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STUDIES ON THE INTERFERENCE OF CALCIUM ABSORPTION BY ITS INSOLUBLE SALTS*

SHI-PING CHU, RU-DONG WEI, TEH-MING KIANG and JOSEPH S. CHEN

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ABSTRACT

Experiments on calcium absorption were carried out in rats by means of radioactive calcium-45. Calcium absorption figures in different groups were compared at 2-hr intervals after feeding. In the presence of oxalate, citrate or sulfate in diet, the net amount of calcium absorbed was significantly reduced. The extent of interference in the absorption of calcium by these salts was in the increasing order of citrate, sulfate and oxalate. Carbonate is unique in that in its presence, the percentage of total calcium absorption and absorption coefficient were not significantly different from those of the controls. The molar solubility of various calcium salts, regardless of their ionizing ability is considered as an important factor for the absorbability of its calcium. However an insoluble calcium salt, as calcium carbonate shown in this study, capable of evolving a gas and shifting the reaction rightward in an acid medium may nevertheless show a high absorbability.

Early studies(1-3) indicated that the calcium supply from the average Chinese diet, mainly vegetable food items, is inadequate in quantity and inferior in quality. Since the report of less availability of calcium in spinach (4), it has been generally agreed that the absorption and utilization of calcium of vegetable origin is inferior to that of milk. However, because of social and economical reasons milk is not a common food item in the Chinese diet. It justifies therefore to evaluate the calcium absorbability of several insoluble calcium salts which commonly occurred in vegetables.

On the basis of their calcium balance tests and observations of calcification of bones, Patton (5,6) and Hayami (7) found that soluble and insoluble calcium are equally available as calcium sources. The interference of oxalate on calcium absorption has been reported by Iwao (8) and Adolph (9), but the influence of citrate, carbonate and sulfate on calcium absorption has not been clarified. In a preliminary study of ours(10) it was observed that in the presence of oxalate and citrate the amount of calcium absorbed was definitely decreased, and that calcium absorption seemed to be directly proportional to the solubility of these calcium salts. This paper will record some additional observations showing the extent of interference on calcium absorption by the presence of oxalate, citrate, sulfate and carbonate and the relation between solubility and absorption in order to shed some light on the search for the available calcium source of vegetable origin.

MATERIAL AND METHODS

Rats and diets. Adult *Long Evans* rats of both sexes, 12 to 18 months old, with mean body weight of 238±47 g were used. Animals were fed on a stock diet of this laboratory (11) with a moderate calcium level of 224.7 mg calcium per 100 g diet for 1 month prior to experimentation, in

* From the Department of Biochemistry, National Defence Medical Center, Taipei.

order to assure the uniformity in calcium status. Then they were divided into five groups, namely the control, oxalate, citrate, carbonate and sulfate groups, each group being tested with one experimental diet. The total calcium content of the basic control diet was adjusted to 230 mg % with 140 microcuries of labeled calcium-45. The oxalate, citrate, carbonate and sulfate diets were the same basic control diet added with one equivalent of their corresponding salt of sodium respectively. All the experimental as well as the control diets were pressed into pellets to avoid scattering.

Feeding plan. Rats were caged separately and restrained from food for 8 hr before experimentation. They were then allowed to feed on the specific experimental or control diet *ad libitum* for 40 min. This procedure was to ensure a rather uniform food intake within the same length of time.

Procedure to determine calcium absorption. At the end of certain hours after feeding, as indicated below, the rats were sacrified under ethyl ether which was claimed without effects on the rate of calcium absorption (12). The gastro-intestinal tract from the cardiac portion of the stomach to the internal anal sphincter of each animal was removed with its contents. These were either divided into five segments as stomach, upper and lower intestine, cecum and large intestine for the first few experiments, or used undivided for the latter 10 hr absorption experiments mentioned below. The feces expelled during experiment was collected and added to the gestro-intestinal tract sample, or the large intestine segment, as may be desired.

The radioactive calcium, obtained from the Oak Ridge National Laboratory, U. S. A; was supplied under the item Ca-45-P-2 processed as $CaCl_2$ in acid solution with a specific activity of 10.2 mc per ml. The radioactivity of samples was determined with a thin end-window (less than 2 g/cm^2) Gerger-Millor counter, and Comar's method (13) was followed with the modification that aliquots of the diluted ashing solutions were used diectly for determination. From the calcium difference between the food offered and left, the amount of calcium ingested was calculated; while from the difference between the ingested and the left-over in the gastro-intestinal tract and feces the amount of calcium absorbed was obtained.

RESULTS AND DISCUSSION

Preliminary study. The first series experiment was run to study the extent of calcium absorption at various intervals after feeding. The gastro-intestinal tracts were divided into five segments as mentioned above. The data in Table I was

TABLE I *Calcium absorption in rat gastro-intestinal tract at different post-ingestion intervals*

Rats no.	Body wt, g	Ca consumption, mg	Interval, hr	% of Ca-45 left in G-I tract					% of total absorption	Absorption coefficient
				S.	U. S. I.	L. S. I.	C.	L. I.		
51	284	4.58	2	11.7	10.9	45.3	13.4	1.7	17.0	2.99
55	250	3.70	2	12.4	14.5	55.9	0.6	0.6	17.0	3.40
59	315	7.86	2	37.2	7.1	27.4	5.9	3.5	18.9	3.00
Mean			**2**						**17.9**	**3.13**
52	300	10.39	4	3.0	2.2	10.7	53.4	4.4	26.3	2.19
57	240	8.61	4	9.8	3.1	17.4	39.6	2.7	27.4	2.85
29	220	2.79	4	9.4	0.6	14.3	46.0	4.1	25.6	2.89
44	255	6.05	4	6.1	1.5	21.8	37.6	3.2	29.8	2.92
Mean			**4**						**27.3**	**2.71**
53	345	8.61	6	0.3	0.3	1.6	31.7	20.5	45.4	2.19
56	242	11.90	6	0.3	0.2	1.4	32.5	17.5	48.1	2.29
58	326	4.52	6	0.4	0.2	1.5	36.6	18.6	43.7	2.24
60	330	8.66	6	2.5	0.9	9.3	32.7	10.9	43.6	2.20
Mean			**6**						**45.2**	**2.28**
49	347	4.25	8	5.8	1.0	1.8	26.8	14.8	49.8	2.10
54	300	5.95	8	5.2	1.4	0.6	19.5	22.5	50.8	2.11
58	280	9.32	8	5.5	0.6	2.5	31.5	11.1	48.8	2.18
Mean			**8**						**49.8**	**2.13**

S.–stomach.
U. S. I.–upper small intestine.
L. S. I.–lower small intesfine.
C.–cecum.
L. I.–large intestine.

constructed as the absorption percentage of the total calcium ingested at different intervals after feeding. Under the condition of our experiment about 45.2% of calcium ingested was absorbed in 6 hrs, and additional absorption later on was relatively small. The distribution of the calcium absorption figure in different segments of gastrointestinal tract was variable, and the difference was insignificant in the present experiments. The absorption coefficient (percentage of calcium absorbed per hour per 100 g body weight) was found to be declining gradually also as reported by Adolph and Liang (14).

Experimental groups. The total amount of calcium absorbed in the presence of oxalate or citrate at different intervals were significantly less ($P<0.001$) then that of the control group. At 6-hr interval, only 10.9 and 25.9% of the ingested calcium were absorbed by the oxalate and citrate groups respectively, in comparison with 45.2% by the control group as shown in Table II and *Fig. 1.*

TABLE II *Percentage of total calcium absorption at different intervals after feeding with or without oxalate and citrate*

Group	Time intervals, hr					P value, t test	
	2	4	6	8	10	1†	2††
Control	17.9±1.1*(3)**	27.3±1.9 (4)	45.2±2.1 (4)	49.8±1.0 (3)	46.3±4.7 (4)		
Oxalate	1.2±0.1 (4)	4.2±0.3 (4)	10.9±1.0 (4)	11.8±1.5 (3)	13.4±2.3 (4)	< 0.001	< 0.001
Citrate		17.4±2.1 (4)	25.9±9.2 (4)		31.3±3.0 (5)	< 0.001	< 0.005

*Mean±Standard deviation.
**Number of rats.
†Calculated from the data six hour after feeding when compared with control.
††Calculated from the data ten hour after feeding when compared with control.

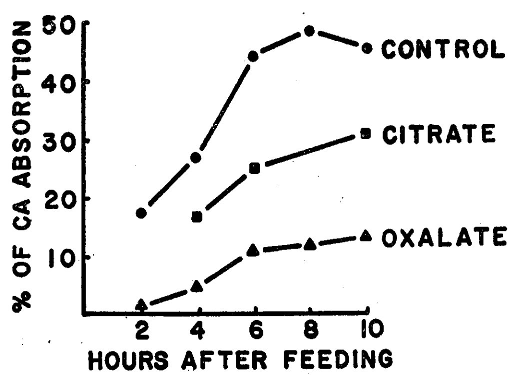

Fig. I: Total Calcium absorption at various post-ingestion intervals with or without oxalate and citrate.

By the end of 10-hr exprimental period, the total amount of calcium absorbed by the experimental groups was again shown to be significcantly less than that of the control: oxalate ($P<0.001$), sulfate($P<0.001$)and citrate($P<0.005$), but insignificant in carbonate ($P<0.1$). Table III shows that the ability to interfere calcium absorption increases in the order of carbonate, citrate, sulfate and oxalate.

It was found that the total amount of calcium absorption was independent of the length of intestine.

The effect of calcium intake and body weight. According to Bronner and Harris (15), the percentage of calcium absorption was inversely proportional to the amount of total calcium intake. On the other hand, Hansard, Comar and Plumlee (16) claimed that in rats the quantity of calcium intake, within the range of 0.28 to 51 mg, had little effect on its absorption. The amount of calcium ingested by all rats in this study fell within this range with the single exception of rat No. 12. So the intake factor may be excluded with reasonable confidence to influence the results. The variation of body weight of rats in this study did not show significant effect either upon the amount of total calcium absorbed.

The interference of oxalate. The interfering action of oxalate on calcium absorption has been reported by many authors (4,8,9,14,17,18,19). Brune (20) claimed, however, that with a large excess of oxalic acid over calcium the wethers might still be in positive calcium balance which led him to conclude that calcium oxalate could be absorbed by sheep. In Adolph's experiment (9) the calcium in spinach, which is rich of oxalic acid, was only 15-20% available in comparison with 100% availability for the milk calcium. In this study, oxalate did show a definite interfering action, but 13.4% of total calcium consumed could still be absorbed in its presence.

TABLE III: *Total calcium absorption from four insoluble calcium salts ten hours after feeding*

Grouping	Rats no.	Body wt, g	Ca ingested, mg	% of Ca left in G-I tract	% of total absorption	Absorption coefficient	P value,* t test
Control	2	189	1.80	60.0	40.0	2.11	
	4	205	9.23	52.8	47.2	2.30	
	6	260	2.68	47.2	52.8	2.12	
	38	208	2.35	54.9	45.1	2.16	
Mean					**46.3**	**2.17**	
Oxalate	9	229	1.92	87.6	12.4	0.54	
	10	190	1.83	89.3	10.7	0.56	
	12	250	0.20	87.9	12.1	0.49	
	13	271	1.83	81.4	18.6	0.69	
Mean					**13.4**	**0.57**	< **0.001**
Citrate	22	220	2.65	63.2	36.8	1.67	
	23	183	0.64	75.9	24.1	1.37	
	24	190	2.60	65.1	34.9	1.83	
	25	190	1.42	67.4	32.6	1.71	
	26	197	2.64	71.7	28.2	1.43	
Mean					**31.3**	**1.60**	< **0.005**
Sulfate	30	156	1.77	73.9	26.1	1.67	
	31	187	4.12	68.7	31.3	1.67	
	32	261	1.61	67.8	32.2	1.23	
	35	217	1.01	67.7	32.3	1.49	
	37	177	2.06	68.7	31.3	1.77	
Mean					**30.6**	**1.57**	< **0.001**
Carbonate	17	214	3.20	56.1	43.9	2.05	
	18	189	1.96	60.5	39.5	2.09	
	19	197	2.13	59.7	40.3	2.04	
Mean					**41.2**	**2.06**	< **0.1**

*** Probability against control.**

The interference of citrate. The effect of citrate on calcium absorption and utilization remains to be clarified. The beneficial effect of citrate in promoting calcium absorption or utilization was reported in rat by Schreier *et al.* (24), and also in human being by Claney and Blunt (21), Hamilton and Dewart (22), and Shohl and Butlar (23). But Day (25) and Mallon and Lard (26) observed no favorable effect of citrate on calcium absorption and retention in rat, and Watson *et al* (27) also failed to observe any demonstrable effect in their humen study. Cramer *et al.* (28), moreover, stated that the absorption of calcium in rat was greatly reduced by the incorporation of citrate, even with the supplement of vitamin D; The absorption of calcium was increased when citrate and vitamin D were omitted. So they proposed that citrate had rachitogenic effect.

The results in this study indicate a definite interference on calcium absorption by the presence of citrate in the diet, but less active than oxalate. In the citrate treated group, about 30% of the total calcium consumed was absorbed in contrast to 45.2% in the control.

Soludility and calcium absorption. The extent of calcium absorption from insoluble calcium salts was known to be definitly less than that from its soluble salts, although its availability remains to be a subject for debate. If the difference in solubility of calcium salts observed *in vitro* was applicable in the gastro-intestinal tract, then the difference in percentage of calcium absorbed between the control and experimental groups observed could be attributed mainly to the solubility of calcium salts used. The molar solubility of calcium in different salts used in this study, as shown in Table IV, were calculated from their gram solubility. With the exception of carbonate group, the calcium absorbability of different salts runs parallelly with their molar solubility. The discrepant result in the carbonate group is probably due to the chemical nature of calcium carbonate. Being less soluble and yet possessing greater absorbability than calcium salts of citrate and sulfate, calcium carbonate is rapidly soluble in diluted acid medium of the stoamach. Thus the rapid loss of carbon dioxide makes the reversible reaction impossible. Calcium

TABLE IV: *Calculated molar solubility of calcium in four insoluble calcium salts*

Salts	Calcium carbonate	Calcium sulfate	Calcium citrate	Calcium oxalate
Formula	$CaCO_3$	$CaSO_4$	$Ca_3(C_6H_4O_7)_2$	CaC_2O_4
Solubility Product*	8.70×10^{-9}	6.10×10^{-5}	**	2.57×10^{-9}
Solubility*	1.40×10^{-3}	2.09×10^{-1}	9.60×10^{-1}	6.70×10^{-4}
Molar solubility of salts	1.40×10^{-4}	1.54×10^{-2}	1.92×10^{-2}	5.23×10^{-5}
Molar solubility of calcium	1.40×10^{-4}	1.54×10^{-2}	5.76×10^{-2}	5.23×10^{-5}

* From Hodgman, C. D.: Handbook of Chemistry and Physics, 1960–61.
** No solubility product of calcium citrate can be found.

citrate is generally believed to be non-ionizable, hence its molar solubility casts the main influence for its calcium absorbability. This leads the authors to speculate that calcium absorbed by gastro-intestinal tract might not be restricted to the ionized form.

A conclusion may be drawn that the molar solubility of calcium salts, regardless of the degree of ionization, plays a major role in determining the absorbability of calcium. However, an insoluble calcium salt which could evolve a gas and shift the reaction rightward in acid medium may be expected to have a high calcium absorbability. If our reasoning were true, calcium carbonate would be an inexpensive and highly absorbable calcium source.

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