FACTORS INFLUENCING THE URINARY EXCRETION OF CALCIUM.[1] I. IN NORMAL PERSONS

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(Received for publication March 8, 1946)

INTRODUCTION

It is common knowledge that the urinary calcium may be markedly altered from normal in many diseases affecting calcium metabolism. The normal range of urinary calcium is very wide, and its limits have never been determined; hence, use of the amount of urinary calcium for diagnostic purposes is limited to those patients whose urinary calcium is extravagantly far from values commonly observed. It is difficult to compare the studies of one investigator made under a given set of dietary conditions with those of another investigator, using a somewhat different regimen. Some attempts to circumvent this difficulty have been made, particularly through drastic limitation of the calcium intake. The effect of the sex and size of the subject on the urinary calcium still remains in doubt.

When the person studied is still in the period of growth, the decision as to whether a given urinary calcium value is normal or abnormal becomes even more difficult. Definite demarcation of the normal range for urine calcium at all ages and intake levels should serve as a useful aid both in diagnosis and in the study of the intermediary metabolism of calcium.

THE EFFECT OF AGE, SEX, AND CALCIUM INTAKE ON URINARY CALCIUM EXCRETION BY NORMAL PERSONS

Sources of data

Even a cursory examination of data on urinary excretion of calcium discloses the wide variability of this component among normal individuals under the same conditions of study. To cite but one example, eight normal girls, 11 to 14 years old, ingested identical diets containing 1.27 grams of calcium daily; the output of calcium in the urine varied from 25 to 217 mgm. a day. With such a wide range of excretion among individuals of the same sex and of limited age range, under identical dietary conditions, it is obviously necessary to use large numbers of data in order to obtain valid conclusions as to possible relationships between the level of excretion of calcium in urine and other factors, such as calcium intake and age, sex, and skeletal size of the subject.

During the course of several years of study of calcium metabolism during growth, a considerable mass of data on urinary excretion of normal infants, children, and adults has been accumulated in this laboratory, much of which as yet is unpublished. Many of these subjects were studied at more than one level of calcium intake. To the findings from this laboratory were added data from studies of normal children and adults reported in the literature (1–44). The recently reported studies of college women (19, 26) afforded the largest contribution of data from this latter source. All studies of patients ill or convalescing from any disease whatsoever were excluded. Excluded also were studies in which the dietary regimen differed in any notable degree from the customary, also those in which the diets were markedly acid in ash. However, it was not possible, for obvious reasons, to exclude all factors known or supposed to influence urinary calcium. Milk was the major source of calcium in all diets except those containing less than 0.3 gram of calcium daily. The total calcium intake was accurately known for all subjects; all figures for calcium intake were obtained by chemical analysis of the diet. Likewise the fecal excretion and retention of calcium were known for 95 per cent of all subjects. Since the emphasis of the present study is on the excretion of calcium in the urine, and because of limitations of space, com-

[1] This paper is condensed from a part of the dissertation submitted by Elizabeth L. Knapp in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Department of Chemistry in the Graduate College of the State University of Iowa. The research was carried out in the Department of Pediatrics under the supervision of Dr. Genevieve Stearns.

TABLE I

Number and age distribution of subjects. Number of studies

Age	This laboratory		Literature		Total	
	No. subjects	No. studies	No. subjects	No. studies	No. subjects	No. studies
Infants under 1 yr.	95	880			95	880
Children 1–9 yrs.	58	112	41	51	99	163
Children 10–16 yrs.	43	104	57	59	100	163
Adults 17–80 yrs.	15	28	224	260	239	288
Total	211	1,124	322	370	533	1,494

plete data are not included, although they are available.[2] A study of the total excretion of calcium of a large number of the subjects included in this analysis has been reported in connection with other studies (19). The relative numbers and age distribution of the subjects studied are summarized in Table I.

McCrudden's method (45), or slight modifications thereof, has been used for the determination of urinary calcium in most of the studies used as source of material; therefore no serious error is introduced by combining the results from the separate studies.

[2] It is obviously impossible to include tabulations of the entire mass of data used for this study. These data are available to the investigator in the form of the thesis "Studies on Urinary Excretion of Calcium," by Elizabeth Knapp (1943), which is on file in the library of the State University of Iowa.

Urinary calcium excretion of individuals from 1 to 80 years of age

The crude relationships between the urinary calcium excretion, age, and calcium intake of the subject are shown in Table II and Figure 1. Table II gives a summary of the number of subjects, the mean values, and the standard deviations for urinary calcium excretion for each 5 years of age for all data from normal individuals from 1 to 80 years of age. In Figure 1 the mean values for four daily intake levels have been plotted and smoothed curves drawn through these points. It is evident that, although the standard deviation is always high, the mean urinary calcium excretion tends to increase both with age and with intake. At any given age the mean urinary calcium increases with the intake of calcium. In adults the amount of increase in urinary calcium with increase in intake appears to be greater than in children; hence, the spread of values becomes greater with increasing age.

The statistical validity of these assumptions was checked by analysis of several groups of data. For a study of the relationship of urinary calcium to calcium intake, it was necessary to limit the data to those studies wherein a given subject was observed with two or more levels of intake; each subject thus served as his own control. The data from 62 subjects studied in this laboratory were chosen for analysis and are summarized in Table III. Three groups, consisting of 42 boys under 12 years of age, 13 women, and 7 men, respectively, were studied at two widely differing levels

TABLE II

Summary of urinary calcium data according to calcium intake and age of subjects

Age	No. subj.	Urine Ca		No. subj.	Urine Ca		No. subj.	Urine Ca		No. subj.	Urine Ca		No. subj.	Urine Ca	
		Mean	S.D.		Mean	S.D.		Mean	S.D.		Mean	S.D.		Mean	S.D.
Ca intake *grams daily*	0–0.299			0.3–0.699			0.7–0.999			1.0–1.399			1.4+		
years		*mgm. daily*			*mgm. daily*			*mgm. daily*			*mgm. daily*			*mgm. daily*	
1–4				13	41	13	34	47	23	42	53	31	6	62	41
5–9	8	28	21	11	71	44	31	79	39	42	96	43	19	104	59
10–14	15	76	41	18	80	46	19	94	49	48	98	55	59	117	67
15–19	3	87		17	145	53	14	178	80	25	178	71	11	237	65
20–24	36	100	48	57	118	57	34	167	68	31	181	65	12	231	53
25–29	13	107	39	22	143	52	5	167	94				9	218	52
30–34	6	121	45	6	182	67									
35–39	6	142	89	5	192	83									
40–44	2	137		2	175										
65 and over	5	147		3	196										

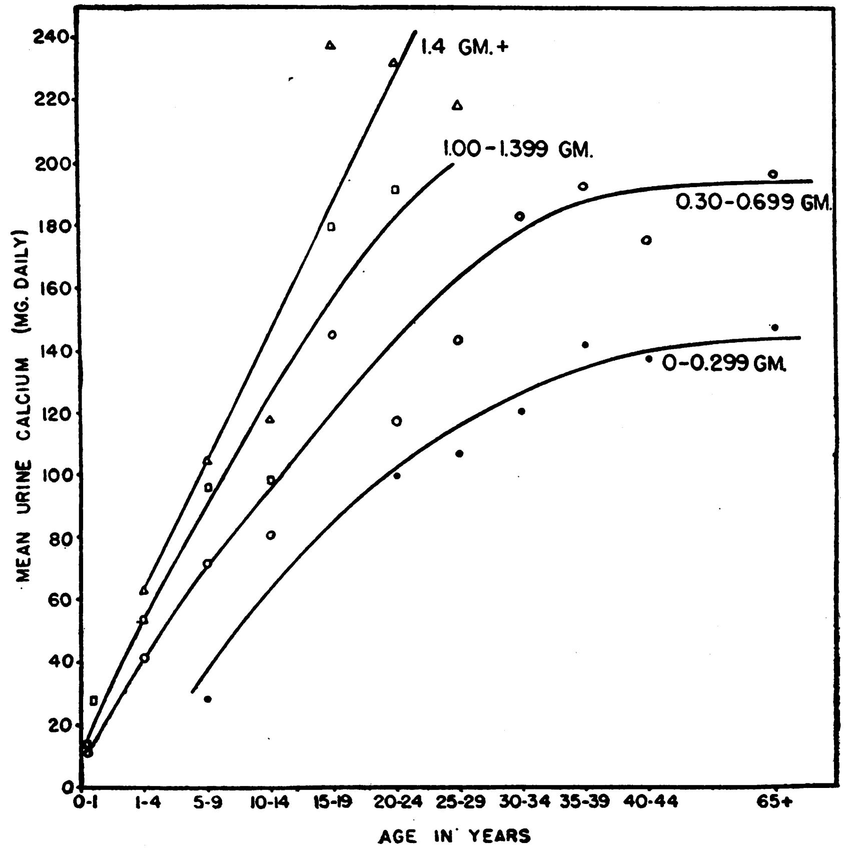

FIG. 1. THE RELATIONSHIP BETWEEN URINARY CALCIUM AND AGE OF THE SUBJECT, AT DIFFERENT LEVELS OF CALCIUM INTAKE

of intake each. Analysis showed "t" [3] to be 4.01, 5.9, and 4.6 respectively, highly significant increases in urine calcium with large increases in calcium intake.

Figure 1 also indicates that, with the daily calcium intake constant, the mean urinary calcium excretion increases with age throughout the period of growth. To determine if these differences in urinary calcium excretion at different ages were significant, the data from a group of 64 boys studied in this laboratory and ranging in age from 1 to 12 years were analyzed (Table IV). The age

TABLE III

Statistical summary of data showing range and mean urinary calcium excretion with changes in age or in calcium intake

Subjects	Number	Age range	Intake mean	Urine mean	Calcium range	Significance of differences*
		years	*grams per day*	*mgm. per day*		
Boys	42	1–12	0.554	56	7–224	+
			1.446	81	10–260	
Women	13	14–35	0.387	96	55–188	+
			1.983	158	80–239	
Men	7	20–25	0.226	131	81–188	+
			2.339	229	130–287	

* + = Significant at 1 per cent level.

[3] If the calculated value of "t" could occur by chance only once in 100 times, the difference is said to be "significant at the 1 per cent level," or highly significant; and if such a value could occur by chance only once in 20 times, the difference is said to be "significant at the 5 per cent level." In other words, when the values for significance are between 1 per cent and 5 per cent, there is a high probability that the differences in the means cannot be accounted for by variations due to sampling; or, as commonly expressed, the means are significantly different. Calculations of "t" were made when only 2 means were being compared, and calculations "F" when more than 2 means were compared. The standards for significance are the same for both values (75).

TABLE IV

Mean urinary calcium values of 64 boys from 1 to 12 years of age and of 23 college women. All received one quart of milk daily

	Age	Number of subjects	Calcium intake Mean	Urinary calcium Mean
	years		*grams per day*	*mgm. per day*
Boys	1	10	1.272	28
	2	7	1.267	62
	3	7	1.300	53
	4	6	1.382	79
	5	7	1.413	85
	6	5	1.397	93
	7–8	6	1.559	115
	9	5	1.574	135
	10–11	11	1.451	111
Women	17–25	23	1.378	210

range chosen covers the interval of most rapid increase of urinary calcium with age as shown in Figure 1. All boys were receiving 1 quart of milk daily in addition to the basal diet; therefore, the daily calcium intake was very nearly constant for all subjects. The number of subjects, the mean daily calcium intake, and urinary calcium for each year of age are shown in Table IV. It is obvious that although the mean calcium intake varies only about 20 to 25 per cent from 1 year to adulthood, the mean urinary calcium increases almost 10-fold during the same period. A value for "F" of 4.31 was obtained, which exceeds the value required for 1 per cent significance.

The mean urinary calcium for the 10- to 11-year old boys (Table IV) was compared with that of 23 college women receiving a similar intake (19, 26). The value of "t" was 3.94, showing that these differences also are highly significant.

It appears probable that the important factor during growth is the increase in skeletal weight of the child. In this country, skeletal weight remains approximately constant at 20 per cent of the total weight throughout life (Scammon) though the relative calcium content of the skeleton increases during growth. To test whether body weight, as a measure of skeletal weight, is the factor responsible for the change in urine calcium with age, calcium intake and urinary calcium per kgm. of body weight were calculated for all subjects for whom weights were available (606 studies). The available data were classified into 6 intake ranges and the mean, maximum, and minimum urinary calcium determined for each range (Table V). The range and mean urinary calcium values for groups 0 to 5 mgm. per kgm. and 5 to 10 mgm. per kgm. intake were so similar (Table V) that these two groups were combined, permitting a study of 148 values, representing an age range of 4 to 80 years. The mean values for urinary calcium for each 5 years of age within these groups are shown in Figure 2, compared with the mean excretion for all groups. Notwithstanding the wide range of values, the means for each age were not significantly different from the mean for the group ("F" = 1.53). A similar analysis of the possible effect of sex on excretion of urinary calcium by 67 men and 128 women showed that the differences, although significant, again were due to differences in weight of the subjects.

TABLE V

Urinary calcium per kgm. in relation to calcium intake per kgm.

Intake range	Age range	Urinary Ca Minimum	Maximum	Mean
mgm. per kgm. per day	*years*	*mgm. per kgm. per day*		
0–5	8–80	0.4	4.7	1.94
5–10	4–80	0.2	4.6	2.03
10–25	1–75	0.6	8.3	3.30
25–50	1–35	0.5	9.3	3.37
50–75	1–15	0.9	10.0	
75+	1–11	0.9	11.4	

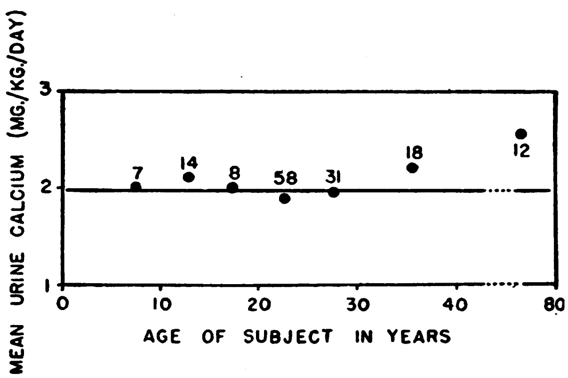

FIG. 2. THE LACK OF RELATIONSHIP BETWEEN URINARY CALCIUM, EXPRESSED IN MGM. PER KGM. DAILY, AND AGE OF SUBJECT

For this figure all data were used wherein the calcium intake was under 10 mgm. per kgm. as the range and mean urinary calcium values for groups with intakes 0–5 mgm. per kgm. and 5–10 mgm. per kgm. were almost identical (Table V). The line represents mean urinary calcium for all ages. Numbers above the dots show the number of subjects in each age group.

Skeletal weight or quantity of bone, as represented by total body weight, is thus the factor responsible for differences in urinary calcium excretion with increasing age and with sex. The relative importance of weight among the various factors affecting urinary calcium was studied by determination of the correlation coefficient, "r," between weight and urine calcium. For 45 boys 10 to 14 years old whose calcium intake was 1.0 to 1.399 grams daily, the value of "r" was 0.149; for 41 boys 10 to 14 years old with calcium intakes between 1.4 and 2.0 grams daily, "r" was 0.302. The figures required for 5 per cent significance are 0.288 and 0.304 respectively. These data tend to confirm the findings of Wang, *et al.* (43) who found no significant correlation between weight and urinary output of calcium for 23 girls, 11 to 15 years old. Thus, while urinary calcium increases significantly with increase in weight of the individual, no direct correlation can be observed between the two. Such a conclusion is to be expected if the skeletal weight is the true factor concerned. Differences in total weight due to differences in fat content of the body would not influence urinary calcium.

In studies of normal adults, McCance (23) found that the amounts of calcium in the urine varied directly with the amount of calcium absorbed and considered that "changes in the urinary excretion of calcium may be used as an index of changes in the amount of calcium absorbed." Examination of the entire group of data from this laboratory shows that in children, at least, urinary calcium level bears no relationship whatever either to calcium absorption or retention. A few illustrative examples of this lack of relationship are shown in Table VI. Apparently during the period of calcium storage, urinary calcium does not reflect the amount of calcium absorption. When storage is complete, increased urinary excretion of calcium would be expected with increased absorption of this element.

TABLE VI

Lack of relationship between calcium absorption and urinary excretion of calcium by children

Name	Age	Calcium			Name	Age	Calcium		
		In-take	Absorp-tion	Urine			In-take	Absorp-tion	Urine
	years	*mgm. daily*				*years*	*mgm. daily*		
R.H.	1	791	78	32	G.M.	2	1,331	209	28*
		791	143	27			1,331	328	27
D.D.	1	698	138	22	C.C.	7	1,373	189	74
		698	281	27			1,373	320	75*
L.R.	1	1,048	178	44	J.D.	7	1,635	702	46*
		1,040	340	37			1,635	422	47*
F.S.	2	1,048	341	128	D.Br.	8	1,641	388	192
		1,065	371	161			1,675	715	50*
R.B.	4	702	87	75	P.E.	8	1,564	404	248
		702	181	71			1,635	827	148*
J.H.	4	685	68	63	P.E.	8	796	286	101
		704	224	53			865	308	230*
F.S.	5	781	88	131	P.Ba.	10	1,603	531	155*
		781	235	129			1,500	502	129*
M.D.	7	1,090	142	50	E.S.	12	1,276	117	34
		1,090	252	53			1,680	348	25*
J.A.	9	809	305	94	V.H.	14	1,262	119	26
		861	285	168			1,680	265	36*
M.K.	9	1,617	367	245					
		1,429	562	73					

* 350 I.U. of vitamin D given daily.

Of the factors thus far considered to affect urinary calcium, two only have been shown to be significant, calcium intake and the weight of the individual. The wide range of values obtained for individuals of the same weight and calcium intake shows that some other factor or factors peculiar to the individual are of major importance in determining the amount of calcium excreted in the urine. In an attempt to find a measure for these unknown factors, presumably endocrine, the urine calcium values were calculated as per cent of intake. The proportion of calcium intake excreted in the urine of a given person is not constant at all intake levels but is highest when the intake is low, rapidly decreasing as the intake is increased. The percentage of any given intake excreted in the urine varied widely among individuals, but all showed a decreasing percentage excreted as the intake increased. The urine calcium values calculated as per cent of calcium intake were then plotted against the calcium intake per kgm. of body weight. By using the per kgm. intake, the weight factor was eliminated and the intake factor retained. The data so compared showed a definite pattern of excretion, the percentage of intake excreted in the urine decreasing at a decreasing rate as the intake rose.

Figure 3 shows the data from 606 studies of persons 1 to 80 years old (2–44, 46, 47). The urine calcium as per cent of intake is plotted against the intake per kgm. body weight, using

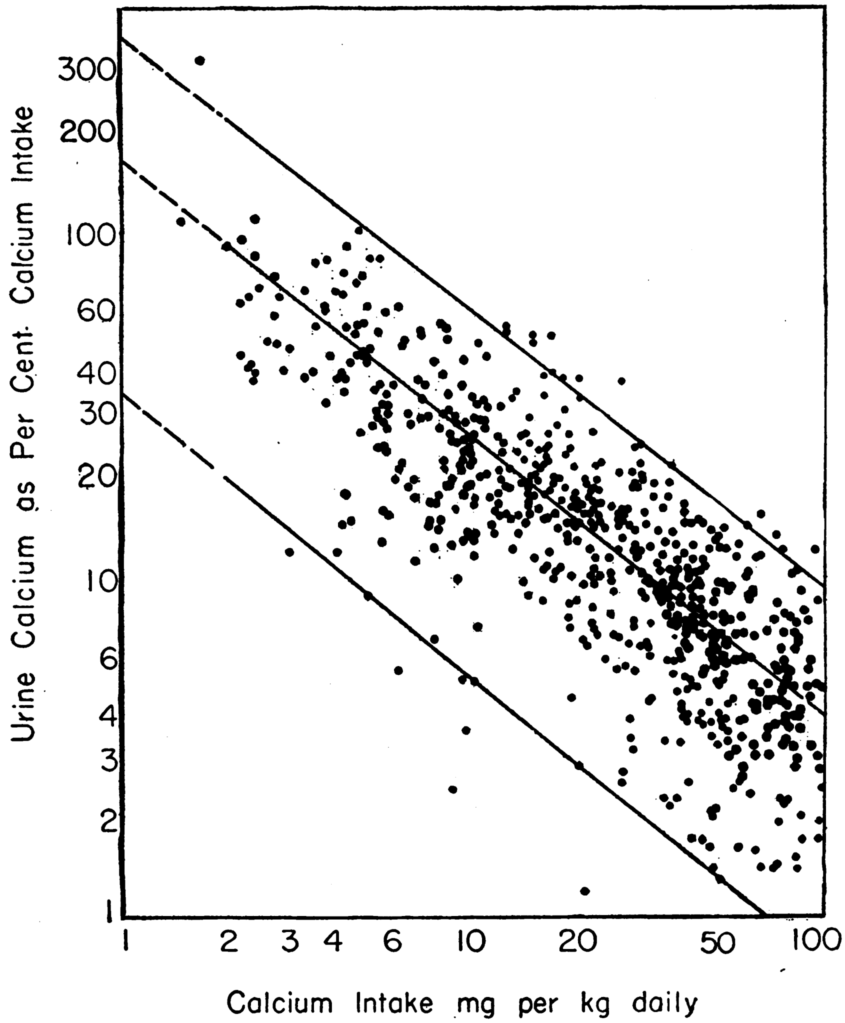

FIG. 3. THE RELATIONSHIP BETWEEN URINARY CALCIUM EXPRESSED AS PER CENT OF INTAKE, AND THE CALCIUM INTAKE IN MGM. PER KGM. DAILY

Logarithmic scales are used for both ordinate and abscissa. Mean, maximum, and minimum normal values are plotted in preference to mean and standard deviation curves. The data include 606 studies from subjects from 1 to 80 years of age (2–44, 46, 47).

a log-log scale. So plotted, it is obvious that $\frac{\text{urine Ca} \times 100}{\text{intake Ca}}$ is an exponential function of the per kgm. intake. The formulas for maximum, mean, and minimum values are

$$\begin{aligned} &\text{maximum } M = 365\ I^{-.801} \\ &\text{mean } \quad y = 158.9\ I^{-.802} \\ &\text{minimum } m = 34.1\ I^{-.835} \end{aligned}$$

I being the intake per kgm. of body weight. The pertinent data for maximum, minimum, and mean lines of Figure 3 are given in Table VII. In preference to standard deviation, maximum and minimum normal values were chosen as being more useful in diagnosis of disease.

Examination of Figure 3 shows that the urinary calcium may normally be greater than the intake when the intake is less than 5 mgm. per kgm. With an intake of only 2 mgm. calcium per kgm., a urine calcium greater than 100 per cent of the intake may not be abnormal, whereas with an intake of 50 mgm. per kgm., the maximum normal excretion is 16 per cent of the intake.

TABLE VII

Urinary calcium as per cent of intake in relation to calcium intake per kilogram of body weight

Calcium intake	Urine Ca as per cent intake		
	Minimum	Mean	Maximum
mgm. per kgm. per day	*per cent*	*per cent*	*per cent*
2	19.1	91.1	210
5	8.9	43.7	101
10	5.0	25.1	58
15	3.6	18.1	42
20	2.8	14.4	33
30	2.0	10.4	24
50	1.3	6.9	16
75	0.9	5.0	12
(100)*	(0.7)	(4.0)	(9)

* Extrapolated value.

It seems probable that the position of urine calcium values of any individual person relative to the mean, as shown on Figure 3, will depend on the endocrine balance of the person. If this is true, the values for any one individual should maintain the same position relative to the mean at all levels of intake. Also, if age *per se* is not a factor, it is expected that the normal range and the mean values should be the same for all ages studied. The validity of these assumptions is shown by Figures 4 to 6. In these figures, individual data are plotted as in Figure 3. When several intake levels of calcium have been studied in one individual these values are connected by dotted lines. $\frac{\text{Urine Ca} \times 100}{\text{Intake Ca}}$ of each individual tends to maintain a constant position relative to the mean at all levels of intake. For each group, the values tend to be distributed about the mean in a manner similar to that of the entire group. The only exceptions to this rule are the values for children 1 to 2 years of age, which tend to lie almost wholly below the normal mean, though

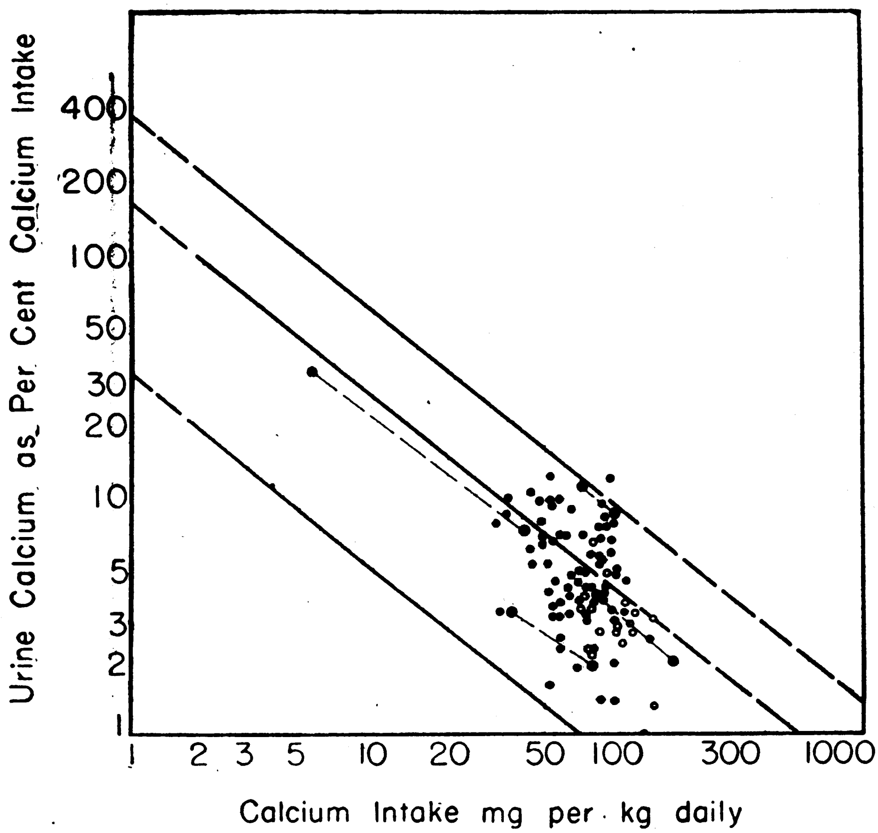

FIG. 4. URINARY CALCIUM VALUES FOR CHILDREN 1 TO 5 YEARS OLD

Solid lines represent mean, maximum, and minimum curves as in Figure 3. The dotted lines connect values obtained from one child at two or more levels of calcium intake. The hollow symbols show urinary calcium values of children 1 to 2 years old.

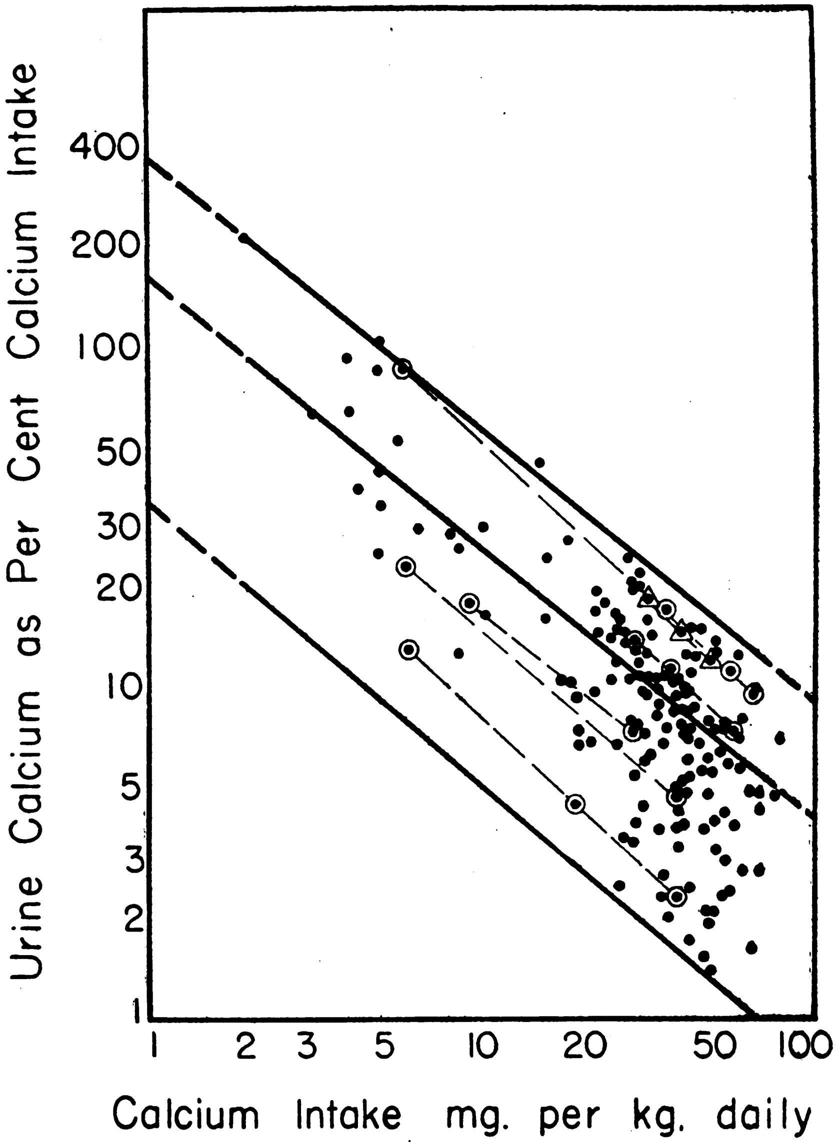

FIG. 5. URINARY CALCIUM VALUES FOR CHILDREN 10 TO 15 YEARS OLD
Solid and dotted lines as in Figure 4.

well within the normal range. The significance of this finding will be discussed later.

The relationships between calcium intake and urinary excretion set forth in Figures 3 to 6 were derived from data on all subjects, irrespective of calcium retention. In order to determine whether a total calcium excretion greater than the calcium intake would modify this relationship, the data for all subjects with negative calcium retentions were plotted as shown in Figure 7. It is obvious that the relationship expressed by the equations for mean, maximum, and minimum excretion and denoted by the lines in Figure 7 is equally valid for subjects in positive and negative calcium balance. In passing, one may note that a calcium intake of 20 mgm. per kgm. seems to be the critical level for negative calcium retentions. Irrespective of age, very few subjects lost calcium from the body when the calcium intake was greater than 20 mgm. per kgm.

A log-log chart of the maximum, mean, and minimum values for urinary calcium has proved

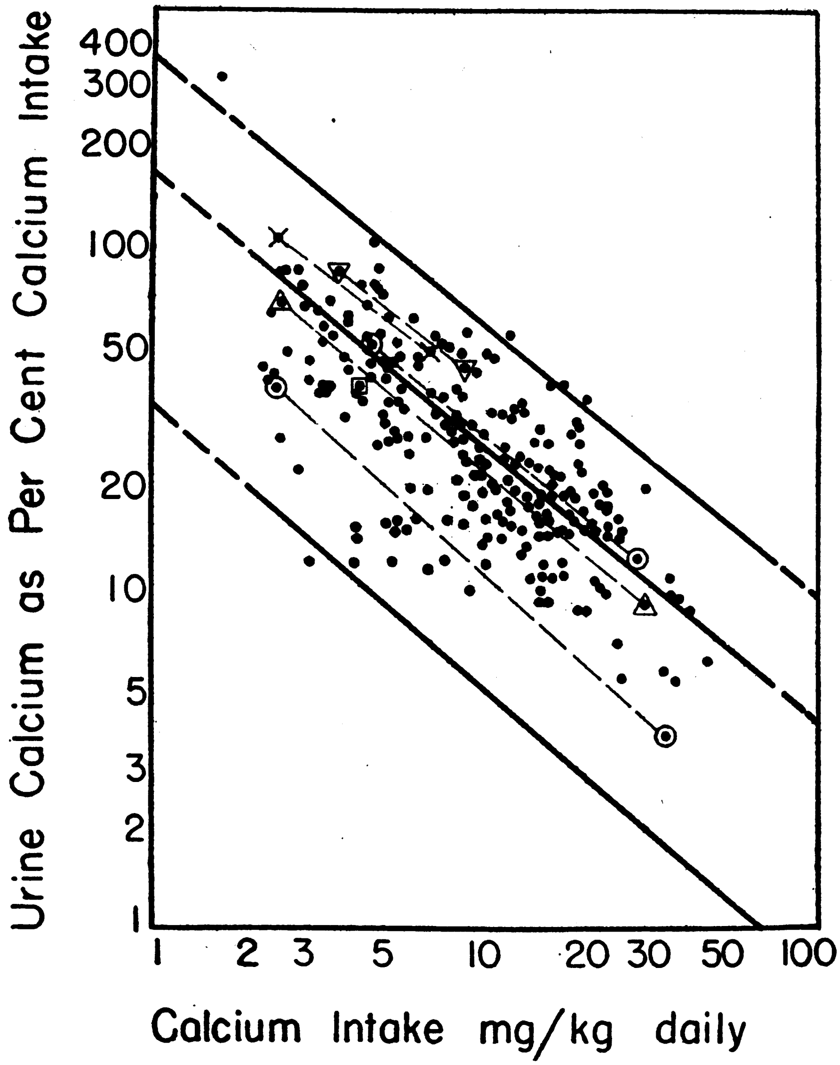

FIG. 6. URINARY CALCIUM VALUES FOR ADULTS 20 TO 80 YEARS OLD
Solid and dotted lines as in Figure 4.

useful in checking the normality of urinary calcium excretion in hospital patients. Three values only are needed for the determination: the daily calcium intake, 24-hour urinary calcium, and the weight of the person. If the milk intake is known, the dietary calcium may be estimated with sufficient accuracy for the large percentage of cases wherein the true values will lie close to the mean. For example, a 20-kgm. child drinks a pint of milk daily and excretes 100 mgm. of calcium in the urine. The true calcium intake would probably lie between 700 and 900 mgm. daily, or 35 to 45 mgm. per kgm. The urine calcium is between 14 and 11 per cent of these intakes. The ranges of these two values outline a square lying above the mean but well within the normal range. Had the urinary calcium been 200 mgm. daily, the values of 22 to 28 per cent of intake would indicate a urine calcium above the normal range, and an accurate study would be indicated.

Urinary calcium in infancy

The urinary excretion of calcium during the first year of life has been considered separately from that of the older subjects. Data were available from 980 3-day studies of 95 normal male infants fed cow's milk and ranging in age from 3 to 52 weeks (46, 48) and for 31 studies of infants under 26 weeks of age given human milk exclusively (49–52).

Each infant given cow's milk received 350 I.U. of vitamin D daily and retained ample amounts of calcium. The daily urine calcium excretion was relatively small, from 1 to 82 mgm. for the infants under 6 months of age and from 3 to 86 mgm. for those over 6 months. The variability of urinary calcium was much greater among the group of infants and for the same infant during the first year of life than was observed with the older children.

The $\frac{\text{urine Ca} \times 100}{\text{intake Ca}}$ of infants fed cow's milk was compared with the intake per kgm. The mean values obtained for each week of age are plotted in Figure 8. The mean values are consistently below the mean noted for the older subjects, but well within the normal range. The values for the youngest infants are lowest and the mean $\frac{\text{urine Ca} \times 100}{\text{intake Ca}}$ tends to approach the mean curve with increasing age and decreasing per kilogram intake of the infants.

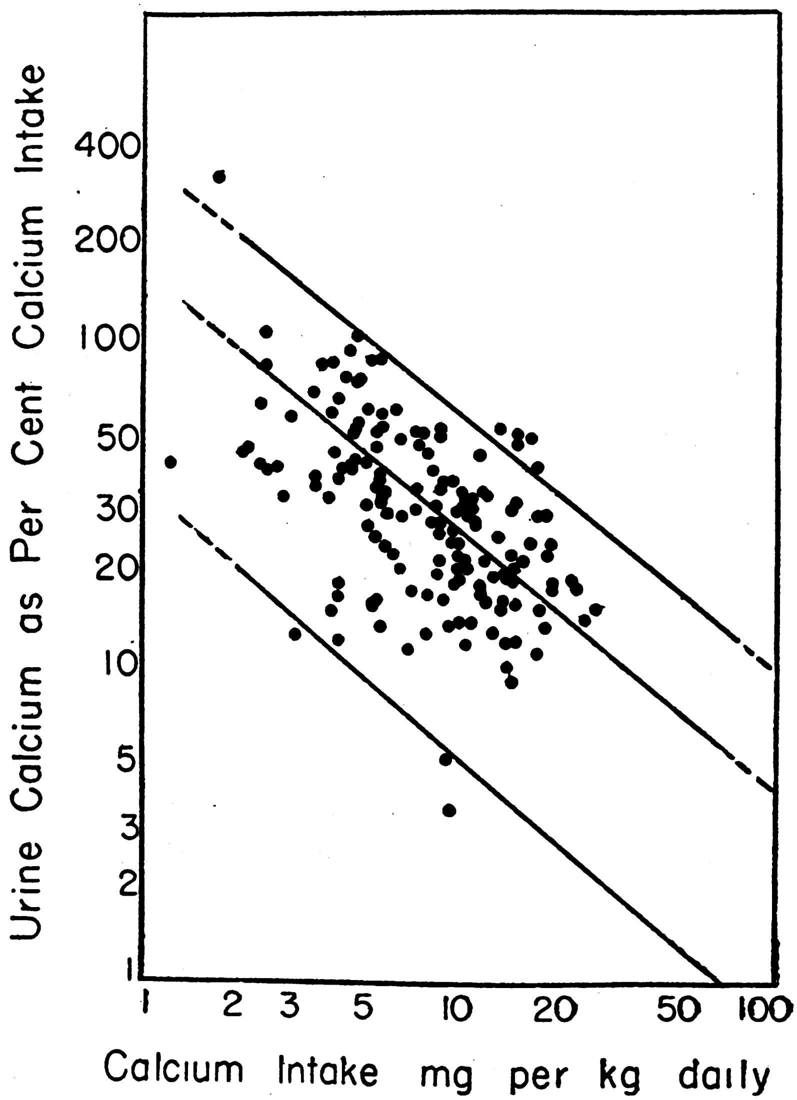

FIG. 7. URINARY CALCIUM VALUES FOR SUBJECTS IN NEGATIVE CALCIUM BALANCE

Solid lines represent mean, maximum, and minimum curves as in Figure 3. The data include 164 studies from subjects 4–76 years old.

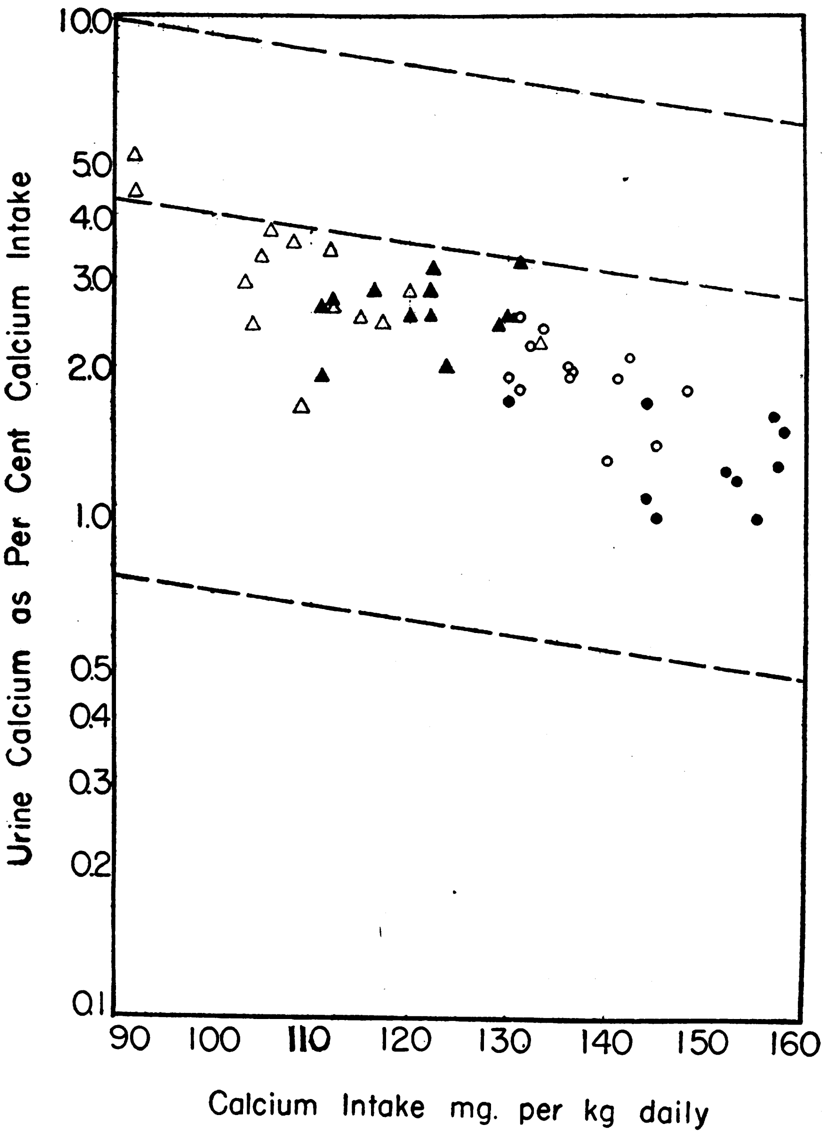

FIG. 8. URINARY CALCIUM VALUES FOR INFANTS FED COW'S MILK (47, 48)

Note that in this figure only the ordinate is on a logarithmic scale. Mean, maximum and minimum curves are extrapolated from those of Figure 3. Values are averaged for each week of age. Solid circles show average values for infants under 3 months old; open circles, those 3 to 6 months old; solid triangles, infants 6 to 9 months old; and open triangles, infants 9 to 12 months of age.

A similar study of the urinary calcium excretion of breast-fed infants whose daily calcium intakes varied from 44 to 125 mgm. per kgm., shows values comparable to those of the infants fed cow's milk. No consistent differences were observed between the values for babies under 3 months and those 3 to 6 months of age (Figure 9).

It thus appears that all normal infants, whether the per kgm. calcium intake be high or low, will excrete an amount of calcium in the urine that is within the range of normal values for older subjects, but below the mean for the latter group. It appears that the decrease in mean excretion is greatest during the first 6 months of life and tends to approach the normal during later infancy.

From the findings in Figure 4, in which the values for $\frac{\text{urine Ca} \times 100}{\text{intake Ca}}$ for children 1 to 2 years of age are still below the mean, it seems probable that the mean level of excretion characteristic for older subjects is not achieved until the child is about 2 years old.

The decrease in urinary calcium observed during infancy has two possible causes. Although individual babies, over a period of several months, tend to maintain a fairly constant level of excretion in relation to the mean, it may be that the endocrine glands, the secretions of which we believe make up the "endogenous factor," function less efficiently in the young infant than in the older child. As an example, hypoparathyroid tetany of the new-born is occasionally observed and appears to be always a transitory disturbance.

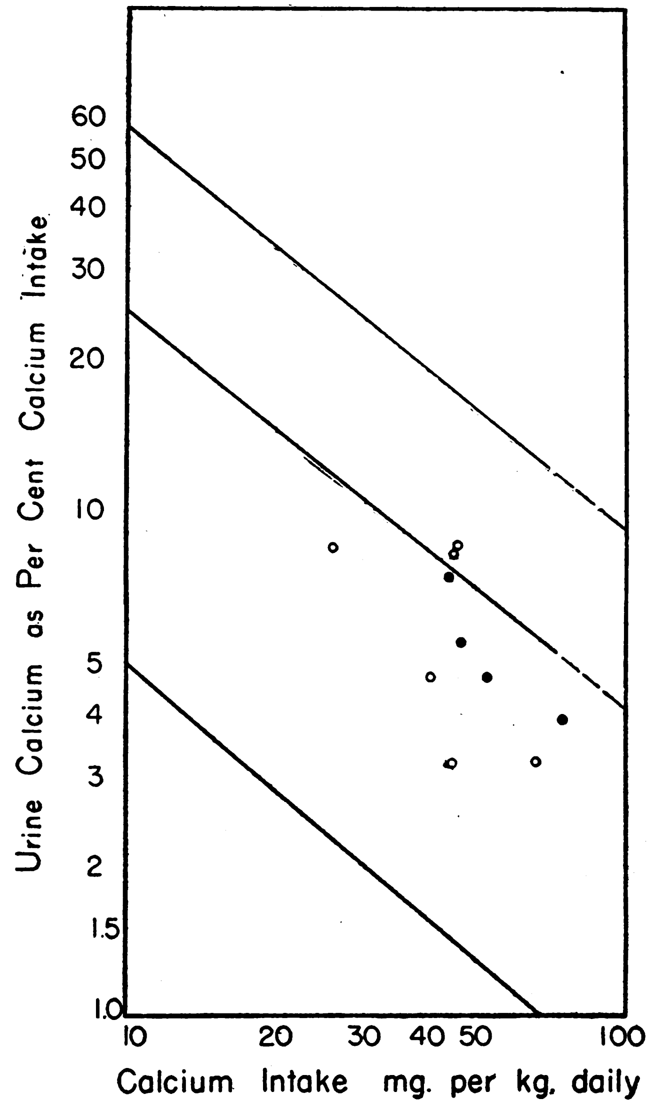

FIG. 9. URINARY CALCIUM VALUES FOR INFANTS FED HUMAN MILK (47, 49–52)

Lines as in Figure 8. The data have been averaged for each week of age. Hollow symbols represent values for infants under 3 months of age; solid symbols, infants 3 to 6 months old.

Also, the relatively marked calcium undersaturation of the skeleton in early infancy may have the same effect on decreasing urinary calcium as does severe calcium undernutrition in older subjects. It appears highly probable that both factors are active in determining the quantity of calcium excreted in the urine of infants.

Undernutrition and overnutrition

During recovery from severe undernutrition, especially calcium undernutrition, the relative unsaturation of bone may be sufficient to cause an unusually large deposition of calcium in bone and result in lowered urinary excretion of calcium. The fact that urine calcium values of infants are below the mean value for older children and adults lends support to this possibility.

Several studies of undernourished children (41, 53) and infants (54) are available for analysis. Figure 10 shows changes in $\frac{\text{urine Ca} \times 100}{\text{intake Ca}}$ occurring as the children gained weight. For the children most seriously undernourished (30 to 45 per cent underweight), urine calcium values were

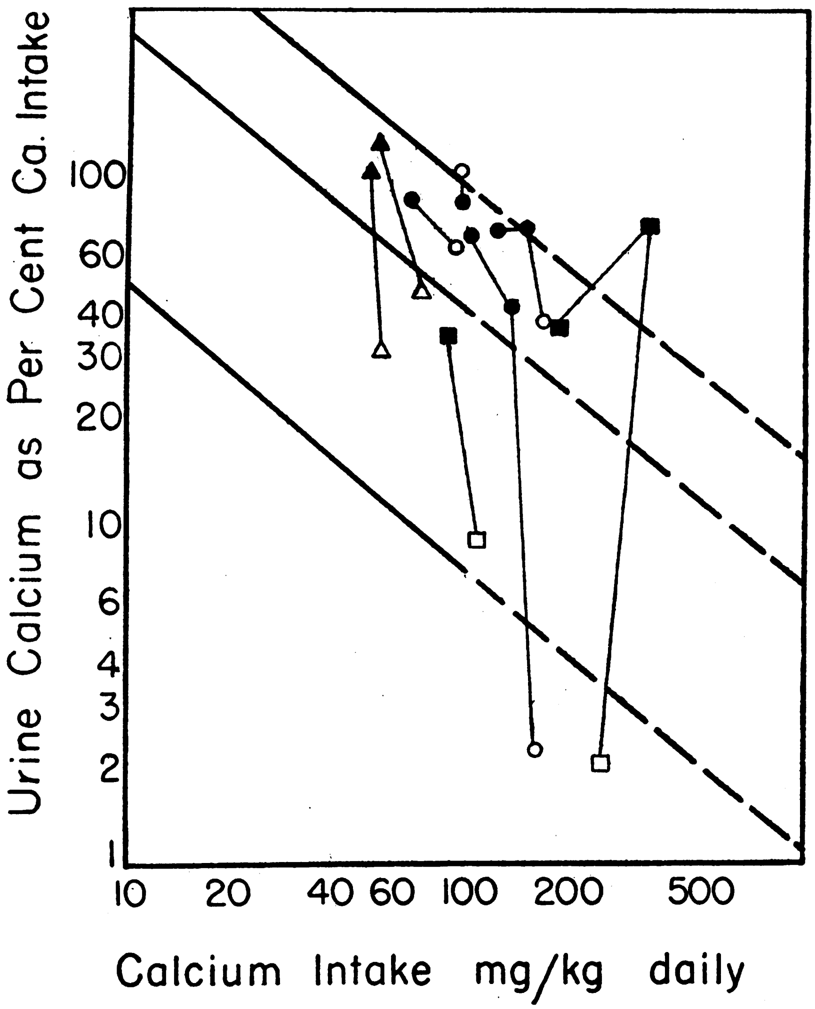

FIG. 10. URINARY CALCIUM VALUES FOR CHILDREN DURING RECOVERY FROM SEVERE MALNUTRITION

The hollow symbols represent the first observation, when the child was most underweight. In most of the cases of severe undernutrition the urinary calcium increased with the gain in weight.

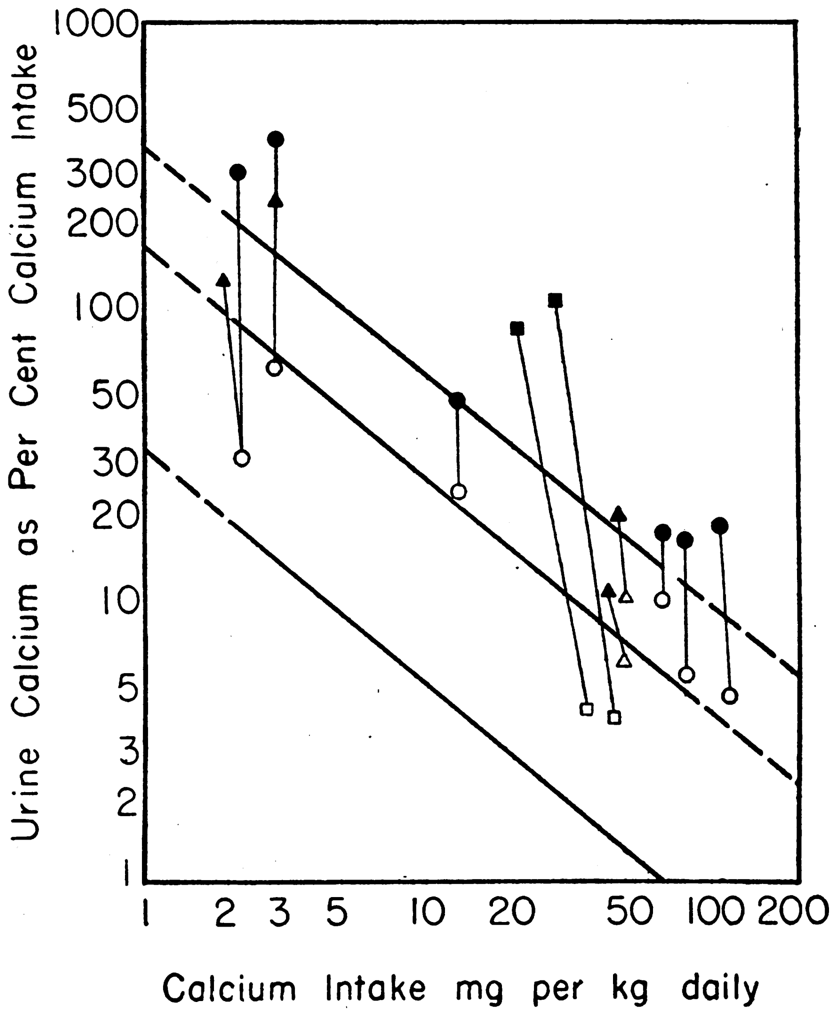

FIG. 11. THE EFFECT OF INCREASING METABOLIC ACIDITY ON EXCRETION OF URINARY CALCIUM

The background lines represent the normal maximum, mean, and minimum $\frac{\text{urine Ca} \times 100}{\text{intake Ca}}$ in relation to calcium intake in mgm. per kgm. Hollow symbols represent control studies with a neutral ash diet; solid symbols, findings after increasing the metabolic acidity by ketogenic diet (■), NH_4Cl by mouth (●), or by ingestion of an acid-ash diet (▲). It is obvious that the first two methods of increasing metabolic acidity can result in increasing urinary excretion of calcium to above normal limits.

uniformly low and increased sharply as the child's weight approached normal. Some children less severely undernourished showed lowered urinary calcium values during the period of undernutrition, but several of this group excreted as much calcium in the urine during the period of undernutrition as when normal weight was attained.

The effect of calcium undernutrition *per se* can be illustrated by a study of a woman of 34 with severe osteoporosis (55). In this subject the presence of a low-grade steatorrhea for over 10 years, although symptomless, had led to a severe osteomalacia even though the milk intake had been adequate. The urinary calcium excretion before treatment was 16 mgm. daily or 1.2 per cent of the calcium intake of 40 mgm. per kgm. This value is minimum normal. She was not retaining calcium. Treatment consisted of cod liver oil, bile salts, and pancreatin in conjunction with a diet high in calcium and phosphorus and low in fat content. After 4 months on this regimen, the patient showed dramatic improvement; she gained weight and the osteoporosis was very nearly healed. The calcium retention at this time approximated 0.5 gram daily. However, the urinary calcium excretion was only 9 to 11 mgm. daily, or 0.5 to 0.7 of the intake of 36 mgm. per kgm., values well below the normal minimum. Practically all the absorbed calcium was being retained in the bones, and very little remained to be excreted in the urine. Similar low values for urine calcium may be observed during recovery from hyperparathyroidism.

In obesity, there seems no reason to assume abnormality of calcium excretion, unless the obesity is accompanied by endocrine disturbance. As it seems probable that the relationship observed between weight of the subject and urinary calcium is due primarily to a relationship between skeletal weight and urinary calcium, the urinary calcium may be expected to be low. Therefore, the use of theoretical rather than actual weight of the obese individual seems preferable in calculating normality of urinary calcium. For example, a boy of 11 during reduction of weight from 124.5 to 99 kgm. excreted 8.5 to 9.3 per cent of his calcium intake of 9 to 14 mgm. per kgm. actual weight, or 26 to 33 mgm. per kgm. theoretical weight. These values are within normal limits for both intake levels, approach the mean value for theoretical per kgm. intake, and are well below the mean value for actual per kgm. intake.

THE INFLUENCE OF DIETARY FACTORS OTHER THAN CALCIUM INTAKE

In addition to the major factors affecting urinary calcium, namely, calcium intake, amount of bone measured by body weight, and endogenous factor, other factors exert lesser effects, which though not great in themselves, must be considered in planning dietary regimens whenever urinary calcium is to be studied. Of these other factors, the increase of acid metabolites in the body, the quantity of protein ingested, the calcium-phosphorus intake ratio, dietary roughage, vitamin D, and the possible effects of intake of other salts have all been discussed as factors affecting urinary calcium (56–73). The relative importance of these factors can now be evaluated.

The increase of acid ions in the body may be accomplished by feeding of mineral acids or acid salts such as ammonium chloride, by producing ketosis through the use of a high fat diet, or by the use of a diet giving an acid ash. Figure 11 shows the effect of these factors on urinary calcium. All data were taken from the literature (27, 56–58), and in every study each subject served as his own control. The calcium intake was nearly constant in all studies except one (27) in which the experimental diet contained less calcium than the control. Ingestion of ketogenic diet (fatty acid: glucose = 2.6) by children resulted in the most marked increase in urinary calcium. Values for $\frac{\text{urine Ca} \times 100}{\text{intake Ca}}$ were increased far above the normal maximum. Ammonium chloride increased the urine calcium excretion of children (27) and adults (56) above the normal maximum. Ingestion of an acid-ash diet by adults (56), although less effective than NH_4Cl, did increase the urine calcium from values near the mean to values near the normal maximum level of excretion. The importance of ingestion of a neutral-ash diet when urinary calcium excretion is studied is thus emphasized.

An additional factor known to influence the quantity of urinary calcium is the ratio of calcium to phosphorus in the diet. The data shown in Figure 12 taken from studies made in this laboratory (46, 61) illustrate the effect of changing the calcium to phosphorus ratio without altering the total calcium content of the diet. The high calcium to phosphorus intake ratios were attained by giving young adults a constant basal diet and providing the dietary calcium as calcium phosphate for the control period and as calcium gluconate or lactate for the experimental periods. The data for the lower calcium intake levels were obtained by giving children a low calcium diet and decreasing the calcium to phosphorus ratio by the addition of sodium glycerophosphate to the diet. The results show consistent increase in $\frac{\text{urine Ca} \times 100}{\text{intake Ca}}$ with increasing calcium to phosphorus intake ratio and constant calcium intake. In some cases, the increases in urinary calcium

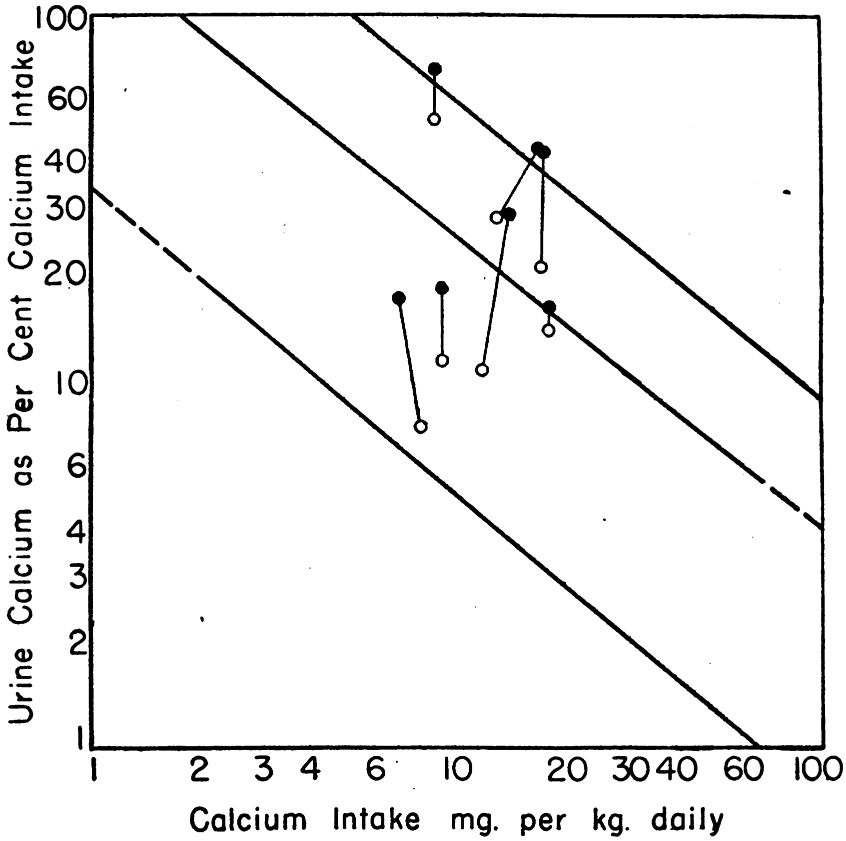

FIG. 12. THE EFFECT OF INCREASING THE CALCIUM TO PHOSPHORUS INTAKE RATIO ON THE EXCRETION OF URINARY CALCIUM

The hollow symbols represent the control studies and always indicate the lower calcium to phosphorus ratio of each pair. Increasing the calcium to phosphorus intake ratio results in variable increases in the per cent of intake excreted in the urine.

were sufficient to bring the values above the normal range.

In a similar study of an infant (62), the dietary calcium to phosphorus ratio was changed from 1.3 to 2.8 and finally to 5.1 by changing the diet from a milk formula to a soybean mixture containing $CaCO_3$. The daily urinary calcium values with the 3 diets were 23, 86, and 286 mgm. respectively.

Another factor affecting urinary calcium is the quantity of dietary protein. The significance of several studies on this subject is obscured by the fact that concurrent changes occurred in acid-base and calcium-phosphorus ratios of the diet. A few studies are available, however, in which these factors were controlled [Pittman and Kunerth (18, 30), McCance *et al.* (59), and Hawkes *et al.* (60)], and wherein each subject served as his control. In these studies and in a study from our own laboratory (46), the diets were controlled by the addition of minerals where necessary, so that the calcium and phosphorus intakes and calcium to phosphorus ratios were held very nearly constant. Also acid-base ratios varied only slightly between the low and high protein diets in all the studies except that of McCance (59), in which no mention is made of possible changes in acidity of the diet on addition of protein. The changes in urinary calcium values obtained in these studies are shown in Figure 13. The figures for high calcium intakes were obtained in pre-school children (60) with relatively minor changes (from 3 to 4 mgm. per kgm. per day) in dietary protein. It appears that increase in dietary protein without notable concurrent increase in acidity of ash of the diet or change in dietary calcium to phosphorus ratio causes a small but consistent increase in urinary calcium. However, the amount of increase was not sufficient to bring the urine calcium outside the normal limits in any of the studies reported.

The question of the mechanical effect of the extra bulk of the cellulose from vegetables on the absorption and excretion of calcium has been studied by several investigators. Particular interest is centered on such vegetables as also contain oxalate. The results of vegetable feeding are somewhat conflicting and the changes in urine calcium are rarely marked. Spinach given to adults (63) decreased both absorption and urinary excretion of calcium. With children, spinach feeding was without significant effect on urine calcium (5), while studies of 4 infants fed small amounts of spinach gave variable results (64). Figure 14 records mean values for urine calcium of infants, children, and adults given diets of low and high vegetable content. In general, the feeding of increased amounts of vegetables appeared to decrease the urinary calcium, particularly when the chief source of intake calcium was vegetables (22, 31, 32, 38, 63), but the effect of the roughage was minor and inconsistent.

The well-known effect of vitamin D in increasing the retention of calcium in the body is usually considered to be the result of increased absorption of calcium from the intestine. Vitamin D given to rachitic infants increases both the absorption and urinary excretion of calcium. However, in amounts of about 400 I.U. daily, vitamin D has small and inconsistent effects on urinary calcium

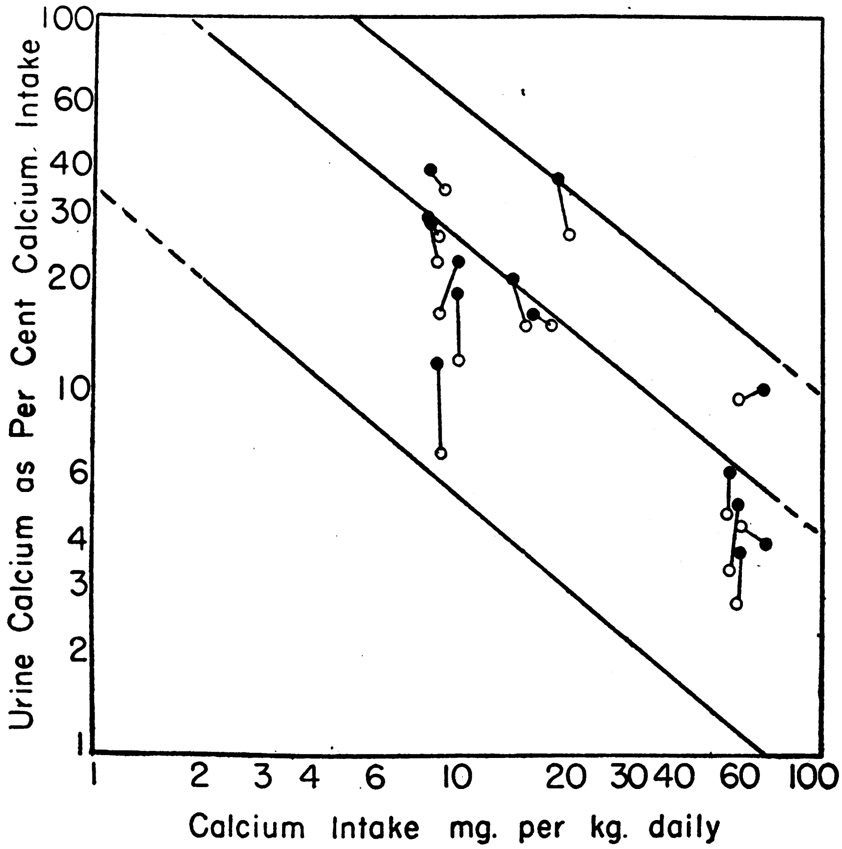

FIG. 13. THE EFFECT OF INCREASING PROTEIN INTAKE WITHOUT INCREASING THE ACIDITY OF ASH OF THE DIET OR THE CALCIUM TO PHOSPHORUS INTAKE RATIO

Background lines and symbols are the same as in Figure 11. Increasing the protein intake results in small increases in urinary calcium excretion which do not exceed the normal limits.

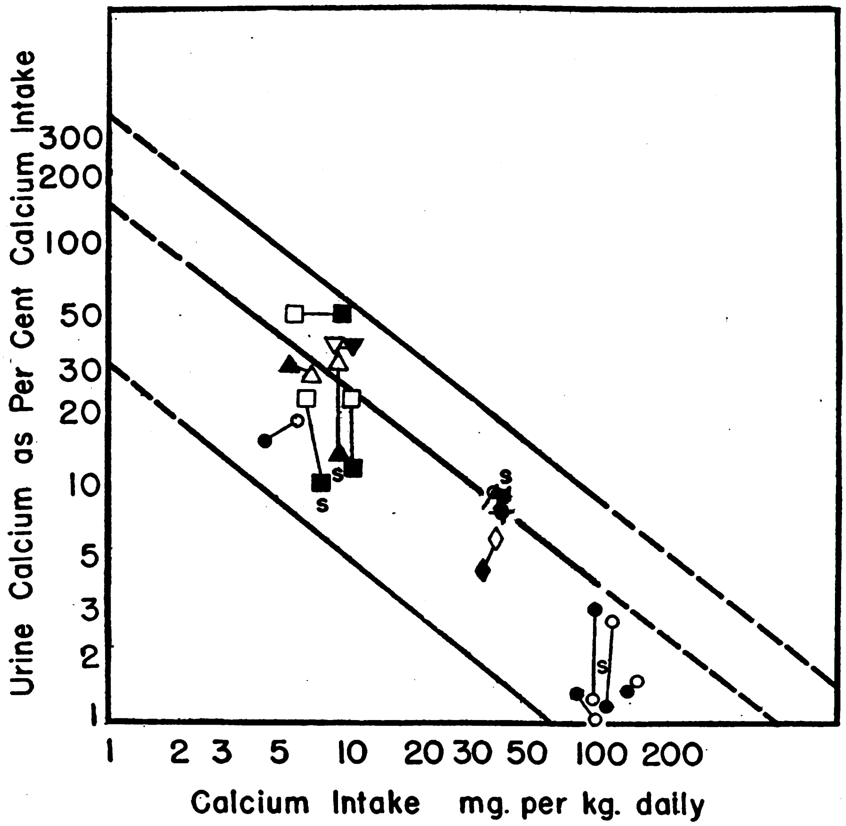

FIG. 14. THE EFFECT OF INCREASED INTAKE OF VEGETABLES ON EXCRETION OF URINARY CALCIUM

Solid symbols represent the higher roughage intake, hollow symbols represent control studies. S indicates that the vegetable studied was spinach.

of healthy children. In one study of 8 children, a consistent slight increase in average urinary calcium was noted (9); in another, no change was observed (66). Data from studies made in this laboratory on boys given 340 I.U. vitamin D as cod liver oil daily at two levels of calcium intake are presented in Table VIII. The administration of vitamin D appeared to increase urinary calcium slightly in 11 of 15 subjects when the calcium intake was moderate. With more ample calcium intake, the changes in urinary calcium were very small and not consistent. Similarly for 8 adolescent girls receiving ample calcium intake (Table VIII), 300 to 400 I.U. of vitamin D daily increased calcium absorption but caused no change in the urinary excretion of calcium. In adults the effect of moderate doses of vitamin D on urinary calcium is negligible (13, 16, 23, 67).

In hypervitaminosis D, the urinary calcium is usually greatly increased above the normal (68).

TABLE VIII

Influence of vitamin D intake on urinary excretion of calcium by children 1–15 years old

Age	No. subj.	Approx. intake	Vitamin D per day: 0 — Urine Mean	0 — Urine Range	300–400 I.U. — Urine Mean	300–400 I.U. — Urine Range
years		*grams per day*	*mgm. per day*		*mgm. per day*	
Boys						
1–3	2	0.750	29	13–45	43	36–50
1–3	4	1.350	36	27–55	38	25–52
4–7	7	0.750	65	33–128	80	24–142
4–7	6	1.450	94	76–165	79	46–181
8–12	6	0.850	82	40–122	174	114–240
8–12	5	1.650	186	83–279	112	60–159
Girls						
11–15	8	1.270	118	26–224	110	25–210

Doses of vitamin D much larger than are physiological, although not necessarily large enough to produce hypervitaminosis, are also reported to increase urinary calcium (67, 69).

Increases in urinary calcium have been noted after ingestion of large amounts of urea (70), magnesium citrate (3), and magnesium lactate (72) by adults. Lactose caused a consistent decrease in urinary calcium of 5 little boys (71). Small increases in urinary calcium were noted after addition of sodium or potassium chloride to the diet of infants studied in this laboratory (61) and in adults (73). Potassium citrate consistently lowered urinary calcium excretion of 8 preschool children (74).

Because dietary factors such as acid-base balance, calcium to phosphorus ratio, and other factors discussed above modify somewhat the level of urinary calcium excretion characteristic for the individual, it is important that they be controlled in any study of the effect of other factors, particularly when small groups of subjects are used.

SUMMARY AND CONCLUSIONS

Through the study of the urinary calcium excretion of 606 normal persons, from 1 to 80 years of age, under standard dietary regulation, the normal range of urinary excretion of calcium has been established and a new tool provided for the study of calcium metabolism.

1. The quantity of urine calcium is dependent on an endogenous factor or factors, presumably endocrine, and on calcium intake per unit of weight. Age and sex are not factors except as they affect skeletal weight. It is thus possible to compare data from subjects of all ages and with varying dietary intakes.

2. Urinary calcium expressed as per cent of calcium intake varies inversely with the intake per kgm. and is an exponential function of the latter. Values for mean, minimum, and maximum normal urinary calcium may be expressed by specific equations.

3. The urinary calcium excretion of subjects below 2 years of age is within the normal range but below the mean value for older children and adults.

4. Dietary factors other than calcium intake have relatively minor effects on urine calcium, with the exception of ingested acids, ketogenic diets, or diets with a high calcium to phosphorus ratio, all of which increase urinary calcium, often above maximum normal limits.

5. The ability to demarcate the normal range of urinary calcium with a considerable degree of accuracy should prove a useful aid in further studies of calcium metabolism, as well as an aid in diagnosis of disease entities associated with alteration of urinary excretion of calcium.

The author wishes to acknowledge receipt of additional data from Drs. Julia Outhouse Holmes, Gladys Kinsman Lewis, Icie Macy Hoobler, Helen Hunscher, Martha Pittman, Margaret Ohlson, and Miss Hughina McKay. Mrs. Helen Kelly and Dr. William Berg carried out the statistical analyses (75).

BIBLIOGRAPHY

1. Bauer, W., Albright, F., and Aub, J. C., Studies of calcium and phosphorus metabolism. II. The calcium excretion of normal individuals on a low calcium diet, also data on a case of pregnancy. J. Clin. Invest., 1929, **7**, 75.
2. Blatherwick, N. R., and Long, M. L., The utilization of calcium and phosphorus of vegetables by man. J. Biol. Chem., 1922, **52**, 125.
3. Bogert, L. J., and McKittrick, E. J., Studies in inorganic metabolism. I. Interrelations between calcium and magnesium metabolism. J. Biol. Chem., 1922, **54**, 363.
4. Bogert, L. J., and Kirkpatrick, E. E., Studies in inorganic metabolism. II. The effects of acid-forming and base-forming diets upon calcium metabolism. J. Biol. Chem., 1922, **54**, 375.
5. Bonner, P., Hummel, F. C., Bates, M. F., Horton, J., Hunscher, H. A., and Macy, I. G., The influence of a daily serving of spinach or its equivalent in oxalic acid upon the mineral utilization of children. J. Pediat., 1938, **12**, 188.
6. Breiter, H., Mills, R., Dwight, J., McKey, B., Armstrong, W., and Outhouse, J., The utilization of the calcium of milk by adults. J. Nutrition, 1941, **21**, 351.
7. Burton, Helen B., The influence of cereals upon the retention of calcium and phosphorus in children and adults. J. Biol. Chem., 1930, **85**, 405.
8. Chaney, M. S., and Blunt, K., The effect of orange juice on the calcium, phosphorus, magnesium, and nitrogen retention and urinary organic acids of growing children. J. Biol. Chem., 1925, **66**, 829.
9. Daniels, A. L., Hutton, M. K., Knott, E., Everson, G., and Wright, O., Relation of ingestion of milk to calcium metabolism in children. Am. J. Dis. Child., 1934, **47**, 499.
10. Davis, N. J., Calcium, phosphorus, and nitrogen retention of children. Effects of acid-forming and

base-forming diets. Am. J. Dis. Child., 1935, **49**, 611.

11. Disch, Anne C., The effect of an acid ash diet upon the calcium and phosphorus excretion of normal adults and kidney stone patients. Thesis, State Univ. Iowa, 1941.
12. Farquharson, R. F., Salter, W. T., and Aub, J. C., Studies of calcium and phosphorus metabolism. XIII. The effect of ingestion of phosphates on the excretion of calcium. J. Clin. Invest., 1931, **10**, 251.
13. Hart, M. C., Tourtellotte, D., and Heyl, F. W., The effect of irradiation and cod liver oil on the calcium balance in the adult human. J. Biol. Chem., 1928, **76**, 143.
14. Herbst, O., Beitrage zur Physiologie des Stoffwechsels in Knaben-alter mit besonderer Berucksichtigung einiger Mineralstoffe. Annales Paediatrici, 1912, **76**, 40.
15. Herbst, O., Calcium and Phosphor beim Wachstum am Ende der Kindheit. Ztschr. f. Kinderh., 1913, **7**, 161.
16. Hunscher, H. A., Donelson, E., Erickson, B. N., and Macy, I. G., Results of the ingestion of cod liver oil and yeast on calcium and phosphorus metabolism of women. J. Nutrition, 1934, **8**, 341.
17. Johnston, J. A., Factors influencing retention of nitrogen and calcium in period of growth. IV. Effect of estrogen. Am. J. Dis. Child., 1941, **62**, 708.
18. Kunerth, B. L., and Pittman, M. S., A long-time study of nitrogen, calcium, and phosphorus metabolism on a low-protein diet. J. Nutrition, 1939, **17**, 161.
19. Leverton, R. M., and Marsh, A. G., One hundred studies of the calcium, phosphorus, iron, and nitrogen metabolism and requirement of young women. Univ. Nebr. Agric. Expt. Sta., Research Bull., 1942, 125.
20. Mallon, M. G., Jordan, R., and Johnson, M., A note on the calcium retention on a high and low fat diet. J. Biol. Chem., 1930, **88**, 163.
21. Mallon, M. G., Johnson, L. M., and Darby, C. R., A study of the calcium retention on a diet containing American cheddar cheese. J. Nutrition, 1932, **5**, 121.
22. Mallon, M. G., Johnson, L. M., and Darby, C. R., The calcium retention on a diet containing leaf lettuce. J. Nutrition, 1933, **6**, 303.
23. McCance, R. A., and Widdowson, E. M., Mineral metabolism of healthy adults on white and brown bread dietaries. J. Physiol., 1942, **101**, 44.
24. McCance, R. A., Widdowson, E. M., and Lehmann, H., The effect of protein intake on the absorption of calcium and magnesium. Biochem. J., 1942, **36**, 686.
25. McClellan, W. S., Rupp, V. R., and Toscani, V., Clinical calorimetry. XLVI. Prolonged meat diets with a study of the metabolism of nitrogen, calcium, and phosphorus. J. Biol. Chem., 1930, **87**, 669.
26. Unpublished data from the study reported by McKay, H., Patton, M. B., Ohlson, M. A., Pittman, M. S., Leverton, R. M., Marsh, A. G., Stearns, G., and Cox, G., Calcium, phosphorus, and nitrogen metabolism of young college women. J. Nutrition, 1942, **24**, 367.
27. Morris, N., and MacRae, O., Metabolic reactions to acidosis produced by ammonium chloride. Arch. Dis. Childhood, 1930, **5**, 207.
28. Owen, E. C., Irving, J. T., and Lyall, A., The calcium requirements of older male subjects with special reference to the genesis of senile osteoporosis. Acta Med. Scandinav., 1940, **103**, 235.
29. Pierce, H. B., Daggs, R. G., Meservey, A. B., and Simcox, W. J., The retention of calcium and phosphorus by pre-school children. J. Nutrition, 1940, **19**, 401.
30. Pittman, M. S., and Kunerth, B. L., A long-time study of nitrogen, calcium, and phosphorus metabolism on a medium-protein diet. J. Nutrition, 1939, **17**, 175.
31. Rose, M. S., Experiments on the utilization of the calcium of carrots by man. J. Biol. Chem., 1920, **41**, 349.
32. Rose, M. S., and MacLeod, G., Experiments on the utilization of the calcium of almonds by man. J. Biol. Chem., 1923, **57**, 305.
33. Sahs, E. B., Dietary factors influencing urinary acidity and stone formation. Thesis, State Univ. Iowa, 1939.
34. Sherman, H. C., Gillette, L. H., and Pope, H. M., Monthly metabolism of nitrogen, phosphorus, and calcium in healthy women. J. Biol. Chem., 1918, **34**, 373.
35. Sherman, H. C., Wheeler, L., and Yates, A. B., Experiments on the nutritive value of maize protein and on the phosphorus and calcium requirements of healthy women. J. Biol. Chem., 1918, **34**, 383.
36. Sherman, H. C., Winters, J. C., and Phillips, V., Efficiency of oat protein in adult human nutrition. J. Biol. Chem., 1919, **39**, 53.
37. Sherman, H. C., Calcium requirement of maintenance in man. J. Biol. Chem., 1920, **44**, 21.
38. Sherman, H. C., and Hawley, E., Calcium and phosphorus metabolism in childhood. J. Biol. Chem., 1922, **53**, 375.
39. Steggerda, F. R., and Mitchell, H. H., The calcium requirement of adult man and the utilization of the calcium in milk and in calcium gluconate. J. Nutrition, 1939, **17**, 253.
40. Steggerda, F. R., and Mitchell, H. H., Further experiments on the calcium requirement of adult man and the utilization of calcium in milk. J. Nutrition, 1941, **21**, 577.
41. Wang, C. C., Kaucher, M., and Frank, M., Metabolism of undernourished children. IV. Calcium metabolism. Am. J. Dis. Child., 1928, **35**, 856.
42. Wang, C. C., Kern, R., and Kaucher, M., Minimum requirement of calcium and phosphorus in children. Am. J. Dis. Child., 1930, **39**, 768.

43. Wang, C. C., Kaucher, M., and Wing, M., Metabolism of adolescent girls. IV. Mineral metabolism. Am. J. Dis. Child., 1936, **52**, 41.
44. Willard, A. C., and Blunt, K., A comparison of evaporated with pasteurized milk as a source of calcium, phosphorus, and nitrogen. J. Biol. Chem., 1927, **75**, 251.
45. McCrudden, F. H., The determination of calcium in the presence of magnesium and phosphates: the determination of calcium in urine. J. Biol. Chem., 1911–12, **10**, 187.
46. Knapp, E. L., Studies on the urinary excretion of calcium. Thesis, State University of Iowa, 1943.
47. Unpublished data; Jeans, P. C., Stearns, G., and others.
48. Jeans, P. C., Stearns, G., McKinley, J. B., Goff, E. A., and Stinger, D., Factors possibly influencing the retention of calcium, phosphorus, and nitrogen by infants given whole milk feedings. I. The curding agent. J. Pediatrics, 1936, **8**, 403.
49. Czerny, A. D., and Keller, A., Des Kindes Ernahrung, Ernahrungsstorungen, und Ernahrungstherapie, Vol. II. Leipzig, 1928.
50. Wang, C. C., Witt, D. B., and Felcher, A. R., A comparison of the metabolism of some mineral constituents of cow's milk and of breast milk in the same infant. Am. J. Dis. Child., 1924, **27**, 352.
51. Lingberg, G., Über den Stoffwechsel des gesunden, naturlich ernährten Sauglings und dessen Beiinflussung durch Frauenmilchfett. Ztschr. f. Kinderh., 1917, **16**, 90.
52. Muhl, Greta, Über den Stoffwechsel des gesunden naturlich ernährten Sauglings und dessen Beiinflussung durch Fettreduktion der Nahrung. Acta Paediat., 1923, **2**, Suppl., 1–140.
53. a. Stearns, G., and Moore, D. L. R., Growth in height and weight, and retention of nitrogen, calcium, and phosphorus during recovery from severe malnutrition. Am. J. Dis. Child., 1931, **42**, 774.
    b. Stearns, G., Jeans, P. C., Catherwood, R., and McKinley, J. B., Diet in malnutrition and celiac disease. J. Pediat., 1941, **18**, 12.
54. Utheim, K., Advanced chronic nutritional disturbances in infancy. Stud. from Dep't Pediat., Wash. U. School Med., 1922, **4**, 1.
55. Miyakawa, G., and Stearns, G., Severe osteoporosis (or osteomalacia) associated with long-continued low-grade steatorrhea. J. Bone and Joint Surg., 1942, **24**, 429.
56. Farquharson, R. F., Salter, W. T., Tibbetts, D. M., and Aub, J. C., Studies of calcium and phosphorus metabolism. XII. The effect of the ingestion of acid-producing substances. J. Clin. Invest., 1931, **10**, 221.
57. Niermann, M., and Winter, M., Stoffwechseluntersuchungen bei Ernährung mit sauren Milchmischugen. Arch. f. Kinderh., 1930, **89**, 290.
58. Nelson, M. V., Calcium and phosphorus metabolism of epileptic children receiving a ketogenic diet. Am. J. Dis. Child., 1928, **36**, 716.
59. Lehmann, H., and Pollak, L., The effect of amino acids on phosphate transfer in muscle extract. Biochem. J., 1942, **36**, 672.
60. Hawks, J. E., Bray, M. M., Wilde, M. O., and Dye, M., The interrelationship of calcium, phosphorus, and nitrogen in the metabolism of pre-school children. J. Nutrition, 1942, **24**, 283.
61. Stearns, Genevieve, and others, unpublished data.
62. Stearns, G., Soybean flour in infant feeding. A study of the relation of the comparative intakes of nitrogen, calcium, and phosphorus on the excretion and retention of these elements by infants. Am. J. Dis. Child., 1933, **46**, 7.
63. McLaughlin, Laura, Utilization of the calcium of spinach. J. Biol. Chem., 1927, **74**, 455.
64. Stearns,, G., and Stinger, D., unpublished data.
65. Edelstein, E., Der Einfluss von Gemüsezulagen auf den Stickstoff und Mineralstoffwechsel des Kindes. Ztschr. f. Kinderh., 1932, **52**, 483.
66. Weld, C. B., and Sykes, J. F., The effect of irradiated ergosterol on calcium and phosphorus retention in children. Trans. Roy. Soc. Canada, Sec. V, 1932, **81**.
67. Bauer, W., Marble, A., and Claflin, D., Studies on the mode of action of irradiated ergosterol. I. Its effect on the calcium, phosphorus, and nitrogen metabolism of normal individuals. J. Clin. Invest., 1932, **11**, 1.
68. Harris, L. J., The mode of action of vitamin D. The "parathyroid" theory: clinical hypervitaminosis. Lancet, 1932, **1**, 1031.
69. Rose, E., Perloff, W. H., and Sundermann, F. W., The effect of low calcium diet and calciferol on calcium and phosphorus metabolism: Studies on 2 parathyreoprivic patients and one "normal" subject. Am. J. M. Sc., 1941, **202**, 691.
70. Aub, J. C., Tibbetts, D. M., and McLean, R., The influence of parathyroid hormone, urea, sodium chloride, fat and of intestinal activity upon calcium balance. J. Nutrition, 1937, **13**, 635.
71. Mills, R., Breiter, H., Kempster, E., McKey, B., Pickens, M., and Outhouse, J., The influence of lactose on calcium retention in children. J. Nutrition, 1940, **20**, 467.
72. Tibbetts, D. M., and Aub, J. C., Magnesium metabolism in health and disease. I. The magnesium and calcium excretion of normal individuals, also the effects of magnesium, chloride, and phosphate ions. J. Clin. Invest., 1937, **16**, 491.
73. a. von Wendt, G., Untersuchungen über den Eiweiss- und Salz-Stoffwechsel beim Menschen. Skandinav. Arch. f. Physiol., 1905, **17**, 211.
    b. Wiley, F. H., Wiley, L. L., and Waller, D. S., The effect of the ingestion of sodium, potassium, and ammonium chlorides and sodium bicarbonate on the metabolism of inorganic salts and water. J. Biol. Chem., 1933, **101**, 73.
74. Watson, E. K., McGuire, E. W., Meyer, F. L., and Hathaway, M. L., Calcium metabolism of preschool children. J. Nutrition, 1945, **30**, 259.
75. Snedecor, G. W., Statistical Methods. Collegiate Press, Ames, Iowa, 1937.