site on the enzyme surface would be large. If in fact, the mechanism of chelate-enzyme interaction changes from metal coordination attachment to charge attraction at elevated pH values, as the data would appear to indicate, it may be possible to identify the specific amino acid(s) responsible for the charge attraction from the estimated pKa. Further investigations are being contemplated to pursue these avenues.

REFERENCES

(1) Mario, E., and Bolton, S. M., *Anal. Chem.*, **37**, 165 (1965).
(2) Bolton, S. M., *J. Pharm. Sci.*, **54**, 373(1965).
(3) *Ibid.*, **54**, 583(1965).
(4) Wilson, C. L., and Wilson, D. W., "Comprehensive Analytical Chemistry," Elsevier Co., New York, N. Y., 1960, p. 351.
(5) Gonick, E., Doctoral dissertation, The Pennsylvania State College, 1951.
(6) Schwarzenbach, G., Maissen, B., and Ackerman, H., *Helv. Chim. Acta*, **35**, 2333(1952).
(7) Bertsch, C. R., Fernelius, W. C., and Block, B. P., *J. Phys. Chem.*, **62**, 503(1958).
(8) Friedenwald, J. S., and Maengwyn-Davies, G. D., in "A Symposium on the Mechanism of Enzyme Action," McElroy, W. D., and Glass, B., eds., Johns Hopkins Press, Baltimore, Md., 1954, p. 154.

Keyphrases

Acetylcholinesterase inhibitors
Cupric chelate synthesis—1,3-diaminopropanol and 1,3-diaminopropane
Dissociation constants—acid
Chelate stability constants
Inhibition, acetylcholinesterase—analysis
pH Effect—acetylcholinesterase inhibition

Comparison of the Absorption and Excretion of Calcium from Precipitated Calcium Carbonate USP XVII and Egg Shells

By ARMEN H. MIRZAIAN and E. BLANCHE SOMMERS

Experiments were conducted to evaluate relative absorption and excretion of calcium from precipitated calcium carbonate USP and powdered egg shells (93.7 percent). Samples from 24-hr. specimens of urine and feces were collected after the fourth day of a repeated daily diet of the same food containing approximately 1 Gm. of calcium. The subjects were continued on the same regimen and samples were collected for 10 consecutive days from each person tested. The calcium content of the samples was determined by means of a flame photometer and a spectrophotometer. This procedure was repeated with addition of 2.5 Gm. of the official precipitated $CaCO_3$ and again with 2.6 Gm. of powdered egg shells to each daily diet. The calcium balance, that is, the difference between the quantity of calcium ingested and the total amount excreted, was positive for each type of experiment. When the diet was supplemented with the precipitated $CaCO_3$ and egg shells, calcium retention was increased 86.3 and 97.9 percent, respectively. Increased calcium absorption was reflected in increased urinary calcium. Egg shells compared favorably with the precipitated calcium carbonate as a source of absorbed calcium.

EXTENSIVE STUDIES have been concerned with nutritional standards for calcium. The National Research Council recommends a daily dietary allowance of 0.8 Gm. of calcium for an adult (1). Goodman and Gilman state that the average adult's daily requirement is about 10 mg./Kg. of body weight (2).

Calcium is the fifth most abundant element in the body, and the major fraction is in the bony structure (99%). It is present in small quantities in the extracellular fluid and to a minor extent in the structure and cytoplasm of cells of soft tissue. In the instance of hypocalcia, daily intake is frequently supplemented with a calcium salt. Wohl (3) prescribed the clinical use of calcium carbonate in preference to other calcium salts since it has a higher percentage of available calcium (40%).

The high calcium content of egg shells—*Gallus domesticus* Temminck (family, *Phasianidae*): calcium carbonate, 93.7%; magnesium carbonate, 1.3%; phosphorus pentoxide, 0.76%; and organic matter, 4.15% (4)—prompted investigation in regard to their value as a source of calcium. Limited studies of this nature have been done. However, they were primarily concerned with the palatability of foods containing powdered egg shells.

The studies presented here are concerned with comparison of calcium balances when the diet is

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supplemented first with precipitated calcium carbonate USP XVII and then with egg shells containing an equivalent amount of calcium.

ABSORPTION AND EXCRETION OF CALCIUM

The problem of defining in a precise manner the relationship between the quantity of calcium ingested and that which is excreted in the stool in terms of absorption alone is exceedingly difficult, owing to the fact that calcium is not only absorbed from the gut lumen but secreted into the luminal contents as well. Therefore, the quantity of fecal calcium represents the net effects of the amounts ingested, absorbed, and secreted. Although considerable progress has been made in understanding some of these interrelations with the use of isotopes of calcium, much remains to be elucidated.

The urinary excretion of calcium appears to be the net effect of the quantity filtered and the amount reabsorbed. There is no evidence of renal tubular secretion of calcium. The mechanisms for the renal absorption of calcium are unknown (5).

According to Best and Taylor (6), the calcium of the feces amounts daily to from 0.4 to 0.8 Gm.; this, though a considerable amount is endogenous, is mainly the unabsorbable calcium of the food. About 150 mg. is excreted daily in the urine. An increase or decrease in the absorption of calcium is reflected in parallel changes in the urinary excretion. The urinary calcium constitutes, therefore, a convenient index of calcium absorption.

Krantz and Carr (7) state that about 30% of the calcium ingested by man is absorbed and that the remainder is excreted in the feces. The excess of the absorbed calcium is excreted in the urine, mainly as calcium phosphate. From 0.1 to 0.4 Gm. of calcium is excreted daily in the urine, dependent upon the dietary intake.

Grollman (8) reports that approximately 80% of ingested calcium is excreted in the feces. The remaining 20% of the ingested calcium in an individual, in calcium equilibrium, is excreted in the urine. With low and moderate levels of intake, 0.1–0.5 Gm. daily, approximately 30–50% is eliminated in the urine, while with high levels, 1 Gm. daily, approximately 25% is excreted. However, considerable deviations from these values occur, dependent upon variable dietary, metabolic, and gastrointestinal factors (8).

EXPERIMENTAL

Ten men ranging from 20 to 25 years of age served as the test subjects. All had normal renal function and no evidence of malabsorption.

Representative samples from pooled 24-hr. specimens of urine and of feces were obtained from each individual. This procedure was initiated after the fourth day of a repeated daily diet of the same food containing approximately 1 Gm. of calcium. The subjects were continued on the same regimen and samples were collected for 10 consecutive days thereafter. After intervals of 10 days, this procedure was repeated first with the addition of 2.5 Gm. of the official precipitated calcium carbonate and then with the addition of 2.6 Gm. of powdered egg shells to each daily diet. Preliminary studies indicated that pretreatment levels of excreted calcium were regained within 10 days after discontinuation of calcium therapy. Accordingly, a 10-day interval before the administration of each of the supplemented diets was allowed. Samples were refrigerated in tightly closed containers until they were assayed for calcium content.

The *Heinz Handbook of Nutrition* (9) was used in the selection of the basic unsupplemented diet which contained approximately 1 Gm. of calcium. Considerations other than calcium content were caloric content, balance of food elements, and food preferences of the 10 subjects. Food portions were weighed and each meal was prepared in the same manner by the same personnel.

This basic diet (Table I) was used for each of the 10 subjects in each of the three phases of the study. During the first phase of the study, it was administered without supplementation. In the second and the third phases of the study, it was supplemented with calcium carbonate and egg shells, respectively. The daily dose of each of these substances was administered in a portion of the milk consumed at breakfast. All of the 10 subjects first received the basic diet, then the calcium carbonate supplemented diet, and finally the egg shell supplemented diet.

A search of the literature concerned with experiments in regard to calcium balance did not reveal control of water intake. Accordingly, this study was not concerned with this aspect. However, total daily urine and feces for each subject were collected and tested for calcium content.

Egg shells used for the experiment were washed and their membranes were removed. The shells after being air dried were reduced to the consistency of the official calcium carbonate, and the powder thus obtained was thoroughly mixed. The official assay specified for calcium carbonate was used to determine the total calcium content of this powder

Table I—Basic, Unsupplemented Diet Containing Approximately 1 Gm. of Calcium

	Weight, Gm.	Calories	Calcium, mg.
Breakfast			
Bread, whole wheat	23	55	22
Butter	7	50	10
Milk	488	332	576
Orange juice, fresh	246	108	47
Coffee, black	230	9	4
Egg	54	77	29
Bacon	32	194	8
Lunch			
Bread, whole wheat	23	55	22
Butter	14	100	20
Potato, baked	100	97	13
Beef, roast	172	378	28
Peas, canned	60	111	24
Tomato, fresh	150	30	16
Peaches, canned	258	175	13
Cola	180	83	...
Dinner			
Bread, whole wheat	23	55	22
Butter	7	50	10
Chicken, roasted	115	227	16
Beans, lima	249	176	67
Salad, tossed green	144	168	38
Pie, apple	220	330	15
Ginger ale	180	63	...
Total		2,923	1,000

TABLE II—EFFECTS ON CALCIUM BALANCE OF EXTRA CALCIUM FROM PRECIPITATED CALCIUM CARBONATE USP XVII AND EGG SHELLS

	Unsupplemented Diet[a] —Calcium, 1,000 mg./Day—				Calcium Carbonate, 2.5 Gm. + Unsupplemented Diet = —Calcium, 1,980 mg./day—				Egg Shells, 2.6 Gm. + Unsupplemented Diet = —Calcium, 1,980 mg./day—			
Subject	Av. Urinary Output, mg./Day	Av. Fecal Output, mg./Day	Av. Calcium Balance, mg./Day	± S.D.	Av. Urinary Output, mg./Day	Av. Fecal Output, mg./Day	Av. Calcium Balance, mg./Day	± S.D.	Av. Urinary Output, mg./Day	Av. Fecal Output, mg./Day	Av. Calcium Balance, mg./Day	± S.D.
1	92	708	+200	±30.4	113	1,566	+301	±10.3	228	1,403	+349	± 22.4
2	110	763	+127	±20.0	183	1,621	+176	±14.6	188	1,458	+334	± 14.5
3	115	754	+131	±16.7	240	1,651	+ 89	± 9.4	165	1,550	+265	± 14.4
4	65	803	+132	±10.0	228	1,598	+154	± 9.6	169	1,573	+238	± 14.0
5	20	850	+130	±11.8	77	1,486	+417	±15.5	215	1,608	+157	± 5.4
6	79	896	+ 25	± 2.8	157	1,590	+233	±24.4	193	1,314	+473	± 28.5
7	141	797	+ 62	± 5.4	181	1,538	+261	±24.1	199	1,610	+171	± 19.5
8	117	683	+200	±17.8	199	1,594	+187	±18.9	160	1,541	+279	± 15.0
9	207	577	+216	±21.9	168	1,530	+282	±27.3	196	1,710	+ 74	± 4.7
10	128	823	+ 49	± 6.2	136	1,573	+271	±28.2	158	1,645	+177	± 14.3
Av.	107	765	+127	±66.1	168	1,575	+237	±91.4	187	1,541	+252	±115.0

[a] The unsupplemented diet (control diet) containing approximately 1 Gm. of calcium was the same in all three cases.

TABLE III—COMPARISON OF CALCIUM RETENTION FROM OFFICIAL PRECIPITATED CALCIUM CARBONATE AND FROM EGG SHELL SUPPLEMENTED DIETS TO CALCIUM RETENTION FROM THE UNSUPPLEMENTED DIET

	Av.[a] Intake, mg.	Av. Output, mg.	Av. Retention, mg.	± S.D.	P[e]	Increase, %
Diet 1[b]	1,000	873	127	± 66.1	...	...
Diet 2[c]	1,980	1,743	237	± 91.4	<.05	86.3
Diet 3[d]	1,980	1,728	252	±115.0	<.05	97.9

[a] Average for the 10 subjects. [b] Unsupplemented diet. [c] Calcium carbonate supplemented diet. [d] Egg shell supplemented diet. [e] Significance determined by subjecting data to student *t* test.

which proved to be 94.1% when calculated as calcium carbonate. The egg shell and calcium carbonate powders were stored in tightly closed containers.

Determination of Calcium—Even though various methods are available for the determination of calcium from organic sources, the assay of some of these compounds is difficult. Urine and feces present difficulties due to the presence of phosphates and coloring matter.

In this study, the determination of calcium in urine was first attempted according to the official direct EDTA titration method specified for calcium carbonate. Interfering phosphate ions of the urine were previously removed by elution of the samples through anion-exchange resin columns containing an exchanger with a high capacity for phosphates. Satisfactory end points could not be obtained so hydroxy naphthol blue, "Indicator of Choice," according to Butler *et al.* (10), was unsuccessfully substituted for the naphthol green screened murexide. Eriochrome black T was also tried as an indicator; however, due to the interference of the coloring matter of the urine, reliable end points were not obtainable.

Flame photometry proved to be a more rapid and accurate method for the determination of the calcium content of urine and feces. The samples were assayed by means of a Coleman model 21 flame photometer and a Coleman model 6D junior spectrophotometer according to the directions of the Coleman operating manual for urinary and fecal calcium (11). Results are shown in Table II.

The calcium retention that occurred when the diet was supplemented first with the official calcium carbonate and then with egg shells was compared with the calcium retention from the basic diet. Results are shown in Table III. The average daily

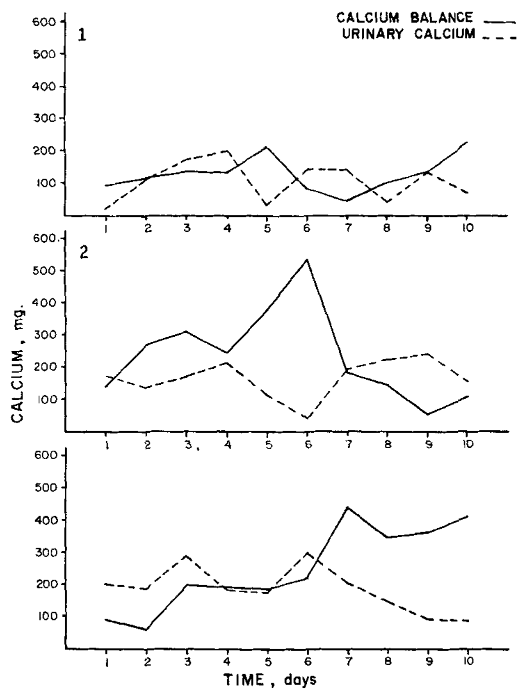

Fig. 1—Comparison of calcium balance with urinary calcium for each of 10 days. Key: 1, unsupplemented diet containing 1 Gm. calcium; 2 and 3, diets supplemented with official calcium carbonate and egg shells, respectively, each containing 1.98 Gm. of calcium (milligrams of calcium represent an average for the 10 cases studied); ——, calcium balance; -----, urinary calcium.

amounts of urinary calcium and daily calcium balance for the 10 subjects studied are shown for the three types of experiments in Fig. 1.

DISCUSSION AND CONCLUSION

Calcium Balance—Based upon the limited number of subjects and the observed values, one cannot definitely say which source of calcium is superior. However, it is obvious that calcium from egg shells is assimilated to approximately the same extent as calcium from the official calcium carbonate. The use of egg shells as a source of dietary and therapeutic calcium warrants further investigation.

Absorption—According to Best and Taylor (6), urinary calcium constitutes a convenient index of calcium absorption. In agreement with their statement, calcium carbonate and egg shell supplemented diets, when compared to the unsupplemented diet, provide greater calcium content in excreted urine.

Some investigations, such as that of Briscoe and Ragan (12), indicate that increased calcium absorption occurs in the instance of increased calcium retention. Briscoe and Ragan also stated, "In studies of calcium balance, if maintained calcium balance is observed, it is assumed that the retained calcium is used for bone mineralization" (13).

Daily Comparison of Calcium Balance with Urinary Calcium—Since Best and Taylor consider urinary calcium a convenient index of calcium absorption (6), the purpose of this phase of the study was to determine if calcium excreted in the urine could also be used as an index of calcium balance.

The experiment concerned with the unsupplemented diet revealed that on days 4, 5, 6, 8, and 10 of the 10-day study increased calcium balances varied indirectly with urinary calcium values. In the instance of the calcium carbonate supplemented diet, this same relationship existed for 8 days of the 10-day period. The diet supplemented with egg shells, except for days 7, 9, and 10, demonstrated direct variation of calcium balance and urinary calcium. The daily calcium balance for this third type of experiment showed an irregular but overall continued increase of calcium balance during the 10-day period.

Little, if any, correlation can be derived for the three types of experiments in regard to the daily averages of calcium balances and calcium excreted in the urine. However, according to Table II, the averages for the total of each of the 10 days for the three types of experiments indicated that calcium balance does vary directly with urinary calcium. According to this study, the average urinary calcium value for a period of time can be used as an index for calcium balance. However, periodic 1-day urinary calcium values can be misleading as a clinically acceptable diagnostic index for calcium balance.

The validity of data obtained in human metabolism studies depends on the constancy of the regimen, an adequate number of subjects, and the length of time covered by the study. These studies were of short but of sufficient duration in order to substantiate the value of egg shells as a source of dietary and/or therapeutic calcium.

REFERENCES

(1) Burton, B. T., "The Heinz Handbook of Nutrition," 2nd ed., McGraw-Hill, New York, N.Y., 1965, p. 138.

(2) Goodman, L. S., and Gilman, A., "The Pharmacological Basis of Therapeutics," 3rd ed., The Macmillan Company, New York, N.Y., 1966, p. 795.

(3) Wohl, M. G., "Diotherapy Clinical Application of Modern Nutrition," W. B. Saunders Co., Philadelphia, Pa., 1945, p. 712.

(4) Jacobs, M. B., "The Chemistry and Technology of Food and Food Products," vol. 1, Interscience Publishers, Inc., New York, N.Y., 1944, p. 540.

(5) Goodman, L. S., and Gilman, A., *op. cit.*, p. 796.

(6) Best, C. H., and Taylor, N. B., "Physiological Basis of Medical Practice," 5th ed., The Williams & Wilkins Co., Baltimore, Md., 1950, p. 825.

(7) Krantz, J. C., and Carr, C. J., "The Pharmacologic Principles of Medical Practice," 6th ed., The Williams & Wilkins, Co., Baltimore, Md., 1965, p. 894.

(8) Grollman, A., "Pharmacology and Therapeutics," 6th ed., Lea & Febiger, Philadelphia, Pa., 1965, p. 1000.

(9) Burton, B. T., *ibid.*, pp. 424–448.

(10) Butler, A. Q., Maurina, F. A., and Morecombe, F. A., *J. Pharm. Sci.*, **54**, 134(1965).

(11) "Operating Directions for the Model 21 Coleman Flame Photometer D-248," Coleman Instruments, Inc., Maywood, Ill., 1960, pp. 27–28, 34–37.

(12) Briscoe, A. M., and Ragan, C. R., *Am. J. Clin. Nutr.*, **19**, 300 (1966).

(13) Ragan, C. R., and Briscoe, A. M., *J. Clin. Endocr. Metab.*, **24**, 386(1964).

Keyphrases

Calcium carbonate

Absorption, excretion—calcium

Egg shell calcium vs. precipitated calcium carbonate USP—absorption, excretion

Colorimetric analysis—excreta

Flame photometry—analysis, excreta