

Calcium in the Intestinal Contents of Rats on Different Calcium Diets* (34121)

THOMAS J. SERNKA¹ AND ANDRE B. BORLE²
(Introduced by William F. Neuman)

*Department of Radiation Biology and Biophysics, The University of Rochester,
Rochester, New York*

Since intestinal calcium is absorbed as free calcium, absorption may be delayed or prevented by the formation of calcium precipitates or complexes (6). Both protein and phosphate are abundantly present in the normal balanced diet and these substances can bind free calcium in the intestinal lumen. Hence an attempt was made to determine the relative fractions of free and bound calcium in the intestinal contents of rats and to relate these fractions to the amount of calcium in the diet.

Methods. Male albino rats (Wistar, 150–300 g) were divided into two groups of 16 each and kept on separate diets for 3 days. The “minimal Ca” group received a diet including vitamin D with no added calcium (Table I). The “tolerable Ca” group was given the same diet with 0.1% calcium by weight in addition. Since the casein present in the diets of both groups may contain as much as 0.2% calcium, the minimal Ca diet was possibly as high as 0.05% and the tolerable Ca diet as high as 0.15% calcium by weight. Both groups were given distilled, deionized (D/D) water to drink and coprophagy was minimized by placing food in covered containers. Food consumed and

TABLE I. Minimal Calcium Diet.

	% Total weight
KCl	1.0
NaCl	0.5
K ₂ HPO ₄	1.8
NaH ₂ PO ₄ · H ₂ O	0.3
Sucrose	67.6
Casein	23.7
Corn oil	4.9
Poly-vi-sol ^c	0.2

^c Mead Johnson and Company; includes 6500 USP units of vitamin D.

weight gains for the 3 days were comparable for the two groups.

Animals were killed by severing the spinal cord with heavy shears. The entire length of small intestine was drawn out by progressively cutting away mesentery. After the length was measured, the intestine was cut into three equal portions: a proximal “duodenum” (D), a middle “jejunum” (J), and a distal “ileum” (I). For a rat of average weight (235 g) the segments were 37 cm long and had weights of 2.65 (D), 2.40 (J), and 2.49 g (I).

Intestinal contents were squeezed out of the lumen using a moistened glass rod and were maintained at 0° until aliquots were taken for calcium analysis. Each segment was washed out with 10 ml of saline and the “rinse” was analyzed separately. An ultrafiltrate of intestinal contents was made using cellulose sacs (Arthur H. Thomas Company, No. 4465-A2 dialyzer tubing) for centrifugation at 1800 rpm for 15 hr (5, 7). The ultrafiltrate was analyzed directly. The time for centrifugation was not so long that ultrafiltration reached completion. Therefore,

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¹ Present address: Gastrointestinal Laboratory, San Francisco General Hospital, San Francisco, California 94110.

² Present address: Department of Physiology, University of Pittsburgh, Pittsburgh, Pennsylvania 15213.

in addition to bound calcium, the sac contained a fraction of calcium which was potentially filterable upon longer centrifugation. This "unfiltered-filterable" fraction would have the same concentration of calcium as the ultrafiltrate. Hence the amount of calcium in this fraction could be calculated as the product of the measured concentration of calcium in the ultrafiltrate and the liquid volume of the sacs after centrifugation. The liquid volume was estimated by the difference between the wet and dry weights of the sac with its contents after centrifugation. The dry weight was obtained by heating the sac with its contents to 110° overnight. Total unfiltered sac calcium was analyzed after the dried, unfiltered portion was dissolved in 25 ml of D/D water with added trypsinogen (Sigma, T20B-068) for 27 hr at 37°. The "bound" calcium was calculated as the difference between the total unfiltered calcium and the unfiltered-filterable calcium.

Calcium was measured using the EDTA titration method of Toribara (8). The concentration of phosphorus also was measured (1) in order to determine if bound calcium was calcium phosphate. A Beckman pH meter, model GS, was used to measure acidity.

Results. The concentration of calcium in the ultrafiltrate ranged from 0.1 mM in the duodenum of rats on a minimal Ca diet to 1.1 mM in the ileum of rats on a tolerable Ca diet. Since the calcium concentration of deproteinized serum is 2.3 mM (8), the ultrafiltrate concentration of calcium was low relative to that of blood. The ultrafiltrate concentration of phosphorus, on the other hand, ranged from 2.2 mM in the ileum of rats on a minimal Ca diet to 8.6 mM in the jejunum of rats on a tolerable Ca diet (Table II). Thus the ultrafiltrate concentration of phosphorus generally exceeded the highest expected serum concentration of 3.5 mM.

Although there was a tendency for the concentrations of calcium and phosphorus to vary inversely in the duodenal and ileal segments, the concentration product was not a constant but ranged from 0.5 mM² in the duodenum of rats on a minimal Ca diet to 2.5 mM² in the ileum of rats on a tolerable Ca diet. The concentration product for calci-

TABLE II. Concentrations of Calcium and Phosphorus and Acidity of Intestinal Ultrafiltrate.

Diet: Segment:	Minimal Ca			Tolerable Ca		
	D	J	I	D	J	I
Calcium ^a	0.09	0.14	0.31	0.19	0.18	1.08
Phosphorus ^a	5.86	8.51	2.18	7.38	8.50	2.28
pH	6.06	6.36	6.41	—	6.41	6.51

^a Average concentrations of 12 rats expressed as mM.

um and phosphorus in the serum is about 5 mM². Hence no calcium phosphate precipitate would be expected for intestinal contents unless the contents were markedly alkaline. In fact, all segments were distinctly acidic (Table II).

The amounts of calcium in the ultrafiltrate, unfiltered-filterable, rinse, and bound fractions are shown in Table III. Since the extremes of calcium concentration were found in the proximal and distal segments, only the duodenum and ileum were considered in detail. The ultrafiltrate, unfiltered-filterable, and rinse fractions together comprised the "filterable" calcium, or the total free calcium of the intestinal contents. Although the filterable fraction was one-half the total for the ileum of rats on a tolerable Ca diet, filterable calcium was the predominant form of calcium only in the duodenum of rats on a minimal Ca diet (Table III).

Except for the duodenum of rats on a minimal Ca diet, however, bound calcium was found to account for one-half or more of the total calcium present in the intestinal contents. Bound calcium as well as total calcium was about ten times as great in the ileum as in the duodenum regardless of diet. The tolerable Ca preparations showed about twice the total calcium as their corresponding minimal Ca preparations.

Discussion. The low concentration and low total amounts of calcium found in the duodenum of rats on both minimal Ca and tolerable Ca diets indicate the efficiency of the intestinal absorption of calcium. The concentration of free calcium in the intestinal contents was well below the serum value in all segments studied. Hence this study does not

TABLE III. Total Amounts of Calcium in Rat Intestinal Contents.

Diet: Segment:	Calcium (μM /segment)			
	Minimal Ca		Tolerable Ca	
	D	I	D	I
Filterable calcium				
Ultrafiltrate ^a	3	9	5	21
Unfiltered-filterable ^b	17	41	19	312
Rinse ^a	1	4	2	26
Total filterable	21	54	26	359
Bound calcium ^b	7	260	39	337
Total calcium	28	314	65	696
Filterable/total	0.75	0.17	0.40	0.52
Bound/total	0.25	0.83	0.60	0.48

^a Mean data for 12 rats.

^b Mean data for four rats.

support the hypothesis that the calcium concentration in the intestinal lumen is regulated to a value near 3 mM, as has been suggested (2).

Relatively small amounts of calcium were found in the duodenum as compared to ileum for rats on a tolerable Ca diet. As a result of calcium absorption in the duodenum, one would expect that the ileum would have a smaller rather than greater total quantity of calcium. Bile, which contains 25 mM calcium, could not selectively increase the calcium in the ileal segment because the bile duct enters the duodenum. Therefore, it is necessary to postulate that calcium be secreted into the ileum in order to account for the increased quantities of calcium found in this segment. The concentration of calcium in the ileum does not exceed the serum value and the electrical potential difference across the ileal mucosa is small and oriented so that the mucosal surface is negative relative to the serosal surface. Therefore, the secretion of calcium into the ileum does not represent the active transport of this ion species.

Previous work has established the presence of comparatively high levels of free calcium in the dog ileum and the dependence of intestinal calcium concentration on dietary calcium (3, 4). In addition, substantial amounts of insoluble calcium have been demonstrated in this segment (6). In the present study the

bound calcium has been found to be the predominant form of calcium in the intestinal contents of rats on a diet with added calcium. As discussed elsewhere (6), the presence of a large fraction of bound calcium can lead to an overestimation of calcium absorption and an underestimation of calcium secretion.

The concentration product for calcium and phosphorus in the intestinal lumen, 2.5 mM² or less, is much larger than the solubility product for calcium phosphate salt. Thus it is possible that much of the bound calcium is precipitated calcium phosphate. The concentration product of these ions in the intestine, however, is less than that of the serum, 5.0 mM². Since there is abundant protein in the intestinal contents, the concentration product of the serum may be more appropriate than the solubility product in estimating the degree to which there is intestinal calcium phosphate precipitation. The calcium phosphate salt is acid-soluble, so that higher concentrations of calcium and phosphorus should have been detected in the more acidic duodenum if a precipitate had formed. On the contrary, the concentration product for the duodenum was relatively low.

The probability that large amounts of calcium are bound to proteins or protein hydrolyzates in the intestine has not been excluded by this study. A plentiful supply of protein was made available in the diet as casein and

the calcium-binding capacity of proteins is widely recognized.

Summary. Free and bound calcium were determined on intestinal contents of rats maintained on minimal and tolerable calcium diets. The concentration of free calcium was less than the serum value for all intestinal segments and for rats on both diets. The amount of bound calcium was greater when calcium was added to the diet and was greatest in the ileal segment for rats on either diet. The results indicate that the complete absorption of calcium from the duodenum may be followed by a small secretion of calcium back into the lumen of the ileum.

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