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G W Bo-Linn, ... , C Santa Ana, J S Fordtran

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### Research Article

Since calcium solubility is a prerequisite to calcium absorption, and since solubility of calcium is highly pH-dependent, it has been generally assumed that gastric acid secretion and gastric acidity play an important role in the intestinal absorption of calcium from ingested food or calcium salts such as CaCO<sub>3</sub>. To evaluate this hypothesis, we developed a method wherein net gastrointestinal absorption of calcium can be measured after ingestion of a single meal. A large dose of cimetidine, which markedly reduced gastric acid secretion, had no effect on calcium absorption in normal subjects, and an achlorhydric patient with pernicious anemia absorbed calcium normally. This was true regardless of the major source of dietary calcium (i.e., milk, insoluble calcium carbonate, or soluble calcium citrate). Moreover, calcium absorption after CaCO<sub>3</sub> ingestion was the same when intragastric contents were maintained at pH 7.4 (by *in vivo* titration) as when intragastric pH was 3.0. On the basis of these results, we conclude that gastric acid secretion and gastric acidity do not normally play a role in the absorption of dietary calcium. Other possible mechanisms by which the gastrointestinal tract might solubilize ingested calcium complexes and salts are discussed.

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## An Evaluation of the Importance of Gastric Acid Secretion in the Absorption of Dietary Calcium

George W. Bo-Linn, Glenn R. Davis, David J. Buddrus, Stephen G. Morawski, Carol Santa Ana, and John S. Fordtran

Department of Internal Medicine, Baylor University Medical Center, Dallas, Texas 75246

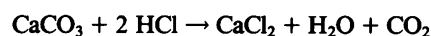
**A**bstract. Since calcium solubility is a prerequisite to calcium absorption, and since solubility of calcium is highly pH-dependent, it has been generally assumed that gastric acid secretion and gastric acidity play an important role in the intestinal absorption of calcium from ingested food or calcium salts such as  $\text{CaCO}_3$ . To evaluate this hypothesis, we developed a method wherein net gastrointestinal absorption of calcium can be measured after ingestion of a single meal. A large dose of cimetidine, which markedly reduced gastric acid secretion, had no effect on calcium absorption in normal subjects, and an achlorhydric patient with pernicious anemia absorbed calcium normally. This was true regardless of the major source of dietary calcium (i.e., milk, insoluble calcium carbonate, or soluble calcium citrate). Moreover, calcium absorption after  $\text{CaCO}_3$  ingestion was the same when intragastric contents were maintained at pH 7.4 (by in vivo titration) as when intragastric pH was 3.0. On the basis of these results, we conclude that gastric acid secretion and gastric acidity do not normally play a role in the absorption of dietary calcium. Other possible mechanisms by which the gastrointestinal tract might solubilize ingested calcium complexes and salts are discussed.

### Introduction

Most dietary calcium is bound in complex formations with other food constituents, e.g., calcium caseinate in milk (1). For this calcium to be absorbed, these complexes must be dissociated by digestive enzymes and the calcium released in a soluble and

probably in an ionized form (1, 2). Moreover, calcium salts (whether preexisting in food or formed from the interaction of different food constituents) are relatively insoluble in water and must therefore be dissolved before calcium absorption can occur (3). Since both the dissociation of food-calcium complexes and the solution of calcium salts are highly dependent on an acid pH, it has generally been assumed that gastric acid secretion plays an important role in calcium absorption (1, 4).

For calcium salts, which are relatively water-insoluble (such as the carbonate and the phosphate), gastric acid secretion is believed to play a critical role in calcium absorption. Clarkson et al. (5) postulated that ingested calcium carbonate reacts with hydrochloric acid to form soluble calcium chloride, part of which is absorbed in the small intestine while the remainder is converted back to calcium carbonate in the lower intestine.



According to this hypothesis, the amount of calcium absorbed after ingestion of  $\text{CaCO}_3$  should be proportional to the acidity of the upper gastrointestinal contents (which is determined mainly by the rate of gastric secretion). In the absence of acid,  $\text{CaCO}_3$  should remain insoluble, and as such, calcium given in this form should be poorly absorbed. Accordingly, one authoritative source states flatly that "calcium carbonate will not be absorbed by patients with achlorhydria" (6).

Although it is logical to presume that gastric acidity and acid secretion play a major role in the absorption of calcium from food and calcium salts, this concept has never been systematically examined. The importance of this issue is apparent when one considers the deleterious effects of calcium malabsorption, the fact that the most popular calcium supplements consist of  $\text{CaCO}_3$ , the high prevalence of hypo- and achlorhydria (especially in elderly women) (7), and the frequency with which potent gastric antisecretory drugs are prescribed (often for long periods of time). To study the role of gastric acid on calcium absorption, we devised a method that permits measurement of net intestinal absorption of dietary calcium after ingestion of a single meal.

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## Methods

### Overview

The method begins with a preparatory washout wherein the subject's entire gastrointestinal tract is cleansed by lavage. The subject then eats a meal, which includes polyethylene glycol (PEG)<sup>1</sup> as a nonabsorbable marker. After 12 h, the intestine is cleansed again by a final washout, and the rectal effluent is combined with any stool (usually none) that was excreted since the meal was eaten. The meal and rectal effluent are analyzed for calcium. The completeness of collection is evaluated by recovery of the nonabsorbable marker. On a separate day, the entire procedure is repeated, except that only water containing the nonabsorbable marker is ingested (i.e., no food or calcium is ingested); this provides an estimate of the amount of calcium in the rectal effluent that is not attributable to eating the meal.

We then calculate net calcium absorption<sup>2</sup> according to the following equation:

$$\text{Net calcium absorption} = \text{Amount of calcium ingested} - (\text{Effluent calcium after meal} - \text{Effluent calcium after fast}).$$

For example, assume that on test day 1, after the preparatory washout, the subject ingested 1,000 mg of calcium, and the rectal effluent from the final washout contained 800 mg of calcium; on test day 2, the subject fasted after the preparatory washout, and the effluent contained 40 mg of calcium. Substituting in the equation,

$$\text{Net calcium absorption} = 1,000 - (800 - 40) = 240 \text{ mg.}$$

### Subjects

16 normal, healthy subjects (12 men, 4 women) were studied. Mean ( $\pm$ SEM) age was  $28 \pm 1$  yr with a range of 21–36. We also studied a postmenopausal woman, age 57, who has pernicious anemia and receives monthly vitamin B<sub>12</sub> injections. After subcutaneous injection of pentagastrin (6  $\mu$ g/kg body weight), she had gastric achlorhydria (lowest pH of gastric aspirate was 7.4). Four patients with chronic renal disease on hemodialysis took part in the validation studies reported in Figs. 1 and 2; these patients did not take part in any of the experiments related to the effect of gastric acid on intestinal absorption of calcium.

This project was approved by the Institutional Review Board for Human Protection at Baylor University Medical Center, and written informed consent was received from each participant.

### Test meals

The low calcium meal consisted of 170 g of sirloin steak, seasoned with salt and pepper, 25 g of white bread with 5 g margarine, 40 g of lettuce with 30 g French salad dressing, 250 ml of tea with 5 g of sugar, and 10 g of PEG. By our measurements, this meal contained  $71 \pm 1$  mg of calcium ( $n = 4$ , range 70–72 mg). The high calcium meal was the same except that 250 ml of skim milk was substituted for the tea and 57 g of Swiss cheese was melted on the steak. The high calcium meal contained  $852 \pm 1$  mg of calcium ( $n = 4$ , range 850–856 mg). A third meal that contained  $290 \pm 3$  mg of calcium ( $n = 5$ , range 284 to 298

1. Abbreviations used in this paper: PEG, polyethylene glycol.

2. Net absorption is defined as dietary intake minus fecal excretion. True absorption exceeds net absorption because some endogenously secreted calcium is reabsorbed.

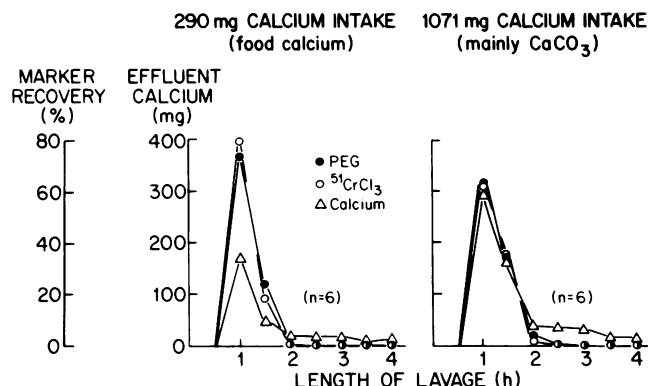


Figure 1. Mean timed recoveries of PEG, <sup>51</sup>CrCl<sub>3</sub>, and calcium in lavage effluents from six subjects after ingesting 290 mg of food calcium (left), and 1,071 mg of calcium as 71 mg of food calcium supplemented with 1,000 mg of calcium as CaCO<sub>3</sub> (right).

mg) was used in validation studies only. This meal consisted of 57 g of sirloin steak, 25 g of white bread, 40 g of lettuce with 30 g of French salad dressing, 30 g of Swiss cheese, 250 ml of tea with 5 g of sugar, and 10 g of PEG.

### Calcium supplements

The low calcium meal was supplemented with either calcium carbonate or calcium citrate. The calcium carbonate was provided in Os-Cal tablets (Marion Laboratories, Kansas City, MO), which did not contain added vitamin D. The source of calcium carbonate in Os-Cal tablets is crushed oyster shell. Os-Cal tablets disperse rapidly in water, but calcium carbonate is negligibly soluble in water. To study the effect of HCl and pH on the solubility of calcium carbonate in oyster shell tablets, we measured the amount of 0.1 N HCl required to maintain pH constant at various set points, when an Os-Cal tablet was placed in a beaker containing 100 ml of water. The amount of HCl required to maintain the pH constant at a given level reflects the amount of calcium carbonate that has gone into solution at that pH (8). As shown in Table I, reducing pH from

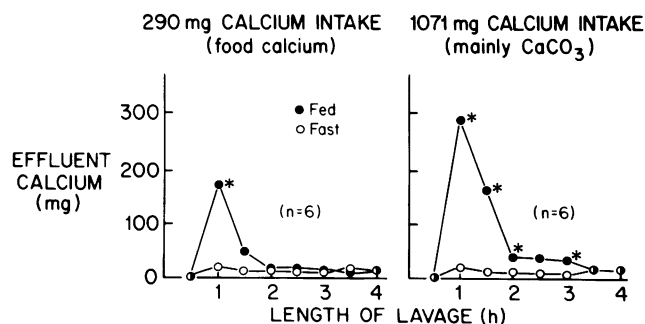


Figure 2. Mean timed recoveries of calcium in lavage effluents from six subjects after fasting and after ingesting 290 mg of food calcium (left) and after 1,071 mg of calcium as 71 mg of food calcium supplemented with 1,000 mg of calcium as CaCO<sub>3</sub> (right). \*,  $P < 0.05$  by paired  $t$  test.

Table 1. Volume of 0.1 N HCl Required to Maintain pH at Various Set Points When One Oyster Shell Tablet Was Added to 100 ml Water

Time	pH set point			
	7.4	6	4	3
min				
10	0	3.1	1.7	5.2
20	0.1	12.3	11.7	27.8
40	0.1	33.9	60.4	91.0
60	0.1	60.3	86.6	140.6
80	0.1	74.3	93.9	172.2
100	0.1	87.6	111.2	190.4
120	0.1	93.0	131.8	202.2

Theoretically, 250 ml of 0.1 N HCl is sufficient to completely solubilize the calcium carbonate contained in one oyster shell tablet (1.25 g of CaCO<sub>3</sub> or 500 mg of elemental calcium). Hence, for example, after 120 min at pH 6, 37% (93/250 × 100) of the calcium carbonate had reacted with HCl and gone into solution as CaCl<sub>2</sub>.

7.4 to 6.0 or 3.0 was associated with a marked increase in calcium carbonate solubility, as assessed by this test.

The calcium citrate supplement originated from effervescent tablets prepared by Miles Laboratories, Inc., Elkhart, IN. These tablets contain calcium carbonate and citric acid. When added to water, they effervesce, thus liberating calcium citrate. The latter is soluble in water, and the calcium is present in ionized form.

The calcium carbonate tablets were swallowed with 200 ml of water. The effervescent tablets were added to 200 ml of water; after effervescence ceased, the subjects drank the calcium citrate solution. Unless otherwise stated, the subjects took the calcium supplements 1 h after beginning to eat the meal.

### Procedure

After an overnight fast, the subjects underwent the preparatory washout with Golytely (Braintree Scientific, Inc., Braintree, MA), a lavage solution that is not associated with either net intestinal absorption or secretion of water and electrolytes (9). We administered Golytely orally (300 ml every 10 min) or by intragastric infusion (30 ml per min) through a mercury weighted, polyvinyl tube that the subject swallowed. After 4 h of Golytely lavage, the rectal effluent was totally clear and contained no unabsorbed dietary nutrients (previous studies from our laboratory). The rectal effluent from the first washout was discarded.

4 h after completion of the first lavage, the subject ate the test meal. 12 h after the meal, without further ingestion of food or drink, the subjects underwent a second (final) lavage, lasting 3 h. The rectal effluent from this final lavage was combined with any stool (usually none) that had been passed since the meal was eaten.

### Analysis of samples

Test meal and rectal effluent were each weighed and then well homogenized in a Waring commercial blender. 10 ml of 2-octonol was added to the rectal effluent as a foam suppressant. 20 ml of homogenate was transferred to acid-washed glass flasks and digested with 20 ml of 16 N

nitric acid to dissolve any insoluble calcium. The homogenate was then brought to 100 ml volume with deionized water in an acid-washed volumetric flask. Duplicate aliquots were analyzed for calcium by atomic spectroscopy (model 2380, Perkin-Elmer Corp., Norwalk, CT). Homogenized but undigested samples were analyzed for PEG by the method of Hyden (10).

### Validation studies

**PEG as a marker for recovery of unabsorbed calcium.** PEG is water soluble, whereas part of the calcium remaining in the gut 12 h after the meal is presumably present as insoluble calcium salts. It was not known whether soluble and insoluble substances would be recovered in rectal effluent at similar rates. To evaluate this, we measured recovery of PEG and <sup>51</sup>CrCl<sub>3</sub> when both were ingested with the test meal. <sup>51</sup>CrCl<sub>3</sub> is believed to become partly insoluble during intestinal transit (11). Six subjects were studied with two levels of calcium intake: 290 mg of food calcium and 1,071 mg of calcium (71 mg of food calcium and 1,000 mg of calcium as CaCO<sub>3</sub>). The results are shown in Fig. 1. It is evident from these results that PEG and <sup>51</sup>CrCl<sub>3</sub> were recovered *pari passu*. Total recoveries of PEG and <sup>51</sup>CrCl<sub>3</sub> were 97.8±2.5% and 97.9±1.3%, respectively, with the lower calcium intake, and 100.0±0.5% and 98.0±1.6%, respectively, with the higher calcium intake. Assuming that part of the <sup>51</sup>CrCl<sub>3</sub> in the gut is insoluble, these results suggest that intestinal lavage removes unabsorbed soluble and insoluble substances at the same rate.

**Extent of recovery of unabsorbed dietary calcium.** Timed recovery of calcium in the rectal effluent is shown in Fig. 1, and it is evident that a small amount of calcium continued to be washed out of the gut by lavage long after complete recovery of the ingested nonabsorbable markers. This continued excretion of calcium in the lavage effluent could represent delayed recovery of unabsorbed meal calcium, recovery of unabsorbed calcium from digestive secretions, or diffusion of calcium from blood into the gut lumen during lavage. To evaluate the source of this continued calcium excretion in the effluent, we performed additional experiments in six subjects.

On one day after the initial lavage, each subject ingested a meal containing 290 mg of calcium. 12 h later, the subjects underwent the final lavage, and all rectal effluent was collected and analyzed for calcium recovery over time. On another day, the subject underwent the same protocol but ingested only water and PEG (instead of eating the calcium-containing meal). The patterns of calcium recovery are shown in Fig. 2 (left). After 2 h of lavage, continued recovery of calcium was similar on the two test days, which suggests that the continued presence of calcium in the lavage effluent beyond 2 h is not due to delayed recovery of unabsorbed dietary calcium. Therefore, it must represent either recovery of unabsorbed calcium from digestive secretions or calcium that diffuses from blood to lumen during lavage, or both.

A similar experiment was performed using a high calcium diet, and the results are shown in Fig. 2 (right). It required 3 h of lavage before calcium excretion in the lavage effluent was similar to that when these same subjects had fasted. These results suggest that 3 h of lavage are necessary to obtain complete recovery of unabsorbed dietary calcium after ingestion of a high calcium diet. When combined with the results shown in Fig. 1, the data suggest that a small amount of unabsorbed dietary calcium may remain in the gut even after virtually all of the nonabsorbable markers in the meal have been recovered. However, 3 h of lavage is sufficient to obtain complete recovery of all unabsorbed dietary calcium, even after the high calcium diet.

It is important to point out that calcium in the lavage effluent after

a fast probably does not directly reflect the amount of unabsorbed calcium from endogenous digestive secretions. This is because the lavage procedure creates electrochemical gradients favoring passive diffusion of calcium from blood to gut lumen, and at least part of the calcium in the effluent after a fast probably is due to net calcium diffusion across intestinal mucosal cell membranes.

**Timing of second lavage.** The 12-h interval between meal ingestion and the final lavage allowed ingested and endogenously secreted calcium to be exposed to absorbing mucosa for a standard period of time. Since transit of chyme through the small bowel is completed within 4–6 h (12), prolonging the interval between meal ingestion and final lavage would increase exposure of the calcium load to colonic but not to small bowel mucosa. To evaluate the extent to which prolonging the exposure time would increase calcium absorption, three normal subjects were studied on two test days with either a 12- or 24-h interval between meal ingestion (852 mg of dietary calcium intake) and final lavage. With the 12-h interval, the lavage effluent contained  $739 \pm 43$  mg of calcium, whereas with the 24-h interval, the effluent contained  $696 \pm 35$  mg of calcium. Since the amount of calcium in the final washout was only slightly less with the 24-h interval, these results are compatible with the concept (13) that most calcium absorption normally takes place from the small bowel rather than from the colon.

**Sensitivity of the technique.** Nine normal subjects were studied on four test days in random order. On the four days they either fasted in between the two lavage periods, or ingested the 71-mg calcium meal plus either 250, 500, or 1,000 mg of calcium as the carbonate. The results for each subject are shown in Table II. With one exception (subject L.Y.), there was a progressive increase in calcium absorption as dietary calcium was increased. Mean absorption was statistically significantly higher with each increment in dietary calcium intake. These

Table II. Calcium Absorption from Diets Containing Different Amounts of Calcium

Subject	Calcium in effluent after fast	Net calcium absorption*		
		321 mg Intake	571 mg Intake	1071 mg Intake
	mg	mg	mg	mg
L.Y.	61	23	152	60
G.E.	27	40	78	184
D.G.	36	-24	196	281
J.C.	52	161	168	489
J.T.	36	39	166	304
S.H.	38	51	107	212
C.S.A.	79	216	356	440
G.C.	34	160	261	383
C.S.F.	29	144	298	507
Mean	44	90	198‡	318§
SD	17	81	90	150
SE	6	27	30	50

\* Calcium absorption calculated according to formula in Methods.

‡  $P < 0.005$  when compared with 321 mg calcium intake.

§  $P < 0.02$  when compared with the 571 mg intake.

results show that the technique can readily detect differences in calcium absorption when small groups of subjects are studied. The reason for the aberrant result in subject L.Y. is unknown.

### Effect of lavage on intestinal absorption

Since all experiments involved intestinal lavage, any effect of lavage per se was constant and hopefully should not influence conclusions about the variable (gastric acidity) being studied. However, with regard to the extent to which this new method for measuring calcium absorption can be considered physiological, it was desirable to evaluate the effect of lavage on absorption. This was done by measuring jejunal absorption with the triple-lumen tube, constant perfusion technique (14–16); subjects were studied twice, once when they had been subjected to lavage, and once when they had not. On the lavage test day, the jejunal perfusion experiments were started 4 h after finishing the lavage, which corresponds to the time when the test meal would have been eaten if dietary calcium absorption had been measured.

As shown in Table III, intestinal lavage had no statistically significant effect on calcium fluxes. The mean value for net calcium absorption was somewhat lower after lavage, but this was mainly accounted for by a large difference in one of the 12 subjects, rather than by any consistent effect. If this one subject were excluded, mean net calcium absorption rates without and with lavage for the remaining 11 subjects were 0.22 and 0.20 mmol/h per 30 cm, respectively.

As also shown in Table III, phosphate, D-xylose, glucose, sodium, and bicarbonate absorption in the jejunum were not affected to a statistically significant extent by gastrointestinal lavage. Water, chloride, and potassium absorption were also unaffected (data not shown).

## Results

**Net calcium absorption from food and from two calcium salts.** Six subjects were studied in random sequence, and the results are shown in Table IV. When calcium intake was zero, the lavage effluent contained 42 mg of calcium. The origin and significance of this calcium was discussed in Methods.

When the ingested meal contained 71 mg of calcium, the amount of calcium in the effluent was 110 mg. After correcting for calcium excretion not related to the meal (41 mg), net calcium absorption after ingesting the 71 mg calcium meal was negligible (3 mg). When calcium intake was 1,071 mg, as supplemented with either calcium carbonate or calcium citrate, net calcium absorption was 255 and 310 mg, respectively (not significantly different by paired  $t$  test). When calcium intake was increased to 852 mg by milk and cheese, 132 mg of calcium was absorbed.

Mean percent recoveries of PEG are shown in the final column of Table IV.

**Effect of cimetidine on net calcium absorption.** To assess the effect of reduced gastric acid secretion on net calcium absorption, we studied these same six subjects with and without cimetidine (600 mg 30 min before the meal was eaten). As shown in Fig. 3, cimetidine had no significant effect on calcium absorption, whether the subject ingested the high calcium meal (852 mg) alone, the low calcium meal plus calcium carbonate (1,071 mg), or the low calcium meal plus calcium citrate (1,071 mg).

Table III. Effect of Gastrointestinal Lavage on Jejunal Absorption Studied with the Triple-Lumen Tube Method

	No. of paired experiments	No lavage	Lavage
Calcium fluxes			
(mmol/h/30 cm)	12		
Net		0.21±0.02	0.17±0.04
Lumen-to-plasma		0.29±0.03	0.24±0.03
Plasma-to-lumen		0.08±0.02	0.07±0.04
Phosphate absorption			
(mmol/h/30 cm)	5	0.37±0.06	0.38±0.04
D-xylose absorption			
(mmol/h/30 cm)	7	1.56±0.14	1.67±0.08
Glucose absorption			
(mmol/h/30 cm)	11	21.7±1.1	22.0±0.8
Sodium absorption			
(meq/h/30 cm)			
Without glucose	7	13.0±2.4	10.6±1.8
With glucose	11	19.7±1.7	19.7±2.7
Bicarbonate absorption			
(meq/h/30 cm)	7	8.1±0.6	8.7±1.2

Calcium absorption was measured during perfusion of 5 mM calcium gluconate, labeled with <sup>47</sup>Ca (14). Phosphate absorption was studied during perfusion of 2.5 mM sodium phosphate (15). Electrolyte and xylose absorptions were measured during perfusion of a balanced electrolyte solution (16). For study of glucose absorption, a 65 mM glucose solution was perfused; the concentration of electrolytes was reduced to make the test solution isoosmolar to plasma. The concentration of perfused solutes and the mean concentration of solutes in the 30-cm test segment without and with lavage were as follows: Calcium (millimolar): 5, 4.10, 4.14; phosphate (millimolar): 2.5, 2.21, 2.19; D-xylose (millimolar): 10, 6.47, 6.41; glucose (millimolar): 65, 31.0, 30.3; sodium (milliequivalents per liter) without glucose: 140, 139, 139; sodium (milliequivalents per liter) with glucose: 105, 121, 121; bicarbonate (milliequivalents per liter): 40.0, 18.5, 19.4. None of these concentration differences were statistically significant, and none of the differences in absorption rates shown in the table were statistically significant.

On separate test days, the extent to which cimetidine reduced gastric acidity was assessed in six subjects (four subjects ingested the low calcium meal alone [71 mg], and two ingested the low calcium meal plus two oyster shell tablets [total 1,071 mg of calcium]). Gastric contents were sampled (via a nasogastric tube) every 15 min for 3 h after the meal was ingested. Mean gastric pH after the low calcium meal increased from 2.79±0.2 without cimetidine to 5.56±0.2 with cimetidine. After the meal plus oyster shell tablets, mean gastric pH increased with cimetidine from 3.70±0.2 to 4.92±0.2.

*Studies in a patient with pernicious anemia.* Since even a large dose of cimetidine did not totally eliminate gastric acidity, the results with cimetidine do not exclude an effect of gastric

acidity on calcium absorption. Therefore, we studied a patient with treated pernicious anemia and proven gastric achlorhydria (pH > 7.4 after maximal stimulation with pentagastrin). The results in this patient are also shown in Fig. 3. Her calcium absorption was modestly higher than the mean value for six normal subjects.

We initially assumed that the pH of gastric contents in this patient would remain near 7.4 after food was eaten. However, this patient's mean gastric pH after she ate the low calcium meal was 5.75±0.09 (methods for measuring gastric acidity identical to that described above). We then suspected that the acidity of the food constituents in the meal itself might be responsible for the acid pH of her gastric contents. Our suspicions were confirmed when we determined in vitro that the pH of the homogenized test meal was 5.4.

Although these results argued against an important role for gastric acid secretion in calcium absorption, they did not rule out a role for the acidity of the gastric contents.

*Studies with in vivo titration to maintain gastric pH at 7.4 and 3.0.* We studied eight subjects on two test days. On each day, the subjects ingested 600 mg of cimetidine. 30 min later, the low calcium meal (homogenized)<sup>3</sup> was infused into the stomach, followed 30 min later by ingestion of two oyster shell tablets (total calcium intake, 1,063 mg). On one test day, the pH of gastric contents was maintained at pH 7.4 for 4 h by in vivo titration (17) with 0.3 N NaHCO<sub>3</sub>. Since acid secretion was suppressed by cimetidine, only 37±6 ml of NaHCO<sub>3</sub> was required to keep gastric pH at 7.4 for the 4-h period. On the other test day, the pH of gastric contents was maintained at pH 3.0 for 4 h by in vivo titration with 0.1 N HCl. The volume of HCl required to maintain pH at 3.0 for the 4-h period was 150±30 ml. Net calcium absorption was measured as described above. The results are shown in Table V. The absorption of calcium was similar on the two test days, suggesting that absorption of dietary calcium is not increased by increasing intragastric acidity from pH 7.4 to pH 3.0. This interpretation of these results is dependent on the assumption that ingested calcium was emptied from the stomach during the 4-h period of intragastric titration to pH 7.4. To evaluate this, five subjects were studied during intragastric titration to pH 7.4 for 4 h. At the end of this time, their stomachs were irrigated for 20 min on four consecutive occasions with 250 ml of a dilute hydrochloric acid solution (pH 2.5). Total calcium recovered in the combined wash solutions averaged 20.9±7.8 mg. Thus, the amount of calcium remaining in the stomach at the end of in vitro titration to pH 7.4 was small compared with the 1,063 mg of ingested calcium, i.e., almost all of the ingested calcium left in the stomach while the intragastric pH was 7.4.

In three additional studies, 1 ml of gastric contents was

3. Lettuce and salad dressing were eliminated from the low calcium meal for these experiments because their inclusion made the homogenate too thick to infuse through the Andersen tube (H. W. Andersen Products, Inc., Oyster Bay, NY).

Table IV. Net Calcium Absorption with Different Dietary Calcium Intakes (n = 6)

	Calcium intake	Calcium in effluent	Net calcium absorption	PEG recovery
	mg	mg	mg	% of intake
Fast	0	42±5	—	100.5±2.2
Low calcium meal	71	110±23	3±22	102.4±2.1
Low calcium meal + oyster shell CaCO <sub>3</sub> tablets	1,071	860±61	255±58	100.9±0.6
Low calcium meal + calcium citrate in solution	1,071	802±48	310±52	100.2±0.5
High calcium meal	852	760±49	132±50	97.9±2.0

Results are given as mean±SEM.

saved each time intragastric pH was measured for intragastric titration (samples taken every 2–3 min); these were pooled at 30-min intervals over the 4-h period, and analyzed for soluble and total calcium concentration. When intragastric pH was maintained at pH 7.4 (two studies), only 3% of gastric content calcium was soluble, whereas at pH 3.0 (one study), 86% of gastric content calcium was soluble.

It will be noted that net absorption of calcium was higher in Table V than in Table IV. There are two possible explanations. First, some of the subjects that took part in the first experiment did not take part in the second, and vice versa. As shown in Table II, absorption in different normal subjects can vary widely. Second, the meal shown in Table IV contained lettuce and salad dressing, and one of these foods may reduce calcium absorption. Finally, the food was homogenized in the experiment shown in Table V, and it is possible that this may enhance calcium absorption.

## Discussion

For reasons enumerated in the Introduction, it seemed important to establish the importance of gastric acid secretion and gastric acidity on intestinal absorption of dietary calcium. None of the

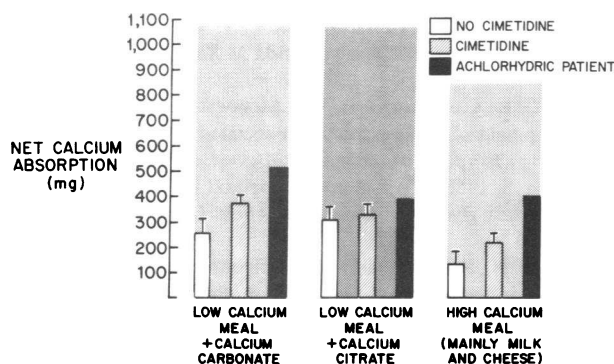


Figure 3. Effect of cimetidine and gastric achlorhydria on net calcium absorption. Six normal subjects and one patient with pernicious anemia and proven gastric achlorhydria were studied. Results as mean±SEM.

previously described methods for measuring calcium absorption seemed suitable for this purpose, being either too time-consuming and expensive (classic balance method) or dependent on the unproven reliability of various isotopic methods that do not actually measure net calcium absorption. We therefore developed and attempted to validate a new method, in which net absorption of dietary calcium is measured after a single meal. The method requires a preliminary and final washout of the gastrointestinal tract by lavage with a special electrolyte solution, and the effect of such lavage on calcium absorption as measured by this technique needs to be considered. However, there is no a priori reason to believe that this lavage would disturb small bowel digestive or absorptive processes, since the lavage fluid is isotonic to plasma and is not associated with net intestinal absorption or secretion (9). The small bowel is normally clear of debris in the fasting state; hence, further cleansing with this inert lavage solution would not be expected to induce physiological derangements. Furthermore, we have recently shown that nutrient absorption measured by a similar method gives almost identical results as are obtained by the classic balance method (18). Finally, as part of our validation experiments (see Methods), we found that lavage did not alter subsequent jejunal absorption of calcium, phosphate, electrolytes, or sugars as measured by the triple-lumen perfusion method. Thus, we do not believe that the lavage procedure as used here alters small bowel absorptive processes. The lavage does remove bacteria from the colon, but colonic bacteria are generally thought not to play an important role in calcium absorption. The method is easy to employ and is well tolerated by normal subjects and by patients with various diseases (unpublished studies from our laboratory).

Using this method, we were not able to confirm the commonly held opinion that gastric acid secretion and gastric acidity play a role in determining the amount of dietary calcium that is absorbed. Thus, a large dose of cimetidine did not alter the amount of calcium that was absorbed by normal subjects, and a patient with gastric atrophy (who secreted no acid) absorbed calcium normally (Fig. 3). These conclusions apply regardless of the form in which calcium was ingested, i.e., milk and cheese, insoluble calcium carbonate, or soluble calcium citrate. Furthermore, absorption of calcium from calcium carbonate was

Table V. Net Calcium Absorption when Gastric pH Is Maintained at 7.4 or 3.0 by In Vivo Intra-gastric Titration

	Calcium intake	Calcium in effluent	Net calcium absorption	PEG recovery
	mg	mg	mg	% of intake
Fast	0	41±4	—	99.3±1.4
Gastric pH 7.4	1,063	645±87	458±87	100.2±1.1
Gastric pH 3.0	1,063	662±63	441±63	100.6±0.9

Results are given as mean±SEM. Calcium intake was 1,063 mg, mainly in the form of calcium carbonate ( $n = 8$ ) (low calcium meal [minus lettuce and salad dressing] supplemented with 1,000 mg of calcium as  $\text{CaCO}_3$ ).

the same when gastric pH was maintained at 7.4 as at 3.0 by in vivo titration, showing that the level of gastric acidity has no discernable effect on absorption of dietary calcium.

Our results raise an interesting but puzzling question. If calcium in calcium carbonate must be solubilized before absorption can occur, how is this critical solubilization achieved in the total absence of gastric acid? One possibility is acid secretion by the mucosal cells of the proximal small intestine. Previous work has revealed that the natural pH of the jejunal contents is ~6.1 (19), and that this is probably due to secretion of hydrogen ions (20). As illustrated by the in vitro experiment shown in Table I, at pH 6 a considerable amount of calcium carbonate will go into solution. Therefore, it is possible that acid secretion by jejunal mucosa, which is not inhibited by cimetidine (21), might serve to solubilize calcium salts and make the calcium available for absorption by intestinal mucosal cells. A second possible explanation is the putative microclimate of intestinal mucosal cells. Isolated human intestinal cells have an electrophoretic mobility consistent with a fixed negative surface charge (22), and it has been suggested that these charges bind hydrogen ions and create a microclimate of high acidity adjacent to the brush border membrane (23). This localized area of high acidity, estimated to be pH 5.3, could also serve to solubilize calcium carbonate. A third possible explanation for the seeming unimportance of gastric acid is that calcium carbonate, while insoluble in water, may become soluble in biliary or pancreatic fluids. Bile acids can increase the in vitro solubility of  $\text{CaHPO}_4$  and enhance the absorption of calcium in chicks independent of the action of vitamin D (24, 25). However, in unpublished results in our laboratory, we have been unable to show that solutions containing pancreatic enzymes and bile acids enhance the solubility of calcium carbonate.

Although the opinion that gastric acidity plays an important role in absorption of dietary calcium is widely held, this has been based more on reasonable logic than on experimental evidence. To the best of our knowledge, the only previous experiment bearing directly on this question was the study of

Ivanovich et al. (4). These workers measured calcium absorption using an isotopic method, and found absorption of  $^{45}\text{CaCO}_3$  to be decreased in four patients with hypo- or achlorhydria when compared to controls. We are unable to explain the discrepancy between this report and our own results. However, we do not believe that these results by Ivanovich et al., using the indirect measurement of calcium absorption, cast serious doubt on our finding that gastric acid secretion and gastric acidity do not normally play an important role in the net absorption of dietary calcium.

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