Journal of Clinical Investigation
Vol. 44, No. 9, 1965

Radioactive Calcium Kinetics during High Calcium Intake in Osteoporosis *

ERNEST SCHWARTZ,† VINCENT A. PANARIELLO, AND JOHN SAELI

(From the Metabolic Unit, Veterans Administration Hospital, Bronx, N. Y., and the Department of Medicine, Cornell University Medical College, New York, N. Y.)

Previously reported positive calcium balances in osteoporotic patients during high calcium intake (1, 2) do not seem attributable to soft tissue deposition (1). If bone is assumed to be the main deposition site, isotopic studies would indicate whether bone accretion rate is increased during positive balance, as inferred from data (3) that did not include serial accretion studies in any one patient, or whether bone resorption is decreased. *In vivo* studies and the analysis of isolated bone have shown reduced skeletal retention of isotopes in man and animals after varying durations of high calcium intake (4–6), representing the converse of increased skeletal isotope retention seen with prolonged calcium deprivation (7–9). However, there has as yet been no detailed presentation of human radiokinetic data obtained during variation of the calcium intake. The purpose of the present studies was twofold: *a*) to correlate with balance data the radioactive calcium bone accretion rates determined at intervals after the commencement of high calcium intake and *b*) to ascertain the duration of positive balance during high intake in osteoporotic patients.

Methods

Clinical data and protocol. Five male subjects were studied, of whom three had classical symptoms and findings of advanced osteoporosis with vertebral compression fractures (B.B., age 62; J.B., age 61; H. S., age 54), one had questionable roentgenographic rarefaction of bone (P.F., age 53), and one was considered to have normal bones (C.M., age 42). Patients B.B. and H.S. had chronic bronchopulmonary disease and emphysema; patient P.F. had Laënnec's cirrhosis and an alcoholic history. In patients J.B. and C.M., there was no significant antecedent medical history. All patients had normal serum calcium, phosphorus, alkaline phosphatase, phosphorus clearance, tubular reabsorption of phosphorus, peripheral hematologic examination, and bone marrow morphology. Urinary excretion of calcium was at high normal levels in patients H.S., P.F., and C.M., and below 100 mg per day in patients B.B. and J.B. Tests of intestinal absorption, including D-xylose excretion, triolein-I^{131} absorption, and total fecal fat excretion, were normal in patients B.B. and J.B. Previously reported studies (2) of high calcium intake in patients B.B. and H.S. had shown sustained positive calcium balances during periods of observation as long as 3 months.

Each of the five patients in the present series underwent a radiocalcium kinetic study during the control dietary periods and a repeat study at 4 to 7 weeks after the addition of approximately 4 g of calcium to the daily intake in the form of dicalcium phosphate ($CaHPO_4$). After an initial 2-month interval of high calcium intake, patient B.B. was given normal calcium intake for approximately 4 months, and then was placed on prolonged continuous metabolic balance on high intake totaling 648 days by July 31, 1964, during which time two more radiocalcium kinetic studies were performed. Patient J.B. underwent 84 days of continuous metabolic balance on high calcium intake followed by 42 days at home on high intake, followed by continuous balance on high calcium intake for the next 318 days. During the last phase, two additional radiocalcium kinetic studies were performed in patient J.B.

Metabolic methods. The metabolic balance procedures employed during high calcium intake have been described in detail elsewhere (2). Urinary and fecal calcium were determined by modifications of the Fiske-Logan method (10) or of an ultramicro EDTA titration method using Cal-red as indicator (11). Fecal calcium and phosphorus recovery averaged 99 to 101% of standard specimens prepared to simulate those obtained during high calcium intake (2). Fecal standards were analyzed with each batch of specimens. For the two long-term studies in patients B.B. and J.B., the graphs were plotted in 30-day periods. Corrections were made for phlebotomy losses of nitrogen, which were especially large during radiocalcium kinetic studies.

Radiocalcium methods. The radiocalcium kinetic studies were done with either $calcium^{45}$ (half-life 164 days) in doses approximating 2 μc or $calcium^{47}$ (half-life 4.7 days) in doses ranging between 14 and 25 μc. In some

* Submitted for publication February 23, 1965; accepted June 3, 1965.

Presented in part at the meeting of the American Federation for Clinical Research, December 13, 1963, New Haven, Conn.

† Address requests for reprints to Dr. Ernest Schwartz, Veterans Administration Hospital, 130 West Kingsbridge Road, Bronx, N. Y. 10468.

of the early studies, the dose was injected as a 2-hour intravenous infusion by a Harvard pump,[1] in order to calculate a rapidly miscible calcium pool (12). In most patients, the entire dose in a small volume was injected quickly intravenously, and a 2-hour miscible calcium pool estimated from extrapolation of the semilogarithmic serum specific activity curve between the second and third hours. Blood was drawn at frequent intervals during the first day and daily thereafter, occasionally twice daily. Calcium^{45} activity of serum, urine, and fecal calcium was determined by liquid scintillation counting (13) with a Packard Tri-Carb system. Calcium^{47} determinations were done with 4-ml volumes by a Picker gamma spectrometer and scintillation well detector. To improve the accuracy of calcium^{47} counting beyond the tenth day of study, calcium oxalate was precipitated (10) from larger portions of serum, urine, and feces and redissolved before counting.

Handling of kinetic data. Kinetic computations were based upon the assumption of a one-compartment homogeneous miscible pool, from which radiocalcium is removed either by bone accretion or by excretion. Although this scheme is only a gross biological approximation, comparisons of serial studies in the same patient may still be meaningful (14). As an internal check, two different methods of graphical calculation based upon a one-compartment calcium pool were employed with the data of each kinetic study. In the method of Bauer, Carlsson, and Lindquist (15), the mean specific activity of calcium removed from the pool and deposited in bone during any time interval is obtained by planimetry under the Cartesian serum specific activity curve. The basic formulation is:

$$\% \text{ dose retained in body at time } T = E\,S_T + A\int_0^T S\,T\,dt,$$

where E is the exchangeable calcium pool in grams, A is the bone accretion rate in grams per day, and S_T is the serum specific activity at time T as per cent of dose per gram calcium. The solution for E and A is obtained from the formulation of two simultaneous equations for two time points, i.e., at the end of 3 and 7 days. Theoretical excretion rates in either urine or stool can be further calculated from the expression: $U \Big/ \int_{T_1}^{T_2} T\,dt$, where U is the total per cent dose excreted in urine or stool in time $T_1 - T_2$. The Bauer Cartesian calculation of accretion rate was also made for 16 days, with time points at the end of the seventh and sixteenth days, in order to obtain qualitative information about the magnitude of reappearance of previously accreted isotope, which would be manifested as a decrease in the 16-day accretion rate. The second graphical method, which is based upon essentially identical mathematical assumptions, was adapted from a modification (16) of the method of Heaney and Whedon (17). This modification embodies a correction for excess excretion of isotope during the mixing period. An extrapolation was made to time zero of the semilogarithmic plasma specific activity curve from 4 to 7 days fitting the

[1] Harvard Apparatus Company, Dover, Mass.

TABLE I

Metabolic balance data: short-term studies

Regimen	Days	Calcium			
		Intake	Urine	Fecal	Balance
				g/day	
		Patient H.S. 9-25-61			
Control: Ca^{45} 2 μc, day 25	1–42	1.07	0.26	0.73	+0.08
$CaHPO_4$: Ca^{45} 3 μc, day 68	43–81	5.02	0.20	4.24	+0.58
Control	82–90	1.07	0.13	0.91	+0.03
		Patient P.F. 1-11-62			
Control: Ca^{45} 2 μc, day 9	1–30	0.89	0.27	0.55	+0.07
$CaHPO_4$: Ca^{45} 2 μc, day 62	31–78	4.86	0.27	3.89	+0.70
Control	79–90	0.89	0.23	1.13	−0.47
$CaHPO_4$	91–96	4.86	0.30	3.88	+0.68
Control	97–108	0.89	0.23	0.82	−0.16
		Patient C.M. 1-31-63			
Control: Ca^{45} 4 μc, day 9	1–30	0.82	0.21	0.71	−0.10
$CaHPO_4$	31–16	4.77	0.23	4.03	+0.51
$CaHPO_4$-Ca gluconate Ca^{47}, 18 μc, day 148	61–168	4.77	0.23	4.22	+0.32
Control	169–180	0.82	0.22	0.92	−0.32

TABLE II

Metabolic balance data: long-term studies

Regimen	Days	Calcium			
		Intake	Urine	Fecal	Balance
			g/day		
		Patient B.B. 1-5-62			
Control: Ca^{45} 2 μc, day 8	1–30	0.87	0.07	0.83	−0.03
$CaHPO_4$: Ca^{45} 2 μc, day 62	31–78	4.83	0.09	4.36	+0.38
Control	79–90	0.87	0.06	0.88	−0.07
$CaHPO_4$	91–102	4.83	0.09	4.50	+0.24
Control	103–114	0.87	0.07	0.79	+0.01
Off study	115–259				
Control	260–289	0.91	0.14	0.75	+0.02
$CaHPO_4$	290–409	4.86	0.14	4.46	+0.26
$CaHPO_4$	410–529	4.86	0.16	4.11	+0.59
$CaHPO_4$	530–571	4.86	0.18	3.88	+0.80
$CaNPO_4$	572–613	4.86	0.14	4.39	+0.33
$CaHPO_4$: Ca^{47} 24 μc, day 656	614–739	4.80	0.15	4.22	+0.43
$CaHPO_4$	740–859	4.73	0.16	4.47	+0.10
$CaHPO_4$: Ca^{47} 16 μc, day 903	860–937	4.74	0.16	4.42	+0.16
		Patient J.B. 3-26-63			
Control: Ca^{47} 12.5 μc, day 25	1–42	0.86	0.05	0.88	−0.07
$CaHPO_4$: Ca^{47} 26 μc, day 94	43–126	4.82	0.06	4.11	+0.65
$CaHPO_4$: at home	127–168	Approximate Ca intake, 4.20 g			
$CaHPO_4$	169–270	4.83	0.05	4.28	+0.50
$CaHPO_4$: Ca^{47} 26 μc, day 289	271–390	4.71	0.05	4.47	+0.19
$CaHPO_4$: Ca^{47} 20 μc, day 430	391–486	4.70	0.06	4.52	+0.12

line to the points by eye. The equation used was: $A = kE - (U + F)$, where A = accretion rate, k = turnover constant ($= 0.693/t_{\frac{1}{2}}$), E = exchangeable pool, and (U + F) = calculated urinary and endogenous fecal excretion rates. Since the Cartesian and semilogarithmic methods were generally in close agreement, i.e., differences in computed accretion rates were usually less than 10%, the arithmetic mean values for all parameters were tabulated. As a means of comparative evaluation, our methods of computation were applied to radiokinetic data from two of Dymling's patients (18) and to the normal reference standard of Heaney and associates (19). These results are listed at the bottom of Table III. Accretion rates and exchangeable pools that we computed from Dymling's data were comparable to those individually calculated by several investigators, as tabulated by Dymling (18). With respect to serial determinations of accretion rate in the same patient, the analysis of sequential studies suggests that reproducibility is probably within ± 16% (± 2 SD) (20).

Calculation of intestinal absorption. After averaging the endogenous fecal excretion rate computed by both graphical methods, per cent absorption of intake was calculated from the formula: % absorption $= (I - I_{un})/I$, where I = Ca intake per day, and I_{un} = unabsorbed intake = fecal calcium excretion − endogenous fecal calcium (21).

Results

Metabolic balance data. Tables I and II present the calcium balances for the five patients. During the initial intervals of high calcium intake, all patients sustained strongly positive calcium balances. In all patients, the actual phosphorus retention and

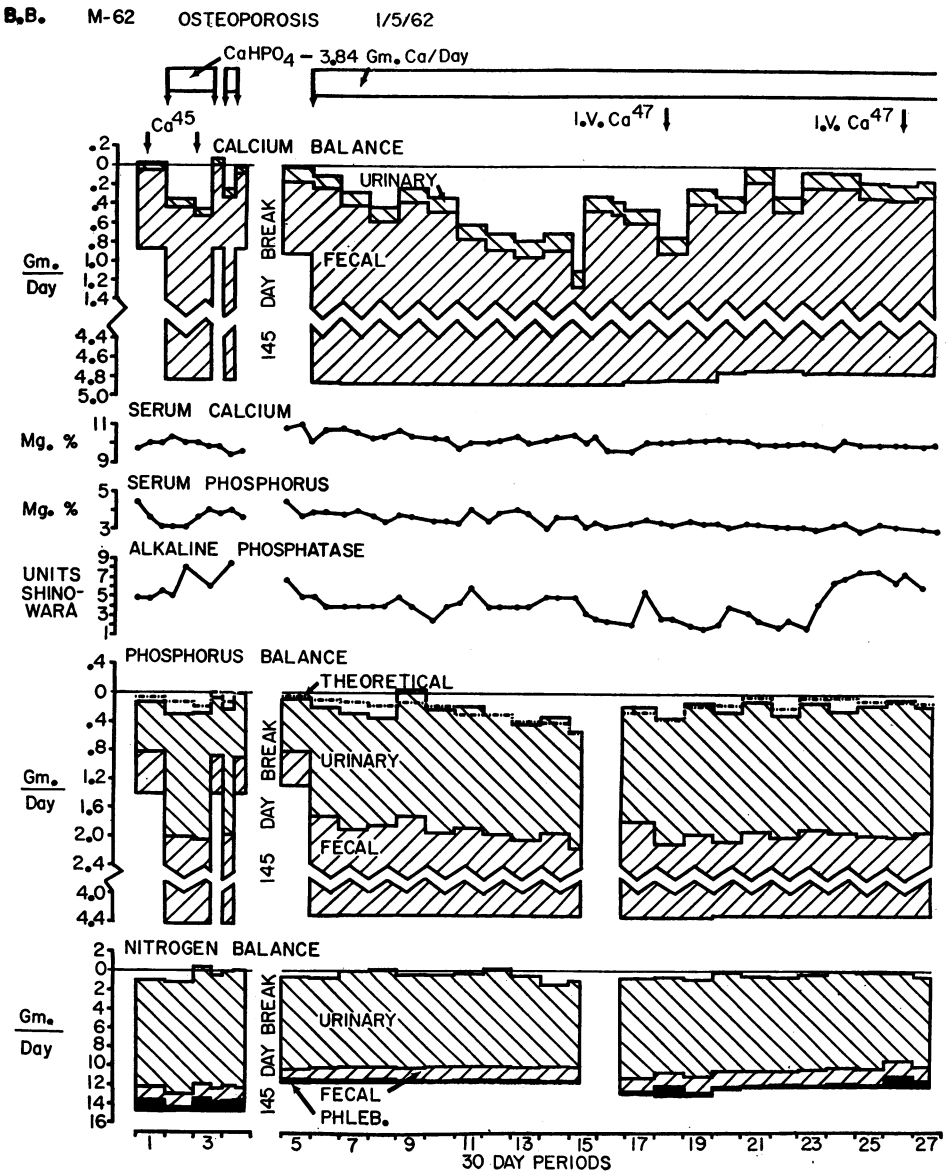

FIG. 1. METABOLIC BALANCE DATA IN PATIENT B.B., A 62-YEAR-OLD OSTEOPOROTIC MALE. The short- and long-term studies were separated by a 145-day interval of normal calcium intake. On day 91 of the short-term study, 29 days after the second Ca^{45} injection, high calcium intake was reinstituted for 12 days in order to see whether suppression of radiocalcium outflow from previously labeled bone would reduce specific activity. The specific activity curve was not altered by this maneuver, continuing to oscillate in the range of 0.4 to 0.8% dose per g calcium.

theoretical phosphorus retention based on calcium and nitrogen balances were in relatively good agreement. These data are depicted graphically for patients B.B. (Figure 1) and J.B. (Figure 2). With continuance of high calcium intake in patients B.B. and J.B., there occurred a gradual reduction of positive calcium balance. Patient B.B. (Table II, Figure 1) showed a decline of positive calcium balance to + 0.10 g per day during days 740 to 859, and + 0.16 g per day during days 860 to 937. Patient J.B. (Table II, Figure 2) showed a decline of positive balance to + 0.19 g per day during days 271 to 390, and to + 0.12 g per day during days 391 to 486. Allowing for

slight losses of calcium output in sweat,[2] on toilet paper,[3] and in the transfer of fecal specimens from steel containers, and for slight losses of calcium intake on dinnerware and glasses, our estimates of the total unmeasured loss at 5 g daily calcium intake ranged from 100 to 150 mg per day, which would be registered as positive calcium balance of the magnitude observed in these two patients during the latter days of their studies. It would therefore appear that patients B.B. and J.B. came into calcium balance after prolonged high calcium intake.

Bone accretion rates, calcium balance, and exchangeable calcium during short-term high intake. Tables III and IV present the kinetic data for the five patients, correlated with the observed calcium balances and calculated calcium absorptions at the time of the radioisotope studies. Figures 3, 4, and 5 show the serum specific activity curves for all the studies. In all five patients, there did not seem to be a significant change in the bone accretion rates or exchangeable pools after 4 to 7 weeks of high calcium intake, despite significantly positive calcium balances. The 16-day total isotope excretion in urine and stool also did not significantly change during the short-term studies. Only one patient, B.B., showed a definite increase in 24-hour urinary calcium excretion. There was relatively good agreement throughout of the observed urinary calcium with the theoretical urinary calcium calculated from isotope data, confirming the accuracy of chemical and isotopic techniques. In four of the five short-term high calcium intake studies, the positive balance achieved was numerically very close to the computed accretion rate. In none of the short-term studies did the positive balance significantly exceed the accretion rate.

Effect of prolonged high calcium intake upon accretion rates. In patients B.B. and J.B., repeat kinetic studies performed at 1 year and 8 months respectively after commencement of high calcium intake showed sharp falls in the accretion rates and a large increase in the total per cent dose excreted over 16 days (Table IV). These findings were confirmed by further kinetic studies in patients B.B. and J.B. at 20 months and 12.7 months,

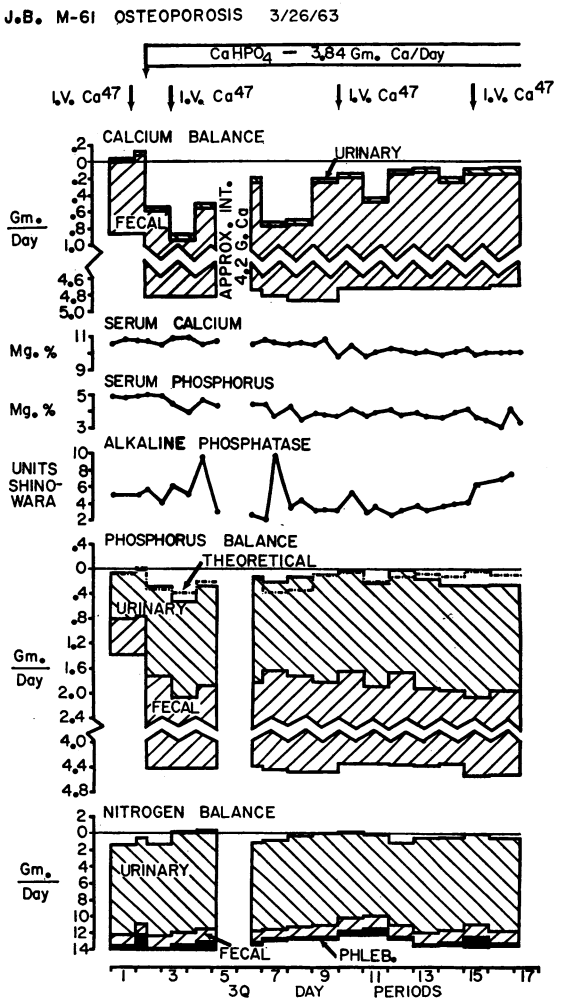

FIG. 2. METABOLIC BALANCE DATA IN PATIENT J.B., A 61-YEAR-OLD OSTEOPOROTIC MALE. In the interval indicated during the fifth and sixth 30-day periods, the patient had a total intake of approximately 4.2 g calcium at home, effected by $CaHPO_4$ supplementation provided in serially numbered weighed packets. By the ninth 30-day period of the long-term study, positive calcium balance had markedly diminished and, despite fluctuations, continued to decrease as the study progressed.

respectively, after starting high calcium intake. The accretion rates were approximately halved, and the per cent dose excreted increased to approximately 150% of the control values. In both patients, the decreases in accretion rates were accompanied by marked decreases in the positive calcium balances. In only one instance, patient B.B., on October 23, 1963, after 1 year of high intake, the accretion rate of 0.21 g per day was substantially exceeded by the observed positive cal-

[2] Sensible sweating was minimized by air conditioning in summer and thermostatic radiator regulation in winter.

[3] Toilet paper losses averaged 10 to 50 mg calcium per day and were disregarded in plotting the balance data.

TABLE III

*Radiocalcium kinetic data: short-term studies**

Patient; diagnosis Date of isotope injection Regimen	Accretion rate and exchangeable pools					Urinary excretion			Fecal excretion			Calcium balance and absorption				
						Urinary Ca										
	Time calculated	A	E	E_{2h}	15-min serum SA†	Measured	Calculated	Urinary excretion	Endogenous fecal Ca	Fecal excretion	Total isotope excretion	Intake	Fecal	Balance	No. days calculated	% Absorption $(I-I_{un})/I$
	days	*g/day*	*g*	*g*	*% dose*	*g/day*		*% dose*	*g/day*	*% dose*	*% dose*		*g/day*			*%*
H.S. (osteoporosis)																
10-19-61	7	0.58	5.7	2.0‡		0.27	0.27	20.3	0.16	8.9	29.2	0.87	0.84	−0.04	42	28
Ca^{45} 2 μc	12	0.43	7.6					25.4		11.2	36.8					
Control																
12-1-61	7	0.61	5.8	2.3‡		0.20	0.17	12.4	0.15	10.3	22.7	5.02	4.24	+0.58	39	19
Ca^{45} 3 μc	16	0.47	7.5					17.6		13.9	31.5					
Ca load 4 weeks																
P.F. (Osteoporosis?)																
1-19-62	7	0.55	7.4	2.9‡		0.27	0.29	19.8	0.17	8.9	28.7	0.89	0.55	+0.07	30	57
Ca^{45} 2 μc	16	0.35	9.7					28.0		16.7	44.7					
Control																
3-13-62	7	0.59	7.0	2.0‡		0.27	0.22	16.4	0.20	13.1	29.5	4.86	3.89	+0.70	48	25
Ca^{45} 2 μc	16	0.54	8.7					22.7		16.1	38.8					
Ca load 1 month																
C.M. (Normal)																
2-28-63	7	0.54	7.4	2.3	57.4	0.21	0.21	14.7	0.17	10.3	25.0	0.82	0.71	−0.10	30	34
Ca^{45} 4 μc	16	0.49	7.2					20.6		14.6	35.2					
Control																
6-27-63	7	0.62	7.0	2.0	63.7	0.24	0.23	16.1	0.19	13.0	29.1	4.77	4.22	+0.32	108	16
Ca^{47} 18 μc	16	0.45	8.7					22.1		18.9	41.0					
Ca load 7 weeks																
Standard cases																
Dymling: Case F-88§ (hypoparathyroid)	7	0.19	4.7				0.33		0.20							
Dymling: Case E-110§ (Ca prostate)	7	7.3	51				0.09		0.25							
Heaney: Standard‖ (Uncorrected data)	7	0.44	5.1				0.13		0.12							

* A = accretion rate; E = exchangeable calcium; E_{2h} = 2-hour calcium pool after rapid injection or slow infusion; $(I - I_{un})/I$ = (intake − unabsorbed intake)/intake, where I_{un} = fecal Ca − endogenous fecal Ca.

† Fifteen-minute serum SA = specific activity of serum calcium at 15 minutes after rapid injection.

‡ Value obtained from slow infusion.

§ Reference 18.

‖ Reference 19.

TABLE IV

Radiocalcium kinetic data: long-term studies

Patient; diagnosis Date of isotope injection Regimen	Accretion rate and exchangeable pools					Urinary excretion			Fecal excretion			Calcium balance and absorption				
						Urinary Ca										
	Time calculated	A	E	E_{sh}	15-min serum SA	Measured	Calculated	Urinary excretion	Endogenous fecal Ca	Fecal excretion	Total isotope excretion	Intake	Fecal	Balance	No. days calculated	% absorption $(I-I_{un})/I$
	days	*g/day*	*g*	*g*	*% dose*	*g/day*		*% dose*	*g/day*	*% dose*	*% dose*		*g/day*			*%*
B.B. (osteoporosis)																
1-12-62	7	0.48	5.1	2.2*		0.07	0.08	6.3	0.21	15.4	21.7	0.87	0.84	−0.04	30	28
Ca^{45} 2 μc	16	0.22	8.5					9.4		25.5	34.9					
Control																
3-7-62	7	0.40	6.6	2.3*		0.08	0.10	8.6	0.31	20.6	29.2	4.83	4.35	+0.39	48	16
Ca^{45} 2 μc	16	0.42	5.5					11.1		26.5	37.6					
Ca load 1 month																
10-22-63	7	0.21	5.4	1.8	58.1	0.17	0.16	15.9	0.26	23.5	39.4	4.82	3.92	+0.74	30	24
Ca^{47} 24 μc	16	0.13	5.9					23.6		38.1	61.7	4.82	44.3	+0.23	30	13
Ca load 1 year																
6-25-64	7	0.30	5.0	1.8	71.2	0.16	0.19	6.9	0.26	25.5	32.4	4.74	4.40	+0.18	30	13
Ca^{47} 16 μc	16	0.19	5.9					21.7		36.7	58.4					
Ca load 20 months																
J.B. (osteoporosis)																
4-19-63	7	0.59	5.0	1.9	65.4	0.05	0.06	4.2	0.21	16.5	20.7	0.86	0.86	−0.05	42	24
Ca^{47} 12.4 μc	16	0.39	7.1					5.8		25.7	31.5					
Control																
6-27-63	7	0.67	5.4	1.8	69.5	0.06	0.06	3.8	0.26	20.0	23.8	4.82	4.09	+0.67	54	21
Ca^{47} 26 μc	16	0.41	8.4					5.3		30.5	35.8					
Ca load 7 weeks																
1-8-64	7	0.35	4.9	1.7	70.6	0.05	0.04	3.4	0.24	24.3	27.7	4.71	4.35	+0.31	48	13
Ca^{47} 26 μc	16	0.25	5.8					5.4		38.0	43.4					
Ca load 8 months																
5-28-64	7	0.24	4.6	1.4	87.8	0.06	0.04	4.3	0.31	29.5	33.8	4.71	4.56	+0.09	30	10
Ca^{47} 20 μc	16	0.18	5.3					7.0		42.4	49.4					
Ca load 12.7 months																

* Value obtained from slow infusion.

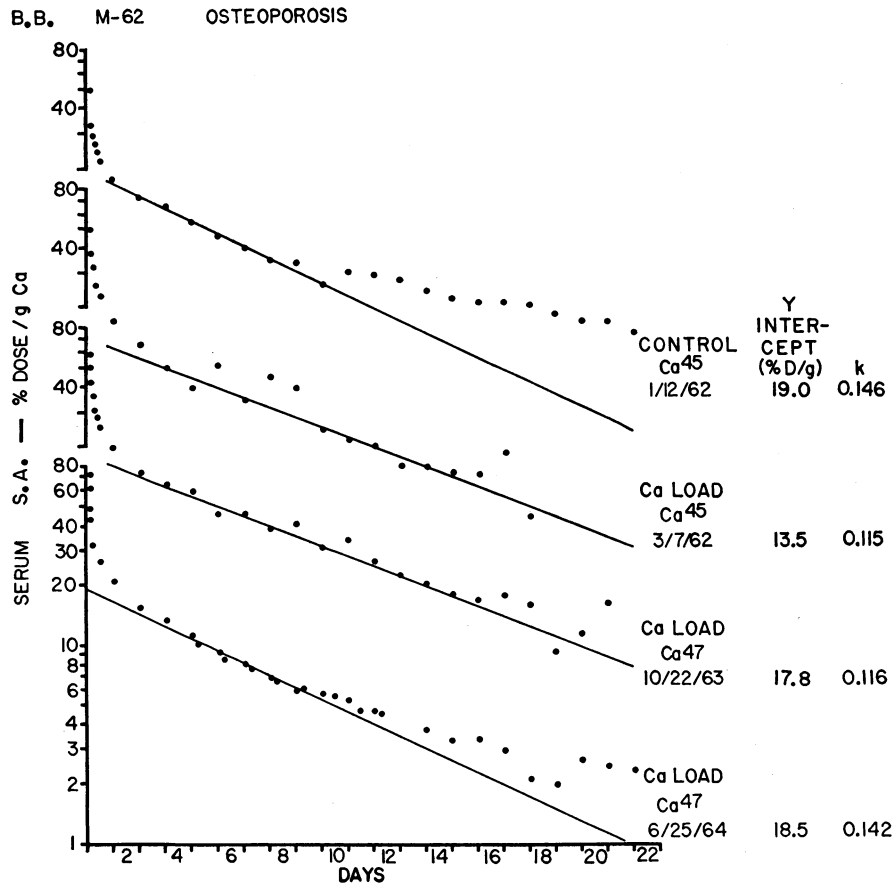

FIG. 3. SERUM SPECIFIC ACTIVITY CURVES IN PATIENT B.B. BEFORE AND DURING PROLONGED HIGH CALCIUM INTAKE. Four- to 7-day slopes are indicated, fitted to the point by inspection. k values represent turnover constants ($0.693/t_{\frac{1}{2}}$).

cium balance of + 0.75 g per day for the first 30 days following the isotope injection, a discrepancy probably due to irregular stool output and to a larger than usual systematic balance error.

Effect of long-term high calcium intake upon slowly and rapidly exchangeable calcium pools. As with short-term high calcium intake, the values for the slowly exchangeable pool, E, showed no consistent alterations. In successive studies, however, there appeared to be a definite increase in the serum specific activity measured at 15 minutes after isotope injection, indicating a decrease in the size of the 15-minute distribution space (Table IV). If the two initial continuous infusion studies in patient B.B. are disregarded, the 15-minute serum specific activities after 1 year and 20 months of high intake were 58.1 and 71.2% dose per g, respectively. In patient J.B., the 15-minute serum specific activities in the first and fourth studies were 65.4 and 87.8% dose per g respectively, associated with a corresponding fall in the 2-hour exchangeable calcium (E_{2h}) from 1.9 to 1.4 g. Since there is evidence that the 15-minute and 2-hour distribution spaces include some part of bone (12, 14), diminution of this space under conditions of sustained calcium storage would imply a progressive saturation of rapidly exchangeable osseous or extraosseous compartments or of both.

Endogenous fecal calcium excretion and intestinal absorption. In three of the five patients (B.B., J.B., and P.F.,) there appeared to be an increase in endogenous fecal calcium on high intake. In all patients, per cent absorption showed substantial declines on high intake, with only transient fluctuations upwards in patients B.B. and J.B.

Patterns of urinary and fecal radiocalcium excretion. The partition of radiocalcium excretion between urine and stool seemed to be characteristic for each patient. As expected, higher urinary calcium excretion was associated with greater isotope excretion by this route. B.B. and J.B. excreted only 27% and 18%, respectively, of the radiocalcium dose via the urinary route in 16 days, whereas H.S. excreted 69% in the urine. Thus, the use of only urinary isotope excretion to approximate bone accretion rates can be erroneous in patients in whom the excretion route is primarily fecal. During the long-term high intake studies, as the serial kinetic data showed increasing total isotope excretion, the route chosen to excrete the increment depended partly upon whether the urinary calcium increased during high intake. In patient B.B. an increased urinary calcium was reflected in increased excretion of radiocalcium by the urinary route as well as the fecal route. In patient J.B., whose urinary calcium did not increase during high intake, the increment in radiocalcium excretion was essentially via the fecal route.

Configuration of serum specific activity curves and comparison of 7-day and 16-day accretion rates. Our families of semilogarithmic curves (Figures 3 to 5) did not consistently manifest sudden change of slope or break in the curve at any specific time, although the construction of a 4- to 7-day slope line occasionally gave this optical impression. In most cases, there appeared to be a gradual, continuous shallowing of slope up to the end of the studies (generally not longer than 22 days). Furthermore, after 7 days, as serum specific activity values became very low, erratic fluctuations of values appeared, probably due to random technical variation. In four of the five control

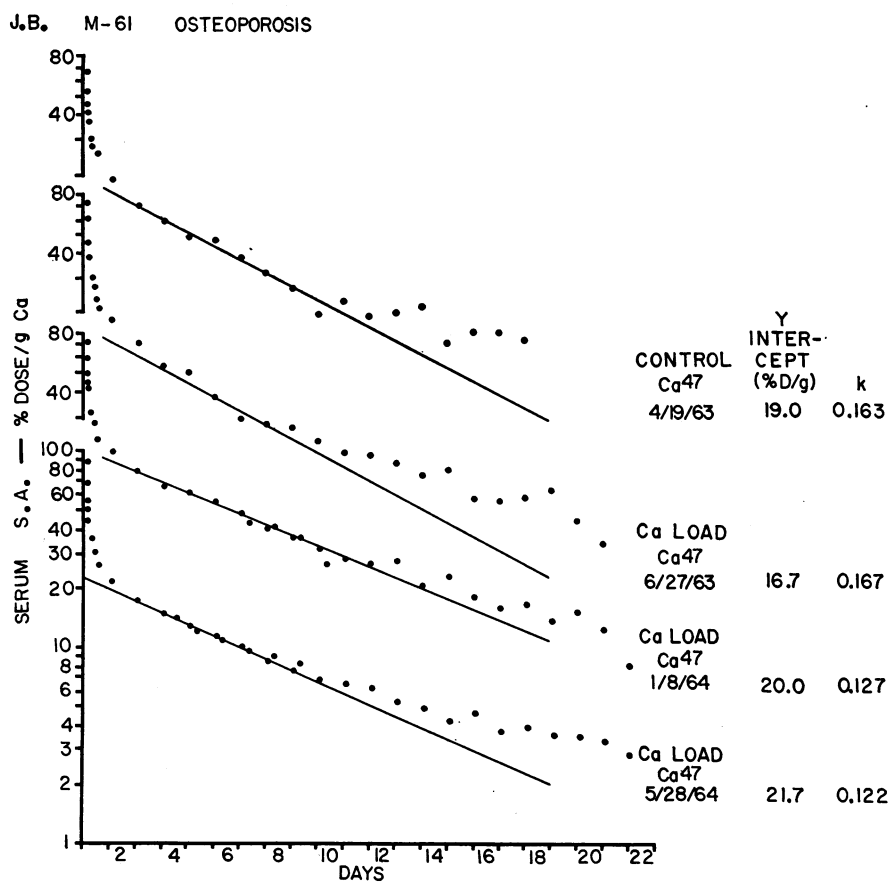

FIG. 4. SERUM SPECIFIC ACTIVITY CURVES IN PATIENT J.B. BEFORE AND DURING PROLONGED HIGH CALCIUM INTAKE. Four- to 7-day slopes are indicated.

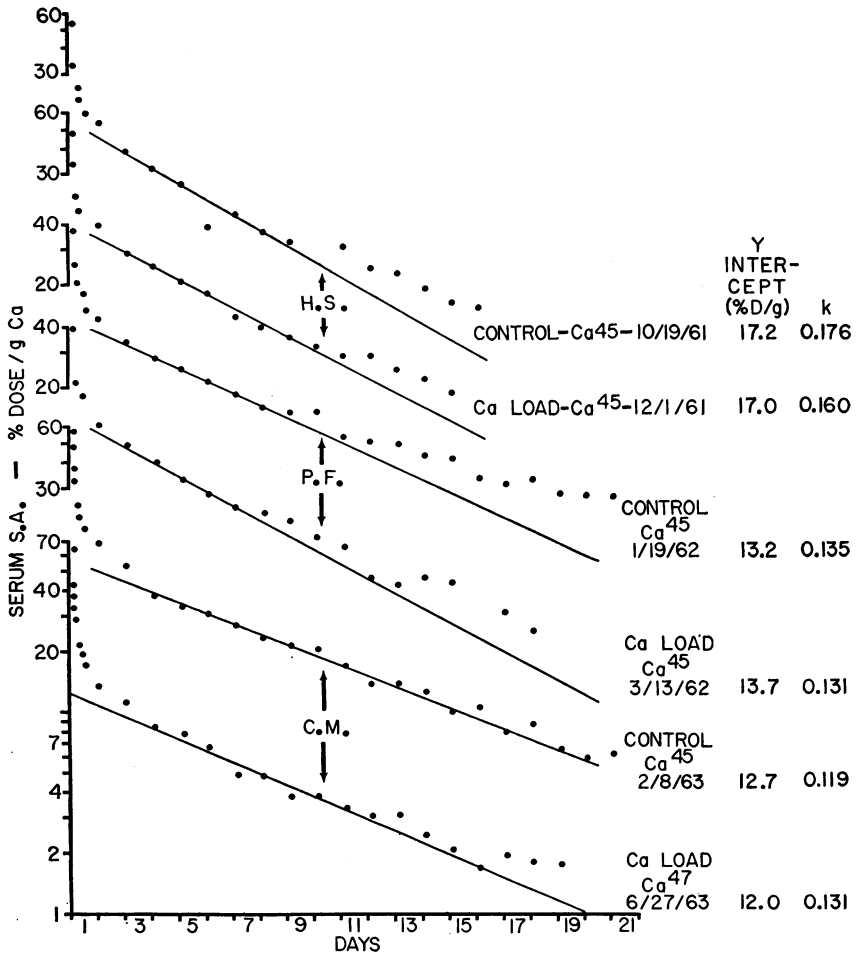

FIG. 5. PAIRS OF SERUM SPECIFIC ACTIVITY CURVES BEFORE AND DURING SHORT-TERM HIGH CALCIUM INTAKE IN PATIENTS H.S., P.F., AND C.M. Four- to 7-day slopes are indicated.

kinetic studies herein reported, the 16-day accretion rates computed by the Bauer Cartesian method were somewhat lower than the 7-day rates. In seven of nine kinetic studies during high calcium intake, the 16-day accretion rate remained proportionately lower than the 7-day rate, suggesting that the process responsible for the difference was not grossly affected by high calcium intake. Using a computational system modified from that of Bauer, Marshall has shown that in any one study, the instantaneous accretion rate diminishes with time, and the exchangeable calcium pool value increases with time in linear power function (log-log) relationships (22). Therefore, the arbitrary selection of the first identifiable prolonged exponential component, for instance from 4 to 7 days, provides a comparatively maximal estimate of total calcium inflow into relatively nonexchangeable bone from the relatively exchangeable space of the one-compartment model. The attribution of shallower slope beyond 7 to 10 days to reappearance of previously accreted isotope is based on experimental data involving simultaneous kinetic study of strontium[85] and calcium[45] (14, 17), upon data from an *in vitro* system suggesting the occurrence of reverse exchange (23), and upon external scanning of cancellous bone (20). The fact that the later portion of the specific activity curve also appears to be a relatively smooth multiexponential function probably indicates the existence of multiple slow exchange and resorptive processes that cannot be individually quantitated by use of the

one-compartment model. Furthermore, experimental confirmation by direct sampling of bone and soft tissue compartments is lacking for numerous proposed multicompartmental model systems. It would therefore seem that the identification and statistical analysis of the 7- to 10-day slope in 10-day studies as an "isotopic resorption index" (24) is not biologically verifiable at present.

Clinical and roentgenographic findings. After relatively short periods of high calcium intake in the five patients, there appeared to be neither significant change in clinical symptomatology nor gross radiologic change. Of the two patients who were on prolonged high calcium intake, one, B.B., showed no changes in the serial roentgenograms of bones from November 1959 to September 1964. However, although there appeared to be no obvious increase in gross bony density, there was also no evidence of further progression of osteoporosis. Clinically, this patient continued to have backache intermittently. The second patient, J.B., during the interval between December 1962 and September 1964, seemed to manifest a suggestive increase in the radiologic density of the cortical end plates in the thoracic and lumbar vertebrae as compared with the vertebral bodies. Nordin (25) claims to have seen this radiologic change after protracted high calcium intake. The remainder of the bones in patient J.B. appeared unchanged. Patients B.B. and J.B. both had areas of aortic calcification in the initial roentgenograms, which did not grossly change after high calcium intake. Of considerable interest in patient B.B. was the appearance on abdominal films taken in January 1965 of gallstones outlined by calcific rims, which had not been visible 1 year previously. By that time, the patient had been consuming a high calcium intake on a metabolic balance regimen for 822 days.

Discussion

During our short-term studies of high calcium intake, in which the accretion rates did not increase, substantial positive calcium balances were observed, apparently indicating inhibition of bone calcium outflow. This conclusion agrees with preliminary data obtained in humans after equilibration during continuous feeding of calcium[45] (26). During high calcium intake, plasma or urinary specific activity, which would normally be diluted by calcium outflow from unlabeled bone, increases, indicating inhibition of unlabeled calcium outflow. Although high calcium intake appears to reduce the increased bone resorptive activity of calcium-deficient adult cats (27), it is not yet clear how much of the depression of calcium outflow in our patients can be attributed to resorption-inhibition.

Biological implications of kinetic analysis. The interpretation of outflow inhibition involves the discrimination of diffuse inflow-outflow processes that appear to be responsible for about one-half the measured total inflow-outflow rate, as estimated by quantitative radioautography of bone (28) and by the comparison of radiocalcium uptake by bone with new bone formation determined by the tetracycline-labeling method (29). The issue is unresolved as to whether the diffuse process is simply long-term ion exchange (28, 30) or biologically controlled, or both, except during active growth in young animals, where the total radiocalcium inflow is shunted largely into bone formation (31). The least restrictive formulation would state:

> Bone calcium balance = calcium inflow − calcium outflow = (diffuse inflow + bone-forming inflow) − (diffuse outflow + resorbing outflow).

Inhibition of calcium outflow would imply inhibition of resorption only if the resorptive outflow component were specifically inhibited. The magnitude of resorption inhibition would therefore depend upon the presently unknown quantitative ratio of diffuse and resorptive outflow components in any individual patient.

From the above formulation, it can be deduced that a patient in calcium balance with a high accretion or inflow rate will have, by definition, an equally high calcium outflow rate. If high calcium intake produces positive balance by inhibiting calcium outflow without initially affecting accretion, a patient with high accretion rate should be capable of greater positivity of balance than a patient with low accretion rate, a correlation observed by others (3, 32) in individual patients on high calcium intake. The observed radiocalcium accretion rates would therefore place a theoretical ceiling upon the magnitude of positive balance obtainable by total outflow inhibition.

Interpretation of reduced accretion rate with prolonged high calcium intake; coupling of bone formation and resorption. Reduction of bone formation rate by inhibition of resorption in the human is suggested by the lowering of radiocalcium accretion rates to 0.30 g per day and 0.24 g per day in patients B.B. and J.B. after prolonged high calcium intake, a phenomenon also observed by Lutwak (6). High calcium intake has also been reported to lower the elevated radiocalcium accretion rates of human vitamin D-resistant rickets (33). All of these experimental situations may reflect the close biological coupling of bone resorption and bone formation, first clearly described by Albright (34), anatomically delineated by Sissons (35), and conceptually integrated by Frost into the "remodelling rate" (36). Additional interpretations of our accretion rate findings, both initially and after sustained high calcium intake, might invoke the presence of calcium-deficient hydroxyapatites (9) or hydroxyapatite-deficient bone in osteoporosis (27), with progressive saturation of bone calcium content. There is as yet no documentation of the presence of calcium-deficient apatite in human osteoporosis although the storage of calcium without proportionate storage of phosphorus has been observed (2, 24). Although markedly decreased ash content per unit of medullary vertebral volume has been demonstrated in senile osteoporosis, the isolated trabecular bone had either normal or increased ash content and normal organic content (37). Since normal bone is not totally calcium saturated, a more likely explanation than those advanced, in the same vein, would postulate the capacity for further secondary mineralization, as suggested by the ability of patients with normal bone, e.g., patient C.M., to retain calcium on high calcium intake (2, 3). In this situation a declining accretion rate would imply inadequate formation of new unsaturated bone to maintain the initial accretion rate, resulting in the establishment of a new radiokinetic equilibrium level at a higher level of calcium saturation.

Mechanisms and significance of resorption inhibition. The postulated inhibitory effect of high calcium intake upon resorption may be reciprocally related to the observed parathyroid dependence of bone rarefaction in calcium-deprived growing rats (38) and may merely represent a state of induced parathyroid suppression. This supposition may be supported by the fact that the accretion rates in patients B.B. and J.B. after prolonged high calcium intake were comparable to those reported by numerous investigators in hypoparathyroid patients. As observed with high calcium intake, the antiresorptive action of estrogen (39, 40) does not seem initially to change the radiokinetic accretion rates (24, 41), but long-term estrogen or androgen therapy has resulted in declining accretion rates (24, 42). If declining accretion rates with high calcium intake or estrogen therapy signify reduced bone formation, it is possible to explain the gradual reduction of positive balance observed in our two long-term patients and in long-term studies by other investigators (43, 44), as well as the failure of bones to recalcify during estrogen or calcium therapy of established osteoporosis. From a therapeutic standpoint, however, one cannot totally exclude the possibility of clinical benefit from inhibition of the relatively excessive bone resorption thought to characterize osteoporosis (27, 45, 46).

Summary

1. Radiocalcium accretion rates in five patients, of whom three had advanced osteoporosis, showed no significant change during the first 1 or 2 months of positive calcium balance induced by high calcium intake. The data of a short-term study in one patient with normal bone were qualitatively similar to those of the osteoporotic patients.

2. In two patients with advanced osteoporosis who were on sustained high calcium intake for as long as 20 months, the 7-day radiocalcium accretion rates declined to about 50 to 60% of the control values, and the total 16-day radioisotope excretion increased to about 150% of the control values.

3. Declining accretion rates in these two patients were associated with substantial declines in positive calcium balance. If we allow for small unmeasured losses at 5-g calcium intake levels, the patients appeared to arrive at approximate calcium balance.

4. In these two patients, the 15-minute serum specific activity value appeared to increase progressively in serial studies during high calcium intake. In one patient, the 2-hour miscible calcium pool successively diminished, suggesting previous filling (saturation) by high calcium in-

take of osseous or extraosseous components of this compartment or of both. There were no consistent alterations of the slowly exchangeable calcium pool.

5. Inspection of semilogarithmically plotted serum specific activity curves of up to 22 days duration did not disclose consistent evidence of sudden change of slope at any point in time. In general, there appeared to be progressive shallowing of slope with time, probably due to multiple slow exchange and resorptive processes. The shapes of the curves did not seem to be grossly altered by high calcium intake.

6. The mechanism of positive balance during high calcium intake appears to involve suppression of bone calcium outflow, probably including inhibition of the bone-resorptive component of calcium outflow, perhaps effected by parathyroid gland suppression. The reduction of accretion rates with sustained high calcium intake may reflect in part a cybernetic coupling of bone formation and bone resorption.

7. There were no unequivocal clinical or gross radiological changes in these patients associated with high calcium intake, but osteoporosis did not seem to progress during the two long-term studies.

Acknowledgments

We are grateful to Dr. Solomon Berson for helpful advice and critical review of the findings; to Drs. Clayton Rich and Melvin Horwith for assistance with the continuous infusion determination of rapidly miscible pools; to Drs. William V. Chokas, Arnold Kopeloff, and Sheldon Sorokoff for help in the care of patients; and to Mr. Charles Iversen, physicist, for help with the radioisotope equipment. We deeply appreciate the dedicated work of the Metabolic Unit staff: Miss Jeanette McLoughlin, Miss Cathleen Hanrahan, Miss Ernesta Albertini, Mrs. Susanne Aldor, Miss Rose Saffo, Mrs. Sarah Gibbs, nurses; Miss Katina Birbil, Miss Catherine Miller, and Mrs. Harriet Kahn, dietitians; Mrs. Loretta Davis and Miss Bernice Middleton, biochemists; Mrs. Evelyn Edwards, dietetic aide; and Miss Josephine Sciucca and Miss Mae Brunelli, secretaries.

References

1. Whedon, G. D. Effects of high calcium intakes on bones, blood and soft tissue; relationship of calcium intake to balance in osteoporosis. Fed. Proc. 1959, **18**, 1112.
2. Schwartz, E., W. V. Chokas, and V. A. Panariello. Metabolic balance studies of high calcium intake in osteoporosis. Amer. J. Med. 1964, **36**, 233.
3. Nordin, B. E. C., J. MacGregor, and M. M. Bluhm. Determination of bone formation rate with radioactive strontium. Clin. Sci. 1963, **24**, 301.
4. Spencer, H., M. Li, and J. Samachson. Effect of high calcium intake on strontium^{85} metabolism in man. J. clin. Invest. 1961, **40**, 1339.
5. Hegsted, D. M., and M. Bresnahan. Dietary strontium and calcium and deposition of Sr^{90} and Ca^{45} in bones of rats. Proc. Soc. exp. Biol. (N. Y.) 1963, **112**, 579.
6. Lutwak, L. High dietary calcium and osteoporosis *in* Dynamic Studies of Metabolic Bone Disease, O. H. Pearson and G. F. Joplin, Eds. Philadelphia, F. A. Davis, 1964, p. 87.
7. Carlsson, A. Metabolism of radiocalcium in relation to calcium intake in young rats. III. The effect of calcium deprivation on the absorption and on the excretion of radiocalcium. Acta Pharmacol. 1951, **7** (suppl. 1), 23.
8. Harrison, M., and R. Fraser. Bone structure and metabolism in calcium-deficient rats. J. Endocr. 1960, **21**, 197.
9. Menczel, J., R. Schraer, G. Pakis, A. S. Posner, and R. C. Likins. Effect of a low calcium diet on bone crystallinity and skeletal uptake of Ca^{45} in rats. Proc. Soc. exp. Biol. (N. Y.) 1963, **112**, 128.
10. Fiske, C. H., and M. A. Logan. Determination of calcium by alkalimetric titration; precipitation of calcium in the presence of magnesium, phosphate and sulphate, with applications to analysis of urine. J. biol. Chem. 1931, **93**, 211.
11. Bachra, B., A. Dauer, and A. E. Sobel. The complexometric titration of micro- and ultramicroquantities of calcium in blood, serum, urine and inorganic salt solutions. J. clin. Chem. 1958, **4**, 107.
12. Rich, C. The distribution of calcium given to human subjects by sustained intravenous infusion. J. clin. Endocr. 1960, **20**, 147.
13. Lutwak, L. Estimation of radioactive calcium-45 by liquid scintillation counting. Analyt. Chem. 1959, **31**, 340.
14. Heaney, R. P. Evaluation and interpretation of calcium-kinetic data in man. Clin. Orthop. 1963, **31**, 153.
15. Bauer, G. C. H., A. Carlsson, and B. Lindquist. Bone salt metabolism in humans studied by means of radiocalcium. Acta med. scand. 1957, **158**, 143.
16. Eisenberg, E., and G. S. Gordan. Skeletal dynamics in man measured by nonradioactive strontium. J. clin. Invest. 1961, **40**, 1809.
17. Heaney, R. P., and G. D. Whedon. Radiocalcium studies of bone formation rate in human metabolic bone disease. J. clin. Endocr. 1958, **18**, 1246.
18. Dymling, J. F. Calculations of calcium kinetics: interlaboratory comparison *in* Medical Uses of Ca^{47}: Second Panel Report. Vienna, International Atomic Energy Agency; New York, International Publications, 1964, p. 169.
19. Heaney, R. P., G. C. H. Bauer, F. Bronner, J. F. Dymling, F. W. Lafferty, B. E. C. Nordin, and C.

Rich. A normal reference standard for radiocalcium turnover and excretion in humans. J. Lab. clin. Med. 1964, **64**, 21.

20. Lafferty, F. W., and O. H. Pearson. Calcium[47] accretion and resorption rates in man *in* Dynamic Studies of Metabolic Bone Disease, O. H. Pearson and G. F. Joplin, Eds. Philadelphia, F. A. Davis, 1964, p. 25.
21. Blau, M., H. Spencer, J. Swernov, and D. Laszlo. Utilization and intestinal excretion of calcium in man. Science 1954, **120**, 1029.
22. Marshall, J. H. Theory of alkaline earth metabolism *in* Medical Uses of Ca^{47}: Second Panel Report. Vienna, International Atomic Energy Agency; New York, International Publications, 1964, pp. 21, 170, 177.
23. Bluhm, M. M., J. MacGregor, and B. E. C. Nordin. In vitro and in vivo studies with bone-seeking isotopes *in* Radioaktive Isotope in Klinik and Forschung. Strahlentherapie Sonderbande 1960, **45**, (suppl.), 29.
24. Lafferty, F. W., G. E. Spencer, Jr., and O. H. Pearson. Effects of androgens, estrogens and high calcium intakes on bone formation and resorption in osteoporosis. Amer. J. Med. 1964, **36**, 514.
25. Nordin, B. E. C. The pathogenesis of osteoporosis. Lancet 1961, **1**, 1011.
26. Nordin, B. E. C., H. Glass, D. Smith, J. MacGregor, J. Nisbet, and H. G. Burns. Bone formation and resorption rates in various clinical states *in* Medical Uses of Ca^{47}: Second Panel Report. Vienna, International Atomic Energy Agency; New York, International Publications, 1964, p. 75.
27. Jowsey, J., and J. Gershon-Cohen. Clinical and experimental osteoporosis *in* Bone and Tooth, H. J. J. Blackwood, Ed. New York, MacMillan, 1964, p. 35.
28. Marshall, J. H., R. E. Rowland, and J. Jowsey. Microscopic metabolism of calcium in bone. V. The paradox of diffuse activity and long-term exchange. Radiat. Res. 1959, **10**, 258.
29. Sissons, H. A., and W. R. Lee. Tetracycline studies of bone turnover *in* Bone and Tooth, H. J. J. Blackwood, Ed. New York, MacMillan, 1964, p. 65.
30. Rowland, R. E. Resorption and bone physiology *in* Bone Biodynamics, H. M. Frost, Ed. Boston, Little, Brown, 1964, p. 335.
31. Marshall, J. H. Microscopic metabolism of calcium in bone *in* Bone as a Tissue, K. Rodahl, J. T. Nicholson, and E. M. Brown, Eds. New York, McGraw-Hill, 1960, p. 144.
32. Bronner, F., L. J. Richelle, P. D. Saville, J. A. Nicholas, and J. R. Cobb. Quantitation of calcium metabolism in postmenopausal osteoporosis and in scoliosis. J. clin. Invest. 1963, **42**, 898.
33. Lafferty, F. W., C. H. Herndon, and O. H. Pearson. Pathogenesis of vitamin D-resistant rickets and the response to a high calcium intake. J. clin. Endocr. 1963, **23**, 903.
34. Albright, F. The process of bone resorption. Trans. Conf. on Metabolic Aspects Conval. 1946, **14**, 24.
35. Sissons, H. A. Experimental determination of rate of longitudinal bone growth. J. Anat. (Lond.) 1953, **87**, 228.
36. Frost, H. M. Bone Remodelling Dynamics. Springfield, Ill., Charles C Thomas, 1963, p. 65.
37. Arnold, J. S. Quantitative study of mineralization of bone as an organ and tissue in aging *in* Medical and Clinical Aspects of Aging, H. T. Blumenthal, Ed. New York, Columbia University Press, 1962, p. 206.
38. Harrison, M., and R. Fraser. The parathyroid glands and calcium deficiency in the rat. J. Endocr. 1960, **21**, 207.
39. Armstrong, W. D., M. Knowlton, and M. Gouze. Influence of estradiol and testosterone propionates on skeletal atrophy from disuse and on normal bones of mature rats. Endocrinology 1945, **36**, 313.
40. McLean, F. C., and A. M. Budy. Further studies on the mechanism of the effect of estrogen on bone. Trans. Conf. on Metabolic Aspects Conval. 1947, **15**, 114.
41. Heaney, R. P. Radiocalcium metabolism in disuse osteoporosis in man. Amer. J. Med. 1962, **33**, 188.
42. Gordan, G. S., and E. Eisenberg. The effect of œstrogens, androgens and corticoids on skeletal kinetics in man. Proc. roy. Soc. Med. 1963, **56**, 1027.
43. Lutwak, L. and G. D. Whedon. Osteoporosis. DM (Chic.) 1963, April.
44. Menczel, J., M. Li, H. Spencer, and J. Samachson. Metabolic effects of added calcium in osteoporosis. Fed. Proc. 1962, **21**, 386.
45. Frost, H. M. Postmenopausal osteoporosis: a disturbance in osteoclasia. J. Amer. Geriat. Soc. 1961, **9**, 1078.
46. Riggs, B. L., J. Jowsey, P. J. Kelly, and F. R. Keating. Bone remodelling in metabolic diseases assessed by quantitative microradiography. Clin. Res. 1964, **12**, 356.