Journal of Clinical Investigation
Vol. 43, No. 11, 1964

The Effect of Prednisone on Calcium Metabolism and Ca^{47} Kinetics in Patients with Multiple Myeloma and Hypercalcemia *

CARL J. BENTZEL,† PAUL P. CARBONE, AND LEON ROSENBERG WITH THE TECHNICAL ASSISTANCE OF MILLICENT BEAN

(From the Radiation Branch, Medicine Branch, and Metabolism Service, National Cancer Institute, Bethesda, Md.)

Hypercalcemia is a frequent complication of multiple myeloma and has been observed in 43% of the 76 patients admitted to the Clinical Center of the National Institutes of Health from 1953 to 1961 (2). The pathogenesis of the hypercalcemia associated with multiple myeloma has been attributed to excessive bone resorption (3). Carbohydrate-active steroids have been successfully used to correct hypercalcemia associated with sarcoidosis (4), idiopathic hypercalcemia of infancy (5), and neoplastic diseases including multiple myeloma (6). In idiopathic hypercalcemia of infancy and sarcoidosis, diseases in which calcium absorption is increased, cortisone has been shown to increase total fecal calcium (4, 5). Steroids may also alter calcium metabolism of bone (7) and modify calcium transport in the renal tubule (8). In a single patient with multiple myeloma and hypercalcemia, corticosteroid administration was associated with net calcium retention and decreased bone resorption (3).

The present study was designed to further study the effects of prednisone [1] on calcium metabolism in patients with multiple myeloma and hypercalcemia. Steroid-induced reversal of hypercalcemia is associated with a decrease in the miscible pool size and a distinct reduction in bone resorption.

Methods

Patients. Of the seven patients studied, five had multiple myeloma (Table I). Two control patients ("normals") without multiple myeloma or bone disease were studied (Table I). All patients were hospitalized in an air-conditioned metabolic ward and were ambulatory throughout the study.

Experimental design. All patients except G.J. were studied in a control phase of 15 to 24 days followed immediately by a treatment phase of 15 to 18 days. In addition, two patients (D.F. and W.G.) with multiple myeloma were restudied after cessation of prednisone therapy. These recovery phases of 6 and 10 days respectively were begun 5 and 10 days after the last dose of prednisone. One patient, E.B., was studied in reverse order, the treatment phase preceding the control phase. Because of adrenal insufficiency that developed on attempted prednisone withdrawal, E.B. was treated with physiological doses of prednisone (7.5 mg per day) during the control phase. Patient G.J. was studied for only 3 days before therapy. Treatment consisted of prednisone, 10 to 20 mg, administered orally every 6 or 8 hours. Except for G.J., Ca^{47} kinetic studies were performed at least twice on each patient, once during the control phase and again 5 to 8 days after institution of prednisone therapy.

Metabolic balance. Patients were given a fixed metabolic balance diet throughout control, treatment, and recovery phases. Three to 10 days of "trial" balance preceded the control phase of study to allow for adjustment to the balance diet. Diets were analyzed four to six times during each study. Sodium and potassium intakes were kept in the range of 40 to 80 mEq Na and 100 to 200 mEq K daily. Total fluid intake was held constant by providing a fixed quantity of distilled water daily. All medications that might influence calcium phosphorus metabolism were avoided (Table I). Medications were analyzed for calcium content, and the necessary additions were made to the daily calcium intake. Refused food and emeses were likewise analyzed and subtracted from the daily intake. Any inconstancy in dietary intake was due to these two factors. Voided urine was immediately pooled in a refrigerated jar containing 10 ml of concentrated HCl. Stools were separated at intervals of 5 or 6 days with carmine or FD & C blue no. 1 markers.[2] All stool determinations were

* Submitted for publication February 11, 1964; accepted July 9, 1964.

Part of this work has appeared in abstract form (1).

† Present address: Biophysical Laboratory, Harvard Medical School, Boston, Mass. 02115.

[1] 17α,21Dihydroxy-1,4-pregnadiene-3,11,20-trione.

[2] Contains FD & C blue no. 1 dye, 50 mg, and methylcellulose, 200 mg per capsule. Prepared by the Pharmaceutical Development Service, Pharmacy Department, Clinical Center, National Institutes of Health.

TABLE I

Clinical and laboratory data in five patients with multiple myeloma and two "normal" subjects

Patient Diagnosis	Age	Sex	Serum calcium	Serum phosphorus	Bone marrow†	Skeletal X ray†	Comments
			*mg/100 ml**	*mg/100 ml*			
G.J. Myeloma	38	F	18.4	1.7	80% abnormal plasma cells	Generalized decalcification, multiple lytic lesions	No anomalous serum proteins, parathyroid glands normal on post-mortem exam
W.G. Myeloma	62	F	12.4	4.6	90% abnormal plasma cells	Moderately severe decalcification, diffuse lytic lesions	β_{2A} serum myeloma protein, Bence Jones proteinuria
E.B. Myeloma	59	M	12.3	3.9	15% abnormal plasma cells	Severe decalcification, multiple lytic lesions, multiple old rib fractures, many old compression fractures of spine	Had received prednisone 15 mg/day for the year before study. Parathyroid glands normal on post-mortem exam. Bence Jones protein in serum and urine
A.M. Myeloma	56	F	11.2	3.8	50% to 90% abnormal plasma cells	Severe decalcification, multiple lytic lesions, two recent rib fractures, compression fracture of T8	γ serum myeloma protein, aspirin (1 to 3 g/day) during first two control periods. 1,600 roentgen X-ray therapy (5 × 3-inch port) to T8 during third control period
D.F. Myeloma	73	F	10.0	4.3	40% abnormal plasma cells	Moderate decalcification, diffuse lytic lesions	Arteriosclerotic heart disease. γ serum myeloma protein
C.R.‡ Epilepsy due to a-v malformation	31	M	9.9	2.8		Normal	
E.R.‡ Squamous cell carcinoma of tongue	60	F	10.3	4.1		Normal	Bilateral submandibular node metastases. Treated with X-ray therapy 4 weeks before study

* Average value: control study.
† Before treatment.
‡ "Normals."

made on pooled 5- or 6-day specimens. Ca, Na, and P were usually analyzed from daily urine samples. Urinary nitrogen (N) was analyzed from 5- or 6-day pooled specimens. The following analytical methods were used: nitrogen, macro-Kjeldahl (9); urinary, dietary, and fecal phosphorus, Taussky and Shorr (10); serum phosphorus, Reiner (11); stool, dietary, and urinary calcium, Kochakian and Fox (12); serum calcium, MacIntyre (13); serum alkaline phosphatase, King and Armstrong (14); and serum and urinary creatinines, Chassen, Grady, and Stanley (15). Diffusible serum calcium was measured under 5% CO_2 at 23° C on previously frozen, stored serum by the method of Toribara, Terepka, and Dewey (16). Sodium and potassium were analyzed on the Patwin flame photometer. Hydroxyproline, determined by the method of Prockop and Udenfriend (17), was measured from daily samples of urine. A minimum of four determinations was made at 2- to 4-day intervals when patients were receiving no therapy and again during prednisone treatment. The patients were not on low hydroxyproline diets. Renal function was evaluated two to four times a week by determining 24-hour endogenous creatinine clearances. All clearance results are presented uncorrected for body surface area.

Intravenous Ca^{47} studies. Ca^{47} (physical half life, 4.7 days) had a specific activity of 20 mc per g. Before administration to patients, a sample of the original Ca^{47} solution was sterilized and then assayed for radioactivity, along with standards prepared at the same time. The error in the radioassay was calculated to be less than 8%. The isotope was administered intravenously in water. Fifteen or 20 μc of $Ca^{47}Cl_2$ in 5 ml of isotonic saline was administered intravenously to each patient (except C.R. and W.G.) during the control phase of study and the same dosage repeated 5 to 8 days after prednisone treatment had been instituted. Patient C.R. received two injections of 6.5 μc each, and W.G. received an additional 14 μc during the recovery period. Fifteen days after the intravenous administration of 15 to 20 μc Ca^{47}, urine, stool, and serum radioactivity had returned to a count rate that was not statistically different from background. All counting was done in a single channel gamma ray spectrometer, with the lower gate set at 400 kev to exclude daughter decay product, Sc^{47}. Serum samples were obtained at 12- to 24-hour intervals, and 2- or 4-ml samples were counted in an automatic well-type scintillation counter.[3] Five-hundred-ml samples of 24-hour urine collections and 500-ml samples of 1- or 2-day homogenized stool collections were counted in quart paint cans in a well-type scintillation counter.[4] Assayed standards were prepared to conform to the geometry of the serum or stool and urine samples and were counted in the same manner. Appropriate correction was made for fecal lag in Ca^{47} excretion. All samples obtained within the first week after the Ca^{47} injection were counted sufficiently long to insure a counting error of less than 2%. Results were expressed as percentages or fractions of administered dose.

[3] Nuclear Chicago Corp. (model C-120), Des Plaines, Ill.

[4] Nuclear Chicago, model 1810.

Oral Ca^{47} studies and calcium absorption. In three patients with multiple myeloma, studies of calcium absorption were performed during the first week of the balance study or during trial balance and were repeated 15 to 20 days after the institution of prednisone therapy. The isotope (0.4 to 0.5 μc) was administered orally during breakfast. Stools were counted until radioactivity returned to background. Absorption was calculated from the equation, net Ca absorption (milligrams per day) = $(1 - F_e) \times$ dietary Ca (milligrams per day), where F_e = fraction of orally administered dose recovered in the stool.

Calcium kinetics. The conceptual model employed to determine the miscible calcium pool (E) and the bone formation rate (BFR) was taken from Heaney and Whedon (18). Serum specific activity was measured from 48 hours until θ and plotted semilogarithmically. θ is defined as the temporal point at which the slope of the Ca^{47} disappearance curve becomes less negative (120 to 216 hours) (18). The ordinate intercept (extrapolation of the linear portion of the specific activity curve to zero time) and the slope (k) of the serum specific activity curve were determined by the method of least squares (19) using five to nine specific activity values from 48 hours to θ. E was determined by dividing the ordinate intercept into the total administered dose. BFR was then calculated by the formula proposed by Heaney and Whedon (18): BFR = Ek $(1 - fu - fs)$, where E = miscible calcium pool, k = fractional rate of loss of Ca^{47} by all routes from E (k =

TABLE II

Metabolic balance of calcium

Patient	Prednisone treatment	Days	Serum calcium	Urinary calcium	Fecal calcium	Calcium intake	Calcium balance
	mg/day		*mg/100 ml*	*mg/day*	*mg/day*	*mg/day*	*mg/day*
Multiple myeloma							
G.J.	C*	1–3	18.4	559	309	127	−741
	80	4–8†	16.7	529	420	145	−804
		9–13	15.0	620	301	150	−654
		14–18	15.7	579	331	191	−719
		Mean	15.8	576	351	162	−726
W.G.	C	1–5†	12.1	348	346	272	−421
		6–13	13.0	381	354	272	−463
		Mean	12.6	368	351	272	−447
	40	14–20	11.1	400	388	272	−516
		21–25†	10.1	320	373	272	−421
		26–30	10.1	288	367	272	−383
		Mean	10.5	343	377	272	−449
	Recovery	47–52†	9.8	55	367	272	−149
		53–58	9.9	51	277	272	− 57
		Mean	9.9	53	322	272	−103
E.B.	7.5	19–24‡	12.1	174	369	234	−309
		31–36†	12.0	129	486	193	−422
		37–42	12.2	144	319	231	−232
		Mean	12.2	149	391	219	−321
	30	1–6	11.7	209	305	219	−295
		7–12†	11.5	191	305	230	−266
		13–18	11.6	178	362	247	−294
		Mean	11.6	193	324	232	−285
A.M.	C	1–6	10.5	172	852	171	−853
		7–12	11.0	326	368	180	−514
		13–18†	11.1	232	693	226	−699
		19–23	11.3	315	297	240	−373
		Mean	11.2	262	553	204	−610
	40	24–30	10.2	261	484	258	−487
		31–36†	10.0	230	376	258	−348
		37–42	9.7	260	386	258	−388
		Mean	9.8	250	415	253	−408

* Control phase of study.
† Metabolic period during which Ca^{47} study was begun.
‡ Following 6 days omitted during stabilization of prednisone dosage.

TABLE II—(*Continued*)

Patient	Prednisone treatment	Days	Serum calcium	Urinary calcium	Fecal calcium	Calcium intake	Calcium balance
	mg/day		*mg/100 ml*	*mg/day*	*mg/day*	*mg/day*	*mg/day*
D.F.	C	1–6	9.9	117	459	504	−72
		7–12†	10.3	122	352	509	+35
		13–18	10.0	127	632	506	−253
		Mean	10.1	122	481	506	−97
	40	19–24	9.9	109	394	499	−4
		25–30†	10.0	118	250	527	+159
		31–34	10.0	116	586	513	−189
		Mean	10.0	114	410	513	+11
	Recovery	55–60	9.7	150	484	463	−171
"Normal"							
C.R.	C	1–5	9.8	86	405	341	−150
		6–10†	10.0	91	405	343	−153
		11–15	9.9	105	229	343	+9
		16–20	10.0	97	256	344	−9
		Mean	10.0	95	324	343	−76
	40	21–25	10.3	129	369	341	−157
		25–30†	9.7	148	163	347	+36
		31–35	10.0	184	301	339	−146
		Mean	9.9	154	278	342	−89
E.R.	C	1–5	10.3	207	301	344	−161
		6–10†	10.2	155	220	344	−31
		11–16		179	344	344	−179
		Mean	10.3	180	292	344	−128
	40	17–25	10.0	260	217	344	−133
		26–30†	10.0	370	141	344	−66
		31–35	10.2	296	251	344	−203
		Mean	10.1	273	205	344	−134

$0.693/t_{\frac{1}{2}}$), and $fu = [Fu\ (t_2 - t_1)]/[e^{kt_1} - e^{kt_2}]$, where Fu $(t_2 - t_1)$ is the total fraction of the administered dose of Ca^{47} excreted in the urine from 48 hours to θ. (To exclude the "mixing phase," t_1 was arbitrarily chosen as 48 hours and $t_2 = \theta$.) $fs = [Fs\ (t_2 - t_1)]/[e^{kt_1} - e^{kt_2}]$, where Fs $(t_2 - t_1)$ = total fraction of the administered dose Ca^{47} excreted in stool from 48 hours to θ. Bone resorption rate (BRR) was determined by the relation, BFR + BRR = calcium balance (20). Calcium balance data used to calculate BRR were obtained from metabolic periods in which isotopic measurements were made.

Results

The effect of prednisone on serum calcium concentration. In the four patients with hypercalcemia and myeloma, steroid administration was associated with a reduction in serum calcium (Table II). A comparison of the average serum calcium concentration measured 4 days before institution of therapy to average measurements obtained during the first 7 days after therapy revealed a mean decrease of 2.2 mg per 100 ml (range, 3.3 to 0.8 mg per 100 ml). In patient E.B., studied initially on higher prednisone dosage, an increase in serum calcium concentration occurred when the prednisone dose was reduced (Table II). In the three normocalcemic patients, prednisone therapy resulted in a decrease in the mean serum calcium concentration of 0.2 mg per 100 ml (range, 0.4 to 0.1 mg per 100 ml).

Effect of prednisone on calcium balance. During the control phase of study, all seven patients were in negative calcium balance (Table II). The hypercalcemic patients with multiple myeloma were in a greater degree of negative balance than D.F. and the two normal patients. The patients with multiple myeloma and hypercalcemia had higher urinary and fecal calcium values than did normal subjects on comparable intakes (21). In four of the five patients with multiple myeloma, the urinary calcium excretion was related to the degree of hypercalcemia, with G.J. showing the

TABLE III

*Results of Ca^{47} studies**

Patient	Prednisone treatment	Weight	E	$t_{\frac{1}{2}}$	k	BFR	BRR	θ	Ca^{47} absorption
	mg/day	*kg*	*g*	*days*	*day^{-1}*	*g/day*	*g/day*	*day*	*% oral dose*
Multiple myeloma									
G.J.	C								13
	80	36.65	8.44	3.67	0.189	1.50	2.23	9	
W.G.	C	48.10	5.37	3.10	0.224	1.08	1.53	6	21
	40	45.63	4.20	2.84	0.244	0.91	1.31	5	19†
	Recovery	46.37	4.43	3.21	0.216	0.92	1.02	7	
A.M.	C	53.92	7.62	3.81	0.182	1.32	1.87	8	28
	40	48.37	4.92	3.54	0.196	0.88	1.25	7	19
E.B.	7.5	61.00	7.93	5.55	0.125	0.92	1.25	9	
	30	61.70	5.74	4.23	0.164	0.84	1.12	8	24
D.F.	C	53.18	3.95	4.47	0.155	0.50	0.61	6	17
	40	51.78	3.27	4.75	0.146	0.39	0.37	6	12
	Recovery								30‡
"Normal"									
C.R.	C	64.00	7.03	4.47	0.155	1.02	1.09	8	45
	40	63.13	6.24	4.17	0.166	0.99	1.10	8	
E.R.	C	63.79	5.05	4.82	0.144	0.65	0.76	8	55
	40	62.50	3.96	3.78	0.183	0.62	0.76	8	

* See Appendix for 95% confidence limits from which E (the miscible calcium pool), k (the slope of the serum specific activity curve), BFR (bone formation rate), and BRR (bone resorption rate) were calculated. θ = the temporal point at which the slope of the Ca^{47} disappearance curve becomes less negative.

† Measurement incomplete.

‡ Measured days after prednisone.

largest daily calcium excretion and D.F., the least (Table II).

In the patients with multiple myeloma (excluding patient G.J., who had only a 3-day control study) prednisone therapy was associated with a decrease in the mean, net calcium loss (A.M., E.B., and D.F.) or no change in mean calcium balance (W.G.). Thus, at a time when serum calcium concentration was decreasing, the changes in balance were in the direction of relative net calcium retention (Table II). In contrast, during prednisone treatment, the two normal patients, C.R. and E.R., demonstrated an increased rate of urinary calcium excretion, with a decrease in stool calcium resulting in a slightly more negative balance (Table II). In these two patients, the enhanced urinary calcium excretion occurred gradually and continued to increase as long as the patients were maintained on prednisone.

Nitrogen and phosphorus balance. In the control phase, all patients were in positive nitrogen balance with the exception of A.M. and W.G. All patients with myeloma, with the exception of D.F., were in negative phosphorus balance during the control phase of study. Prednisone therapy was associated with a net P and N loss. The two patients without bone disease showed similar increased losses of P and N during the treatment phase. Serum Na, K, P, and alkaline phosphatase concentrations did not change during prednisone treatment.

The effect of prednisone on intestinal absorption of calcium. The absorption of Ca^{47} in patients with myeloma averaged 22% during control periods and 18% during prednisone therapy (Table III). The normal patients, E.R. and C.R., absorbed 55 and 45%, respectively, while on no treatment. In D.F., studied after cessation of prednisone therapy (recovery phase), oral Ca^{47} absorption increased from 12 to 30%. In those patients on low calcium intakes (200 to 272 mg per day), net changes in absorbed calcium during prednisone therapy were less than 10 mg per day.

The effects of prednisone on renal function

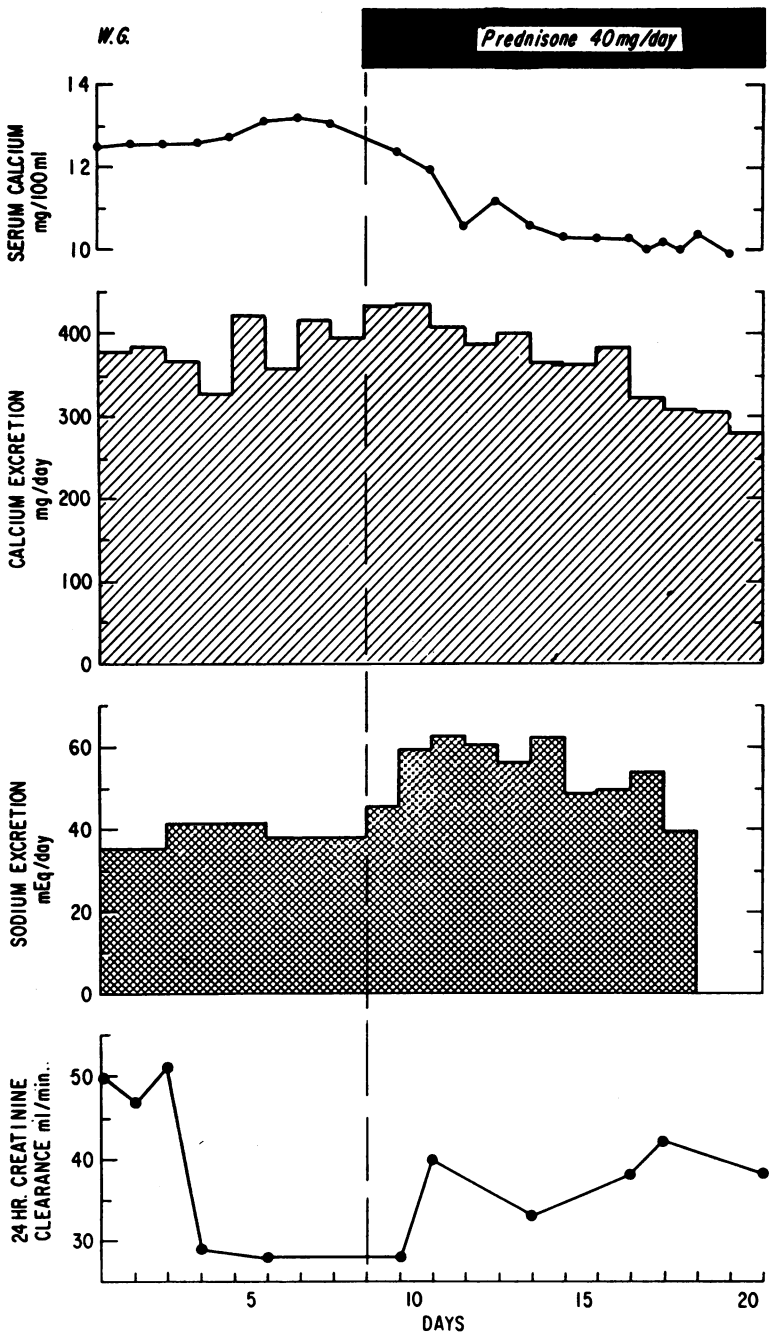

FIG. 1. URINARY CALCIUM AND SODIUM EXCRETION AND 24-HOUR CREATININE CLEARANCE IN PATIENT W.G. BEFORE AND DURING PREDNISONE THERAPY. Changes in serum calcium concentration are represented at the top of the graph. Sodium excretion was determined from pooled 2- or 3-day urine collections. Sodium intake was 45 mEq per day.

and on the renal excretion of calcium and sodium. The reduction in serum calcium concentration after institution of prednisone therapy was not associated with an increase in calcium excretion in the urine (Figures 1 and 2). In three of the five patients, sodium excretion rose significantly

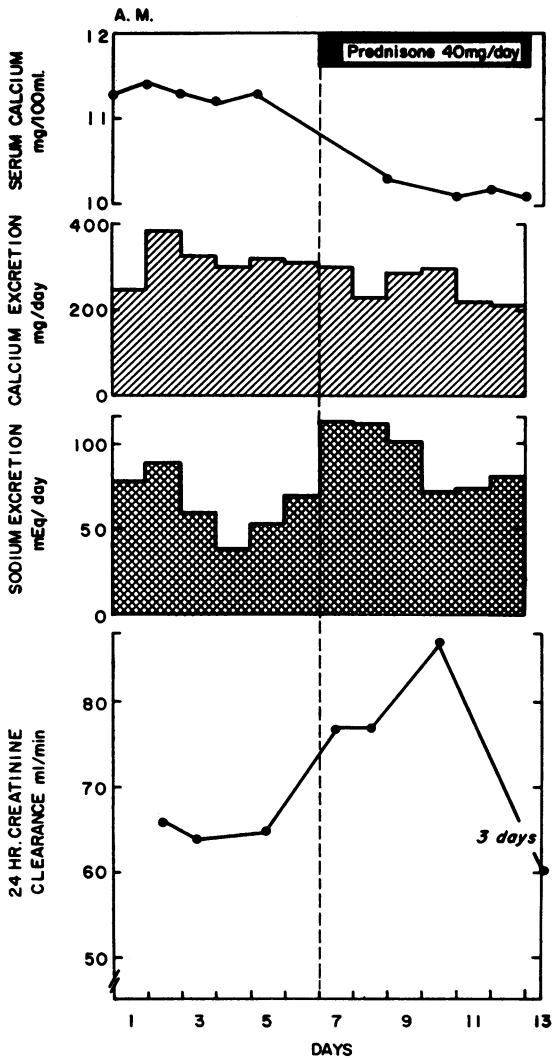

FIG. 2. URINARY CALCIUM AND SODIUM EXCRETION AND 24-HOUR CREATININE CLEARANCE IN PATIENT A.M. BEFORE AND DURING PREDNISONE THERAPY. Sodium intake was 62 mEq per day.

during the first week of prednisone therapy. In two patients, A.M. and W.G., an increase in creatinine clearance was observed after the institution of prednisone therapy (Figures 1 and 2). In patient D.F., sodium excretion increased from 61 to 89 mEq per day within 3 days after prednisone therapy. However, no change in serum calcium concentration (10.4 to 10.0 mg per 100 ml), calcium excretion (115 to 117 mg per day), or creatinine clearance (55 to 50 ml per minute) occurred.

The effect of prednisone on serum protein-bound calcium. Of the five patients with multiple myeloma, three (A.M., D.F., and W.G.) had an increased total serum protein concentration > 9.0 g per 100 ml (Figure 3). Prednisone therapy was associated with reductions in both dialyzable and total serum calcium. Therefore, the percentage of bound calcium remained essentially unchanged (Figure 3). In three patients, W.G., A.M., and D.F., reduction in the total serum protein concentration, 1 to 1.5 g per 100 ml during prednisone therapy, was due to a decrease in the myeloma protein fraction (Figure 3).

Effect of prednisone on Ca^{47} kinetics. *A*) *Miscible calcium pool* (*E*). In four patients with hypercalcemia, the miscible Ca pool size (E) measured during the control phase was either elevated or at the upper limits of normal (22) (Table III). During prednisone therapy, the four patients with multiple myeloma (G.J. not included) had a mean reduction in pool size of 25% (range, 17 to 35%, $p < 0.05$) (19). Expressed in grams, patient A.M. demonstrated the largest reduction (2.70 g) and D.F., the least change (0.68 g). In the two normal patients the decrease in E was 11 and 22% ($p < 0.2$).

The 95% confidence limits for the determination of the ordinate intercept and the slope for each of the serum specific activity curves are given in the Appendix. Although all patients showed a similar directional change, there was overlap in the 95% confidence limits in most patients, especially in determination of the ordinate intercept.

B) *Bone formation rate* (*BFR*). Two of the four hypercalcemia patients had an elevated BFR during their initial study. All four patients with multiple myeloma had a reduction in BFR as a result of prednisone therapy (Table III).

C) *Bone resorption rate* (*BRR*). During the control period, BRR was greater than BFR in all patients (Table III). Hypercalcemic patients with multiple myeloma had the highest BRR and also the greatest differences between BRR and BFR. During prednisone therapy, BRR was reduced in all patients with multiple myeloma ($p < 0.05$). The normal patients showed no significant change in BRR (Table III).

In Figure 4 the changes in E, BFR, and BRR during prednisone treatment are expressed as percentage of control. In the patients with multiple myeloma, the mean decrease in BFR was 20%.

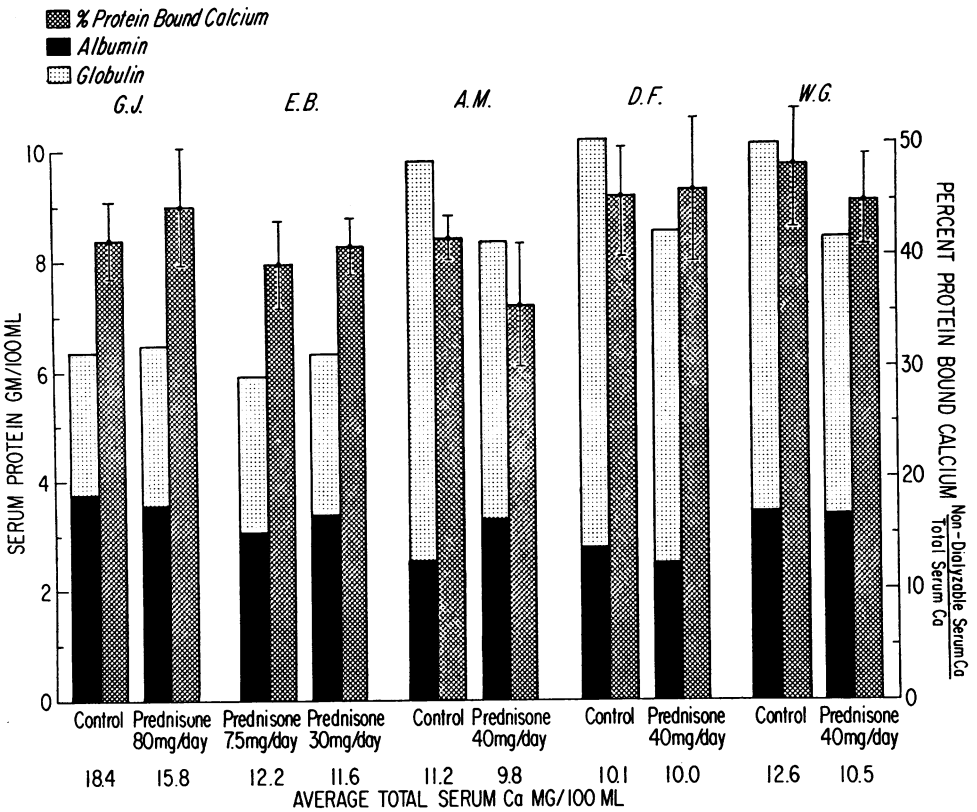

FIG. 3. THE EFFECT OF PREDNISONE THERAPY ON SERUM PROTEIN CONCENTRATION AND PERCENTAGE OF PROTEIN-BOUND SERUM CALCIUM IN PATIENTS WITH MULTIPLE MYELOMA. The mean serum calcium concentration given at the bottom of the figure was determined during the period in which dialyzable serum calcium was measured. The vertical bars at the top of the percentage of bound serum calcium column represent 1 SD.

The change in BRR was relatively greater (mean decrease, 24%). The normal patients had very little change in either BFR or BRR.

In Figure 5 calcium balance data are shown for W.G. in relation to the Ca^{47} kinetic studies. During control, hypercalcemia was associated with an elevated urinary calcium excretion, and E was 112 mg per kg (5.37 g). After prednisone therapy, the serum calcium returned to normal within 4 days, and urinary calcium excretion gradually decreased. The second Ca^{47} study was performed on day 21, and by this time, calcium balance was less negative than during control. E was reduced from 112 to 92 mg per kg, or a total reduction of 1.19 g. The calcium loss from E is not accounted for by balance measurements. After cessation of prednisone therapy, calcium balance was only slightly negative, and serum calcium concentration remained normal.

Hydroxyproline excretion. In all patients with multiple myeloma, the mean hydroxyproline excretion measured during the control phase was higher than that measured during the treatment phase (Table IV). However, only two patients showed reductions of more than 1 SD. The two normal patients showed a slight increase in hydroxyproline excretion during prednisone therapy. Although there was no quantitative relationship between the change in percentage of BRR and the change in hydroxyproline excretion, all patients with myeloma who had a decrease in BRR also demonstrated a mean decrease in hydroxyproline excretion. Reductions in bone formation rate also occurred in all patients with relatively less change noted in the two patients, E.R. and C.R. (Table IV).

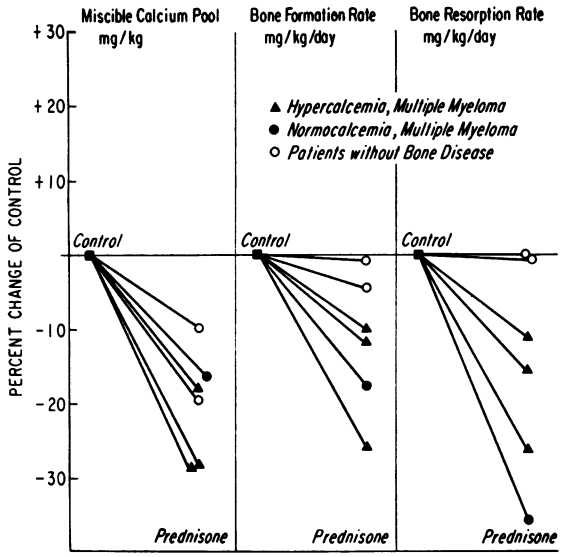

FIG. 4. CHANGES IN MISCIBLE CALCIUM POOL SIZE (E), BONE FORMATION RATE (BFR), AND BONE RESORPTION RATE (BRR) ASSOCIATED WITH PREDNISONE THERAPY.

Discussion

Evaluation of the method. The model system for calcium metabolism employed in these studies was suggested by Heaney and Whedon and others (18, 20). Since reviews have recently been published evaluating calcium kinetic methods and their application to metabolic balance techniques (20, 23), only those aspects pertinent to our studies will be discussed. It is assumed that the serum calcium is readily exchangeable within the miscible calcium pool and that changes in serum calcium specific activity with time, after a single injection of a tracer dose of Ca^{47}, can be expressed mathematically by a series of exponential terms (20). In most human studies, including patients with multiple myeloma (24), the serum specific activity decreases monoexponentially from 36 to 210 hours after a single intravenous injection of a calcium isotope. During this time, the miscible calcium pool, the bone formation rate or accretion rate, and if calcium balance is included, the bone resorption rate can be determined (20). In repetitive studies on the same patient, determination of E by the simplified method (extrapolation of the linear portion of the specific activity curve to 0) is justified if E is relatively small and if changes in disease activity or calcium metabolism between the two studies are not great (25). In these studies the patient served as his own control. Similar portions of the specific activity curve were analyzed during each phase of study, and control studies immediately preceded or followed treatment studies. However, in performing Ca^{47} studies in close time sequence, even though serum radioactivity is within background counting error, it is possible that isotope fixed to bone from a previous injection might recycle due to bone resorption and influence the serum specific activity curve. Total body counting studies demonstrate a continued loss of previously injected Ca^{47} for periods up to 1 month, but the rate of loss, even in multiple myeloma, is less than 2% per day (26). Recycling of previously administered isotope would cause a reduc-

TABLE IV

Mean urinary hydroxyproline excretion: relationship to bone resorption rate determined by Ca^{47} and calcium balance studies

	Patient	Hydroxyproline excretion		Hydroxyproline excretion	Bone resorption rate
		Control*	Prednisone*		
		mg/day	*mg/day*	*% change of mean†*	*% change of mean‡*
Multiple myeloma					
	G.J.§	77.7 ± 2.1	66.0 ± 10.6	−15.1	
	G.W.	41.5 ± 4.7	32.3 ± 3.4	−22.2	−14.4
	E.B.	33.8 ± 1.6	27.1 ± 0.5	−19.8	−10.4
	A.M.§	69.7 ± 5.3	58.0 ± 17.0	−17.8	−33.2
	D.F.	22.8 ± 1.3	21.8 ± 2.4	− 4.6	−39.3
"Normal"	C.R.	57.6 ± 1.4	63.7 ± 4.3	+10.6	+ 0.9
	E.R.	49.5 ± 3.9	58.8 ± 6.2	+18.8	0

* Mean ± 1 SD.
† [(1 − mean excretion, prednisone) × 100]/[mean excretion, control].
‡ [(1 × BRR, prednisone) × 100]/[BRR, control].
§ Gelatin, 20 mg per day, in diet.

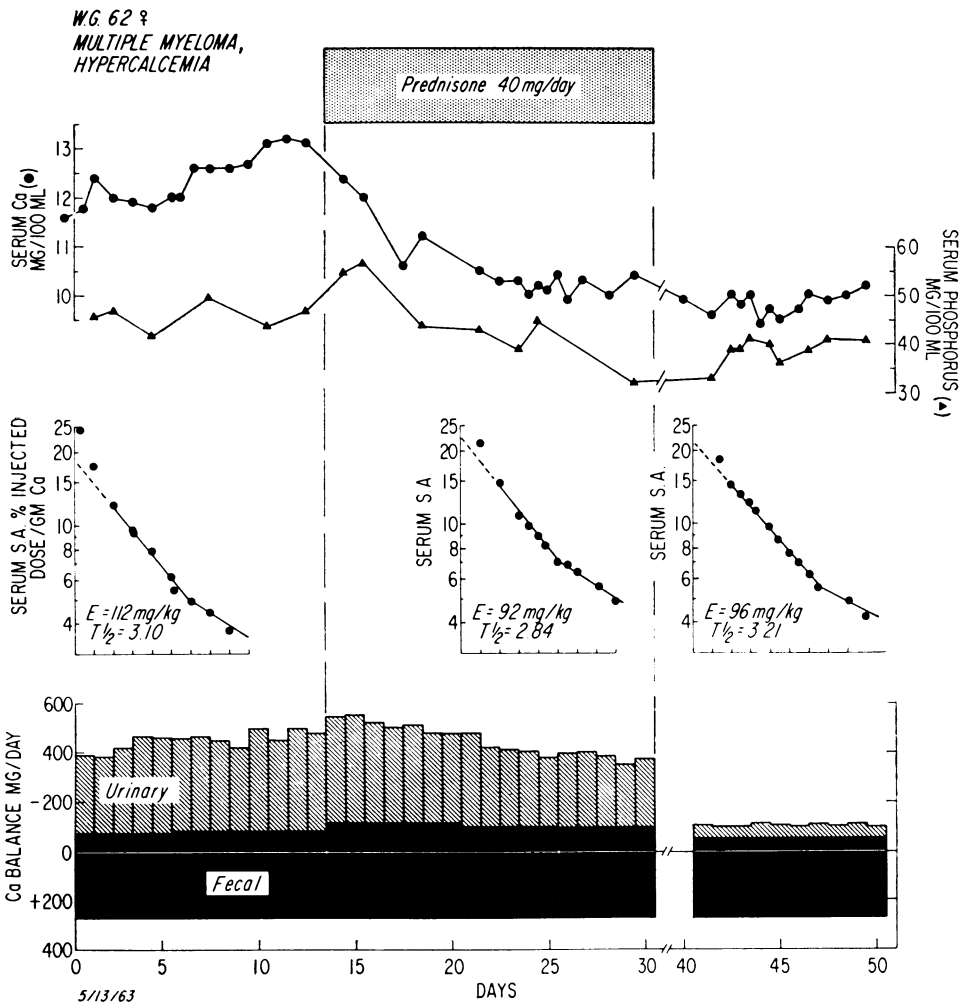

FIG. 5. CALCIUM BALANCE AND Ca^{47} SERUM SPECIFIC ACTIVITY CURVES IN PATIENT W.G. BEFORE, DURING, AND AFTER PREDNISONE THERAPY. The serum specific activity curve is expressed as percentage of injected dose Ca^{47} per g of serum calcium. The measurements of E and $t_{\frac{1}{2}}$ were calculated by the method of least squares using a minimum of six determinations taken between days 2 and 6. The decrease in negative slope of the specific activity curve at day 5 or 6 formed θ. In the balance graph, intake is plotted downward; fecal excretion (solid area) and urinary excretion (crosshatched area) are plotted upward.

tion in the negative slope of the second serum specific activity curve. However, in four of five patients studied, the slope of the serum specific activity curve became more negative rather than less negative in the second study performed during prednisone treatment. In repetitive studies in dogs using Ca^{47}, a 15-day interval between studies was considered sufficient to exclude significant error from previous isotope administration (27).

Effect of prednisone on calcium metabolism. In all hypercalcemic patients with multiple myeloma, a reduction in serum calcium concentration and miscible pool size was observed during prednisone therapy. According to the model of calcium metabolism used in these studies, calcium may leave the miscible pool by excretion through the gastrointestinal tract or kidney or through accretion into bone (bone formation). Calcium enters the miscible pool through bone resorption or gastrointestinal absorption. If the primary effect of prednisone in these patients were to increase excretion of calcium from the pool through the kidney or gastrointestinal tract, calcium balance should become more negative during predni-

sone treatment. An inhibitory effect on calcium absorption by prednisone would also increase total fecal calcium, thereby increasing negative calcium balance, providing endogenous fecal excretion remained unchanged. However, calcium balance either did not change or became less negative during treatment, and in no instance could balance changes account for pool size reduction.

Negative calcium balance in multiple myeloma, even on high dietary intakes, is a common observation (24, 28), and an amelioration of negative calcium balance in patients with myeloma during therapy with carbohydrate-active steroids has been previously observed (29, 30). In the present balance studies, it seems unlikely that the improvement in the degree of negative calcium balance was due to adaptation to a low calcium intake, which in normals requires 2 to 3 months (21). Also, it seems unlikely that spontaneous improvement in disease activity could account for observed calcium balance changes, since control studies were performed immediately before treatment.

Balance data indicated that major changes in gastrointestinal calcium absorption did not occur during prednisone therapy. This impression was confirmed in three patients with multiple myeloma studied by oral Ca^{47} administration. Prednisone produced a small decrease in Ca^{47} absorption in two of the three patients, but in each case the change could account for only a minute fraction of the change in pool size. In the third patient, no change in Ca^{47} absorption was noted after institution of prednisone therapy. Since the method used measured total fecal Ca^{47} activity, it measured both unabsorbed Ca^{47} and absorbed Ca^{47} recycled through endogenous secretion. However, the latter component has been shown to account for a small fraction of the recovered isotope (31). The percentage of absorption measured by oral administration of Ca^{47} agreed within $\pm$ 10% with values obtained using a total body counter (32). Calcium absorption ranged from 12 to 30% in the patients with myeloma, compared to values of 40 to 70% observed in normal subjects in the present study and previously reported (20, 33).

In patients with myeloma and hypercalcemia, prednisone resulted in a prompt fall in serum calcium and a gradual, inconstant reduction in urinary calcium excretion, suggesting an increase in calcium clearance. Such an increase could result from decreased tubular reabsorption, increased glomerular filtration, or both. The latter possibility was suggested by increased endogenous creatinine clearances in two patients, but small changes in tubular reabsorption may also have occurred. A transient rise in renal calcium clearance following institution of prednisone therapy could account for the rapid reduction in serum calcium concentration observed in the hypercalcemic patients with multiple myeloma. This explanation would, by necessity, separate the mechanism of the fall in serum calcium from that of the reduction in miscible pool size and would contend that the latter observation resulted from induced alterations in exchange between the miscible pool and skeletal calcium stores.

Alternatively, the effects of prednisone observed in these patients can be ascribed to changes in skeletal metabolism alone. Sissons reported that the decreased bone formation observed in patients with Cushing's syndrome was due to impaired osteoblastic activity in the presence of normal osteoclastic function (7). Clark, Geoffroy, and Bowers found that hydrocortisone decreased Ca^{45} uptake by the rat femur (34). Other studies have been reported which show that adrenal carbohydrate-active steroids may also alter the rate of bone resorption (35–38). Lazor and Rosenberg, reporting data obtained from a single patient with multiple myeloma and hypercalcemia, observed a reduction in bone formation rate and bone resorption rate when hypercalcemia was corrected by prednisone (3). Similarly, in our four patients with multiple myeloma who had control and treatment Ca^{47} studies, prednisone therapy was associated with a decrease in both the bone formation rate and bone resorption rate. To explain the reduction in miscible calcium pool size in these patients according to the conceptual model, it is necessary to conclude that the reduction in the bone resorption rate was relatively greater than the reduction in bone formation rate. We propose, therefore, that prednisone modified existing equilibrium conditions in bone, favoring a reduction in miscible pool size, which in turn, was reflected as a decrease in the serum calcium values. If hydroxyproline excretion is a measure of bone resorption, data demonstrating a mean

decrease in hydroxyproline in all myeloma patients during prednisone therapy are in agreement with the Ca^{47} data (39).

Hypercalcemia in multiple myeloma may occur in the absence of anomalous serum proteins, as in G.J., or when serum protein concentration is normal, as in E.B. and G.J. The latter observation was frequently noted in the series compiled by Gutman, Tyson, and Gutman (40). Previously reported evidence for abnormal serum protein binding of calcium in multiple myeloma is conflicting (41, 42). The average percentage of bound calcium in the five patients with myeloma in the present study was 43%, and prednisone therapy resulted in no change in binding. The reduction in serum calcium during treatment was, therefore, due to a decrease in total calcium and diffusible calcium.

A specific antitumor effect of prednisone on the myeloma cells might account for the beneficial effects of prednisone on the hypercalcemia. Cortisone and prednisone have not increased life expectancy in multiple myeloma (43). However, prednisone may exert a transient effect on tumor cell activity in some patients as shown by improvement in bone pain, marrow morphology, hemoglobin, and a decrease in anomalous urine and serum protein (28, 30). In our patients, anomalous serum protein concentration decreased in all in which it was initially elevated (Figure 4). A decrease in percentage of plasma cells was observed in the bone marrow in all three of the patients in which it was examined. Assuming that the high rate of bone resorption is due to myeloma cell activity, an antitumor effect of prednisone would decrease the rate of calcium loss into the miscible pool, accounting for the observed differences between the myeloma and the normal patients.

Summary

Five patients with multiple myeloma, four of whom were hypercalcemic, and two "normocalcemic" patients without bone disease or multiple myeloma were studied by combining metabolic balance and Ca^{47} kinetic studies to determine the effect of prednisone on calcium metabolism. Prednisone therapy resulted in a reduction in serum calcium concentration in all hypercalcemic patients but was without effect in the patients with normal serum calcium concentrations. In patients with multiple myeloma, prednisone therapy was associated with a decrease in the degree of negative balance or no change in calcium balance. Miscible calcium pool size, bone formation rate, and bone resorption rate as determined by Ca^{47} kinetic studies were elevated in the hypercalcemic myeloma patients and were reduced by prednisone therapy. Only small changes in calcium absorption and renal calcium transport were observed during prednisone therapy. No change in serum calcium binding during prednisone treatment was observed. It is, therefore, proposed that prednisone altered calcium exchange in and out of bone and favored the establishment of a new equilibrium at reduced pool size, in part through an antitumor effect on malignant plasma cells.

Acknowledgments

The authors wish to express their appreciation to Miss Elaine Gaddis, Miss Eileen Jones, and their respective staffs on the metabolism unit of the National Cancer Institute for their assistance in the metabolic studies. We also wish to thank Mr. William H. Briner and his staff in the Radiopharmaceutical Service for their careful preparation of the isotope. We are indebted to Drs. Albert Sjoerdsma, Harry R. Keiser, and E. Carwile LeRoy for the hydroxyproline measurements, to Miss Jean Epley and Dr. Ingborg C. Radde for the serum dialyzable calcium measurements, and to Dr. Norman H. Bell, Mrs. Lillian Fisher, Dr. Nathaniel I. Berlin, and Dr. J. Robert Andrews for their guidance and advice.

APPENDIX

95% confidence limits for ordinate intercept (a) and slope (b) from Ca^{47} serum specific activity curves

Patient	Prednisone treatment	a	b
	mg/day		
Multiple myeloma			
G.J.	80	2.472 ±0.229	0.189 ±0.009
W.G.	C	2.925 ±0.194	0.224 ±0.008
	40	3.170 ±0.225	0.244 ±0.011
	Recovery	3.117 ±0.059	0.216 ±0.005
A.M.	C	2.574 ±0.128	0.182 ±0.011
	40	3.011 ±0.169	0.196 ±0.019
E.B.	7.5	2.534 ±0.328	0.125 ±0.023
	30	2.858 ±0.350	0.164 ±0.024
D.F.	C	3.232 ±0.216	0.155 ±0.032
	40	3.421 ±0.169	0.146 ±0.023
"Normal"			
C.R.	C	2.655 ±0.166	0.155 ±0.013
	40	2.774 ±0.060	0.166 ±0.017
E.R.	C	2.985 ±0.155	0.144 ±0.011
	40	3.229 ±0.541	0.183 ±0.051

References

1. Lazor, M. Z., L. Rosenberg, and C. J. Bentzel. Mechanism of adrenal steroid reversal of hypercalcemia in multiple myeloma. Clin. Res. 1963, 11, 226.
2. Carbone, P. P. Unpublished observations.
3. Lazor, M. Z., and L. E. Rosenberg. Mechanism of adrenal-steroid reversal of hypercalcemia in multiple myeloma. New Engl. J. Med. 1964, **270**, 749.
4. Anderson, J., C. E. Dent, C. Harper, and G. R. Philpot. Effect of cortisone on calcium metabolism in sarcoidosis with hypercalcemia. Possible antagonistic actions of cortisone and vitamin D. Lancet 1954, **2**, 720.
5. Morgan, H. G., R. G. Mitchell, J. M. Stowers, and J. Thomson. Metabolic studies on two infants with idiopathic hypercalcemia. Lancet 1956, 1, 925.
6. Howard, J. E., and W. C. Thomas, Jr. Clinical disorders of calcium homeostasis. Medicine (Baltimore) 1963, **42**, 25.
7. Sissons, H. A. The osteoporosis of Cushing's syndrome. J. Bone Jt Surg. 1956, **38B**, 418.
8. Laake, H. The action of corticosteroids on the renal reabsorption of calcium. Acta endocr. (Kbh.) 1960, **34**, 60.
9. Pierce, C. P., and E. L. Haenisch. Quantitative Analysis. New York, John Wiley & Sons, 1950, p. 140.
10. Taussky, H. H., and E. S. Shorr. A microcolorimetric method for the determination of inorganic phosphorus. J. biol. Chem. 1953, **202**, 675.
11. Reiner, M. Standard Methods of Clinical Chemistry. New York, Academic Press, 1953, p. 84.
12. Kochakian, C. D., and R. P. Fox. Microdetermination of calcium by titration of the oxalate with ammonium hexanitratocerate. Indust. Eng. Chem. (analyt. ed.) 1944, **16**, 762.
13. MacIntyre, I. The flame-spectrophotometric determination of calcium in biological fluids and an isotopic analysis of the errors in the Kramer-Tisdall procedure. Biochem. J. 1957, **67**, 164.
14. King, E. J., and A. R. Armstrong. A convenient method for determining serum and bile phosphatase activity. Canad. med. Ass. J. 1934, 31, 376.
15. Chassen, A. L., H. J. Grady, and M. A. Stanley. Determination of creatinine by means of automatic chemical analysis. Amer. J. clin. Path. 1961, 35, 83.
16. Toribara, T. Y., A. R. Terepka, and P. A. Dewey. The ultrafiltrable calcium of human serum. I. Ultrafiltration methods and normal values. J. clin. Invest. 1957, **36**, 738.
17. Prockop, D. J., and S. Udenfriend. A specific method for the analysis of hydroxyproline in tissues and urine. Analyt. Biochem. 1960, 1, 228.
18. Heaney, R. P., and G. D. Whedon. Radiocalcium studies of bone formation rate in human metabolic bone disease. J. clin. Endocr. 1958, **18**, 1246.
19. Snedecor, G. W. Statistical Methods: Applied to Experiments in Agriculture and Biology. Ames, Iowa State College Press, 1946, pp. 54–137.
20. Aubert, J.-P., F. Bronner, and L. J. Richelle. Quantitation of calcium metabolism. Theory. J. clin. Invest. 1963, **42**, 885.
21. Malm, O. J. Calcium requirement and adaptation in adult men. Scand. J. clin. Lab. Invest. 1958, **10** (suppl. 36), 1.
22. Bell, N. H., J. R. Gill, Jr., and F. C. Bartter. On the abnormal calcium absorption in sarcoidosis: evidence for increased sensitivity to vitamin D. Amer. J. Med. 1964, **36**, 500.
23. Heaney, R. P. Evaluation and interpretation of calcium-kinetic data in man. Clin. Orthop. 1963, **31**, 153.
24. Lazor, M. Z., L. Rosenberg, and P. Carbone. Studies of calcium metabolism in multiple myeloma with calcium^{47} and metabolic-balance techniques. J. clin. Invest. 1963, **42**, 1238.
25. Aubert, J.-P., and G. Milhaud. Méthode de mesure des principales voies du métabolisme calcique chez l'homme. Biochim. biophys. Acta (Amst.) 1960, **39**, 122.
26. Cohn, S. H., S. W. Lippincott, E. A. Gusmano, and J. S. Robertson. Comparative kinetics of Ca^{47} and Sr^{85} in man. Radiat. Res. 1963, **19**, 104.
27. Collins, E. J., E. R. Garrett, and R. L. Johnston. Effect of adrenal steroids on radio-calcium metabolism in dogs. Metabolism 1962, **11**, 716.
28. Adams, W. S., E. D. Mason, and S. H. Bassett. Metabolic balance investigation of three cases of multiple myeloma during ACTH administration; exchanges of calcium, phosphorus, nitrogen and electrolytes. J. clin. Invest. 1954, 33, 103.
29. Skoog, W. A., and W. S. Adams. Metabolic balance study of a patient with multiple myeloma treated with dexamethasone. Amer. J. Med. 1963, **34**, 417.
30. Skoog, W. A., W. S. Adams, and N. S. MacDonald. Metabolic balance and Sr^{85} study of a patient with multiple myeloma treated with corticosteroids and androgens. Metabolism 1962, **11**, 421.
31. Blau, M., H. Spencer, J. Swernov, and D. Laszlo. Utilization and intestinal excretion of calcium in man. Science 1954, **120**, 1029.
32. Bentzel, C. J. Unpublished observations.
33. Bronner, F. Experimental studies of calcium absorption in man. Nutr. et Dieta (Basel) 1962, 3, 22.
34. Clark, I., R. F. Geoffroy, and W. Bowers. Effects of adrenal cortical steroids on calcium metabolism. Endocrinology 1959, **64**, 849.
35. Eisenberg, E. Effects of steroid on calcium dynamics *in* Biological Activities of Steroids in Relation to Cancer. New York, Academic Press, 1960, p. 189.

36. Milhaud, G., W. Remagen, G. Dematos, and J.-P. Aubert. Étude du métabolisme du calcium chez le rat a l'aide, du calcium[45] II action de la cortisone. Rev. franç. Étud. clin. biol. 1960, **5**, 354.
37. Grollman, A. Effect of cortisone on serum calcium, magnesium, and phosphate levels in nephrectomized dogs. Proc. Soc. exp. Biol. (N. Y.) 1954, **85**, 582.
38. Garrett, E. R., R. L. Johnston, and E. J. Collins. Kinetics of steroid effects on Ca^{47} dynamics in dogs with the analog computer II. J. pharm. Sci. 1963, **52**, 668.
39. Dull, T. A., and P. H. Henneman. Urinary hydroxyproline as an index of collagen turnover in bone. New Engl. J. Med. 1963, **268**, 132.
40. Gutman, A. B., R. L. Tyson, and E. B. Gutman. Serum calcium inorganic phosphorus and phosphatase activity in hyperparathyroidism, Paget's disease, multiple myeloma and neoplastic disease of the bones. Arch. intern. Med. 1939, **57**, 378.
41. Glueck, H. I., L. Wayne, and R. Goldsmith. Abnormal calcium binding associated with hyperglobulinemia, clotting defects, and osteoporosis: a study of this relationship. J. Lab. clin. Med. 1962, **59**, 40.
42. Walser, M. The separate effects of hyperparathyroidism, hypercalcemia of malignancy, renal failure, and acidosis on the state of calcium, phosphate, and other ions in plasma. J. clin. Invest. 1962, **41**, 1454.
43. Mass, R. E. A comparison of the effect of prednisone and a placebo in the treatment of multiple myeloma. Cancer Chemother. Rep. 1962, **16**, 257.