthy subject.

injection. After 18 days the retention curves are parallel, so that the greater sensitivity of the chair position (about 4000 cpm/μc) makes it possible to extend the retention curve beyond the time at which the 4.7 day Ca^{47} has decayed below detectable limits using the one meter arc. Simultaneous measurements on both chair and arc showed the two curves to be parallel after 12 to 18 days.

After the initial whole body count, which is performed within five minutes after injection in order to establish a 100 per cent point, the patient is counted again later in the first day, then daily for several days, and finally at intervals of seven days. A patient with high calcium retention and an initial dose of 25 μc can be counted with sufficient accuracy 42 days after injection, but excretion from the body and radioactive decay of Ca^{47} preclude counting beyond this time. Good gamma ray resolution and a pulse height analyzer are essential for accurate measurements late in the study, because all Ca^{47} so far used has contained a variable small amount of Zn^{65}, which often contributes counts in the range of the Ca^{47} photopeak to about the same degree as the patient's K^{40}. After all of the Ca^{47} has decayed, a final count must be made to obtain a background including the Zn^{65} which is subtracted from previous counts. Since only the counts under the Ca^{47} photopeak are used, there was effectively complete discrimination against other isotopes such as Sc^{47} or I^{131}.

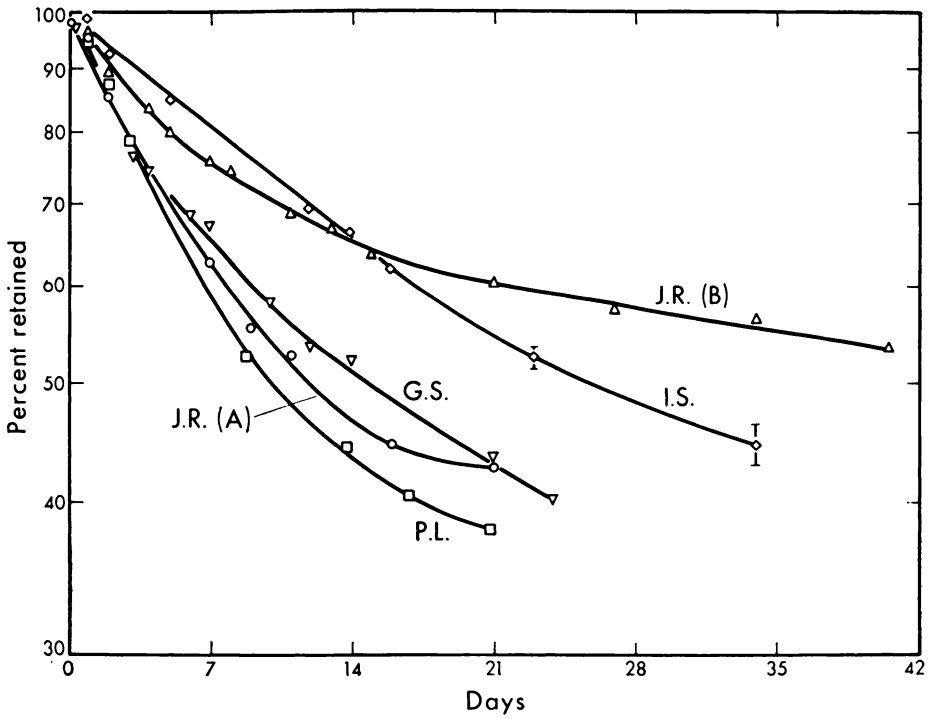

Fig. 2. Radiocalcium retention in four women with metastatic mammary carcinoma. Three patients had osseous metastases of varying degrees. Patient J.R. was studied before treatment (A) and after treatment (B). No osseous metastases were demonstrable in patient I.S.

Blood samples were counted in a standard well counter with pulse height discrimination against the Sc^{47} daughter of Ca^{47}, and against the I^{131} which some patients had received from iodine uptake tests. One liter samples of urine from each 24-hour collection, and the entire stool, were each counted on top of a 2 inch crystal, with a standard suitably diluted to correct for self-absorption. Urine data were plotted as specific activity (cpm Ca^{47}/mg Ca). Stool specimens were not assayed for calcium, so the data are plotted as cpm Ca^{47}/gm stool; since the diets were reasonably constant in calcium, this is equivalent to specific activity so far as the slope of the curves is concerned.

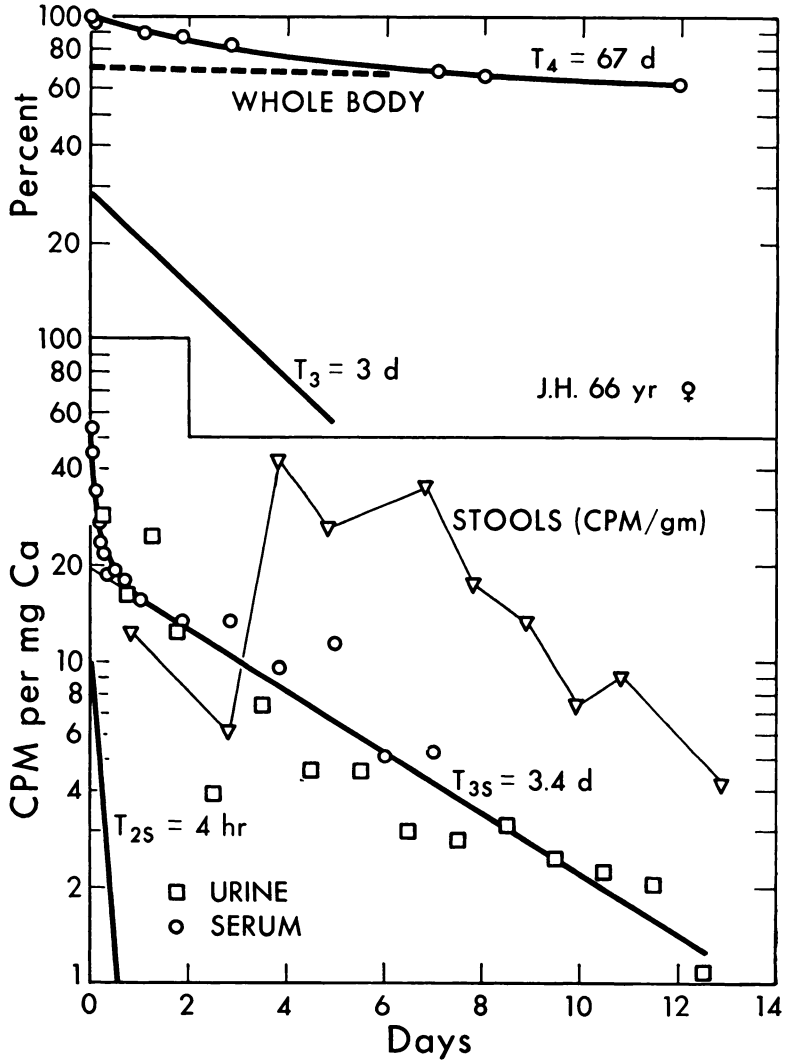

Fig. 3. Retention of whole-body radiocalcium (above) and specific activity of urine, stools and blood (below) of patient J.H., a 66 year old woman with acromegaly. These data are typical of those for the other patients with acromegaly which are summarized in Table 1.

RESULTS AND DISCUSSION

In view of the current uncertainty as to the suitability of the various mathematical models which have been proposed for calcium metabolism, no complex mathematical treatment is attempted here. A simple graphical analysis is used, based on the assumption that retention can be expressed by a sum of exponentials. The interpretations given here are with the reservation that better models will undoubtedly be derived in the future.

All of the Ca^{47} retention curves are plotted on semi-log coordinates, on which straight lines represent exponential functions. By extrapolation of a straight line fitted by hand to the last segment of the curves, a "final" slope can be subtracted from the retention curve and the exponential components resolved consecutively in the usual way. When plotted on log-log coordinates the curves are convex upward demonstrating that the data are best fitted by exponential functions rather than by a power function. This interpretation of the curves is in agreement with the results of the first 30 days of Sr^{85} data of Cohn *et al* (8), who found that after 30 days the data were best fitted by a power function to which an equivalent exponential could be fitted for purposes of resolving the exponentials of the earlier part of the curve.

I. Normal Bone Metabolism

The first group of patients, whose Ca^{47} retention curves are shown in Figure 1, were chosen because their calcium metabolism was believed to be normal. Only one of these subjects, the 33 year old male, was actually without any known disease. As can be seen, the three adult patients who had, respectively, diabetes, breast cancer (without demonstrable bone involvement), treated sprue, and the normal male do not exhibit retention curves markedly different from each other and have initial half-times of 28 to 30 days. There is another component appearing between the 24th and the 30th day; it has not been possible to measure its half-time in normals with the administration of the small amount of Ca^{47} used here, and thus no values for either component can be given.

II. Mammary Carcinoma with Osseous Metastases

Calcium retention curves from four female patients with metastatic mammary carcinoma are shown in Figure 2. All of the patients were premenopausal at the time their breast lesions were detected, and had been oophorectomized prior to receiving pituitary ablation with 900 mev alpha particles from the 184 inch cyclotron (9).

Patient I.S. was a 47 year-old housewife who had extensive cutaneous and pleural metastases. There were no obvious osseous metastases. The alkaline phosphatase, serum calcium, and 24 hour urinary calcium excretion were normal. Her calcium retention curve was considered to be normal, and is shown in Figure 1 and as I.S. in Figure 2.

Patient P.L. was a 48 year old telephone operator who had extensive osseous and hepatic metastases. She had marked hypercalciuria and developed severe

hypercalcemia (16.0 mg per cent) while undergoing pituitary irradiation, and died shortly after treatment. Our study was terminated by her death. Her calcium curve showed an extremely rapid turnover.

Patient G.S. was a 49 year old housewife with carcinoma en cuirasse, and also had metastatic involvement of the dorsal and lumbar spine. She had a normal alkaline phosphatase, mild hypercalcemia (13.0 mg per cent), and hypercalciuria. Her calcium curve was slightly less steep than the former patient, but distinctly different from patient I.S.

Two retention curves are presented on patient J.R. who was a 56 year old housewife in whom only osseous metastases were clinically detectable. The serum calcium and alkaline phosphatase were normal. The first curve (A) was obtained prior to treatment and resembles that of the preceding patients. The second curve (B) was obtained after she was in clinical remisison following combined use of pituitary irradiation and chemotherapy. There is a striking difference between the two curves. The shapes of the curves are similar, but before treatment the turnover rate of calcium was much greater. The curve after treatment probably represents deposition of calcium into areas of the skeleton which were previously being destroyed by osteolytic lesions.

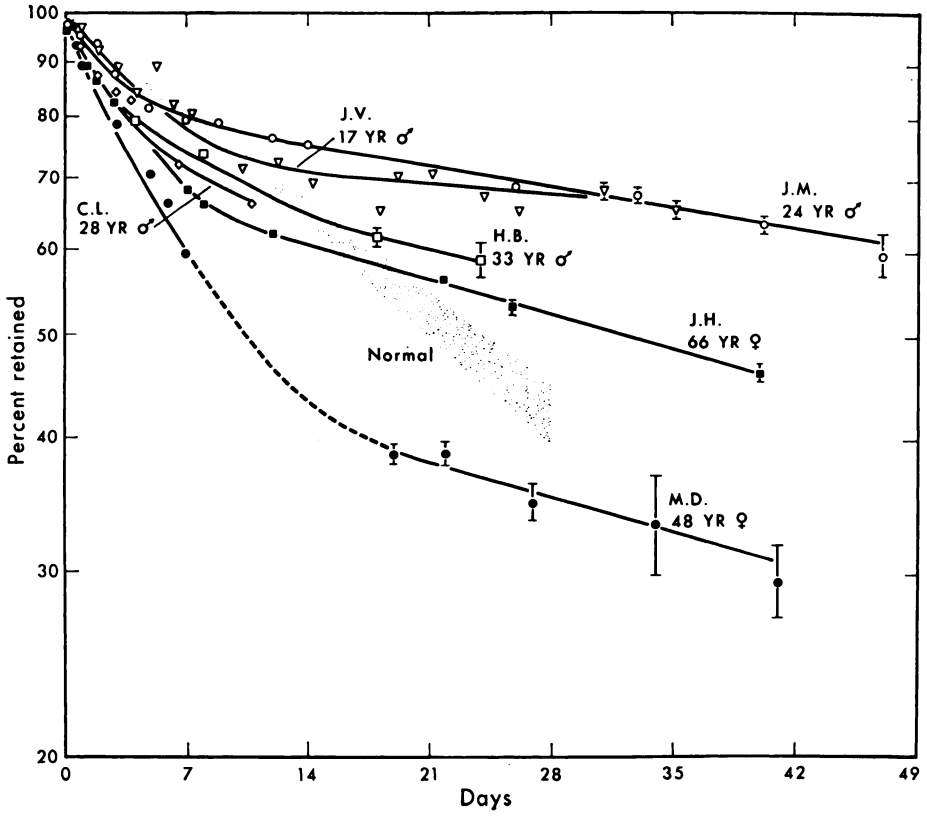

Fig. 4. Radiocalcium retention in six patients with acromegaly of varying degrees of severity.

III. Acromegaly

The profound effects of growth hormone on cartilage and enchondral bone formation are responsible for the skeletal changes which are characteristic of pituitary gigantism and acromegaly. Marked disturbance in calcium metabolism is not a clinically important feature of these disorders except in those relatively rare cases with multiple endocrine tumors and coexisting parathyroid adenomata (10). However, hypercalciuria and hyperphosphatemia are frequently seen in active acromegaly. Bauer and Aub (11), using the calcium balance technique, found hypercalciuria and negative calcium balance in four acromegalics and concluded that the degree of negative calcium balance correlated with the clinical degree of severity, and improved with pituitary irradiation. More recent studies (12) employing similar techniques showed that the hypercalciuria was associated with increased intestinal absorption of calcium, and calcium balance remained positive. Furthermore, the administration of primate growth hormone to human subjects produces hypercalciuria, but does not induce negative calcium balance (13,14). A study of total body calcium turnover should provide a clearer picture of calcium dynamics in acromegaly and also make it possible to determine whether abnormalities in calcium dynamics correlate with clinical findings.

Tracer studies of bone metabolism in acromegaly are not numerous. Stable strontium has been used to demonstrate an increase in the exchangeable calcium pools in acromegalics (15), but strontium is handled differently from calcium in the body and only short term studies can be carried out with non-radioactive tracers. In these studies, the whole body counter was used in conjunction with measurements of Ca^{47} in blood, urine, and stools on a group of six acromegalics who were considered to have varying clinical degrees of activity.

An example of the data is shown in Figure 3, in this case patient J.H. In not all cases did the specific activity curves of the blood and urine coincide as well as here, where a single curve has been fitted by hand to points of both blood and urine. In all cases, however, the blood and urine curves were parallel, as was the fecal activity when calculated per gram of stool. The serum curves were resolved graphically into a sum of exponentials, and the half times of each component are shown in Table I. The half times T_{s1}, T_{s2}, T_{s3} are calculated from serum data and correspond to the rate constants k_1, k_2, and k_3 of Bauer *et al* (5) where $k = 0.693/T$. Analyses such as these are based on the assumption that the longest half-time, T_{s3}, is the final one which is not valid. Serum activity has not been measured for periods of time sufficiently long to determine longer half-times except in one study (7).

A similar analysis of whole body retention data revealed a half-time T_3, which had approximately the same value as T_{s3} in each patient, but in addition showed a longer half-time, T_4. The half-times T_{s1} and T_{s2} represent mixing with various calcium pools in the body (5), and do not appear in the whole body retention curve, because there is no net loss from the body. T_3 is generally considered (5,6,7,8) to represent the exchangeable calcium pool of the body.

Table I also shows the patients' symptoms and the physician's total impression of the clinical degree of activity. The cases are arranged in order of their decreasing clinical severity. While the half-times T_{s3} (serum) and T_3 (whole

body) are in reasonable agreement in each patient, there is no apparent correlation with the clinical severity of the disease. Figure 4 shows the retention curve for each of these patients. All the curves have very similar shapes, and are strikingly different from the normals in Figure 1. Since the smallest slope (longest half-time T_4) is presumed to represent turnover of the tracer which has been deposited in bone (8), it can be seen by qualitative examination of the curves that the patients with acromegaly deposit far more calcium into this compartment than normals. Although, as mentioned previously, T_4 does not represent the final slope of the curve, extrapolation of its slope to injection time yields an intercept which is a fair representation of the amount of tracer which entered this compartment. The normals could not be followed long enough to see this longer half-time, but it is obvious from the curves that its intercept would be much lower than in acromegaly. This longer half-time component must exist in normals because it has been found with Sr^{85} in patients with no known bone disease (8). In contrast to the shorter half-times shown in Table I, the T_4 intercept appears to

TABLE I

COMPARISON OF CLINICAL FINDINGS WITH SERUM AND WHOLE BODY TURNOVER OF Ca^{47}.

Patient (age, sex)	*Clinical Findings*			*Serum*			*Whole Body*		
	Enlargement of Sella	*Serum Phosphorus*	*Degree of Clinical Activity*	T_{S1} *min.*	T_{S2} *hours*	T_{S3} *days*	T_3 *days*	T_4 *days*	T_4 *Intercept %*
J.M. 24 ♂	large	5.8	very active	102	8.5	2.7	1.7	102	82%
J.V. 17 ♂	large	5.2	active	45	7	5.5	5	265	73%
H.B. 33 ♂	moderate	4.2	moderately active	24	7	3.5	3.1	77	75%
C.L. 28 ♂	normal	5.4	moderately active					70	70%
J.H. 66 ♀	slight	5.2	moderate (also arthritis, osteoporosis)	16	2.8	3.4	3.0	85	67%
M.D. 48 ♀	slight	3.5	inactive (hysterectomy, arthritis)	28	3.8	3.2	4	57	49%

correlate with the severity of the disease, the higher intercepts occurring in the more severe cases.

Since the degree of activity in acromegaly is difficult to assess clinically, these preliminary studies indicate that the whole body counter not only provides a highly satisfactory method of studying abnormal calcium metabolism in acromegaly, but may also be of value in determining the degree of activity and the effectiveness of therapy. Follow-up studies of patients who have received treatment for their acromegaly should make such studies more complete.

It is highly desirable to extend the retention curves beyond the relatively short time possible with Ca^{47}, so that the curves may be analyzed accurately and completely for half-times and compartment sizes. The only feasible way to extend the retention curve is to administer Sr^{85} and Ca^{47} simultaneously to the same patients. It is known that strontium is not a perfect tracer for calcium, and that the body discriminates against strontium in favor of calcium (2). By using both isotopes simultaneously, however, it should be possible to normalize and fit the data of Sr^{85} to obtain an equivalent long-term retention curve for calcium. Future studies using both Ca^{47} and Sr^{85} are planned.

SUMMARY

A whole body counter has been used to study Ca^{47} turnover in normal subjects, in patients with metastatic breast cancer, and in patients with acromegaly. Patients with osseous metastases of breast cancer have greatly increased turnover rates. Patients with acromegaly have increased retention of calcium, presumably in bone, and the extent of increased retention seems to correlate with the severity of the disease.

BIBLIOGRAPHY

1. Krane, S. M., Brownwelt, G. L., Standbury, J. B. and Corrigan, H.: The Effect of Thyroid Disease on Calcium Metabolism in Man. *J. Clin. Invest.* **35**:874, 1956.
2. Spencer, H., Li, M., Samachson, J., and Laszlo, D.: Metabolism of Strontinum-85 and Calcium-45 in Man. *Metabolism* **9**:916, 1960.
3. Corey, K. R., Kenny, P., Greenberg, E., Payianos, A., Pearson, O. H., and Laughlin, J. S.: The Use of Calcium-47 in Diagnostic Studies of Patients with Bone Lesions. *Amer. J. Roentgenol.* **85**:955, 1961.
4. Fraser, R., Harrison, M. and Obbertson, K.: The Rate of Calcium Turnover in Bone. *Quart. J. Med.* **19**:85, 1960.
5. Bauer, G. C. H., Carlson, A. and Linquist, B.: Bone Salt Metabolism in Humans Studied by Means of Radiocalcium. *Acta Medica Scand.* **158**:143, 1957.
6. Heaney, R. P. and Whedon, G. D.: Radiocalcium Studies of Bone Formation Rate in Human Metabolic Bone Disease. *J. Clin. Endocrinol.* **18**:1246, 1958.
7. Aubert, J. P. and Milhaud, G.: A Method of Measuring the Principal Routes of Calcium Metabolism in Man. *Biochem. Biophys. Acta* **39**:122, 1960.
8. Cohn, S. H., Spencer, H. S., Samachson, J., and Robertson, J.: The Turnover of Strontinum-85 in Man as Determined by Whole Body Counting. *Radiation Research* **17**:173, 1962.
9. Lawrence, J. H., Tobias, C. A., Born, J. L., Wang, C. C. and Linfoot, J. A.: Heavy Particle Irradiation in Neoplastic and Neurological Disease. *J. Neurosurg.* **19**:717, 1962.
10. Underdahl, L. C., Woolner, L. B. and Black, B. M.: Multiple Endocrine Adenomas:

Report of 8 Cases in which the Parathyroids, Pituitary and Pancreatic Islets Were Involved. *J. Clin. Endocrinol.* 13:20, 1953.

11. BAUER, W. AND AUB, J.: Pituitary in Calcium and Phosphorus Metabolism. *J. Clin. Invest.* 20:295, 1941.
12. JACKSON, W. P. U. AND DANCASTER, C.: Mechanisms Concerning Hypercalciuria. *J. Clin. Endocrinol.* 19:658, 1959.
13. BERGENSTOLL, D. M. AND LIPSETT, M. B.: Metabolic Effects of Human Growth Hormone and Growth Hormone of other species in Man. *J. Clin. Endocrinol.* 20:1427, 1960.
14. BECK, J. C., MCGARRY, E. E., DYRENFURTH, I., MORGEN, R. O., BIRD, E. D. AND VENNING, E. H.: Primate Growth Hormone Studies in Man. *Metabolism* 9:699, 1960.
15. EISENBERG, E. AND GORDON, G. S.: Skeletal Dynamics in Man Measured by Nonradioactive Strontinum. *J. Clin. Invest.* 40:1809, 1961.