Correlation Between ^{47}Ca Absorption and Intestinal Calcium-Binding Activity in the Golden Hamster[1] (36081)

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A vitamin D-induced calcium-binding protein (CaBP) has been identified in the intestinal mucosa of the chick (1), and an analogous CaBP was shown to exist in the intestinal mucosa of a number of other species, including the rat (2), monkey (3), dog (4), and golden hamster (5). In addition to its presence in the intestine, the protein has also been found in the kidney of the chick (6) and in the shell gland of the laying hen (7). Investigations have shown that CaBP: (a) avidly binds calcium; (b) appears in the intestinal mucosa of the vitamin D-dosed rachitic chick at the time when calcium absorption is significantly enhanced (8); and (c) varies in concentration in the intestinal mucosa in direct relationship to the magnitude of the absorptive capacity of the gut (6). Such findings indicate that this protein may be involved, either directly or indirectly, in the mechanim of action of vitamin D in enhancing intestinal calcium absorption.

An exception to these correlations has been suggested by Schachter *et al.* (5, 9). Based on the results of *in vitro* studies of calcium transport in the intestine of the rat and golden hamster, they indicated that, in both species, the level of intestinal calcium-binding activity was greater in the duodenum than in the ileum. However, while calcium transport was greater in duodenal than in ileal segments of the rat, the reverse was true in the golden hamster, *i.e.*, in this species, calcium transport was greater in ileal than in duodenal gut sacs. Based on these data, the golden hamster was represented as a species in which the general direct relationship between calcium-binding activity and calcium absorption did not pertain (5, 9). It was, therefore, deemed of importance to compare calcium-binding activity levels with the degree of calcium absorption from the duodenum and ileum of the golden hamster using *in vivo* procedures, recognizing the possible error associated with the relatively nonphysiological everted gut sac method.

Materials and Methods. Male golden hamsters, weighing about 85 g, were obtained and fed a commercial hamster chow containing 1.09% Ca and 0.72% P for 1 week. After an overnight fast, each animal was anesthetized with ether and a midventral laparotomy was performed. When the duodenum was studied, this segment was exposed and a single cotton suture was placed around the duodenum about 8 cm distal to the pylorus and tied securely. A second cotton suture was placed loosely around the duodenum about 0.5 cm distal to the pylorus. For the ileal gut segments, a single cotton suture was placed around the ileum approximately 8 cm proximal to the cecum and tied securely. A second suture was then placed loosely around the ileum about 0.5 cm proximal to the cecum. In either case, the needle of a 1 ml hypodermic syringe was inserted from outside the gut segment into the lumen so that the loose suture could be drawn tightly about the needle. After this was done, 0.5 ml of a solution containing 10 mM Ca Cl_2 and 0.2 μCi of ^{47}Ca in 0.9% NaCl solution (pH 6.8) was injected into the gut loop. The needle was withdrawn while tension was maintained with the suture, and the suture was then tied. The gut segment was replaced in the abdominal cavity and the incision was closed with Michelle wound clips.

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TABLE I. Comparison of Intestinal Radiocalcium Absorption *in Vivo*, Femoral Deposition of ^{47}Ca, and Mucosal Ca Binding Activity in the Golden Hamster.[a]

Intestinal segment	No. of animals	Intestinal absorption of ^{47}Ca (%)	Femur deposition of ^{47}Ca (%)	^{45}Ca binding activity of mucosal supernatant[b] (%)	Sp act of ^{45}Ca binding (%/mg of supernatant protein)
Duodenum[c]	6	65.8 ± 6.1[d]	0.61 ± 0.16	35.9 ± 2.3	33.1 ± 1.8
Ileum	5	36.1 ± 2.0	0.30 ± 0.40	27.0 ± 1.1	23.0 ± 0.8

[a] A duplicate experiment resulted in essentially similar data.

[b] Determined by the ion-exchange resin assay (11).

[c] For all measurements, duodenal values were significantly greater than ileal values, as determined by Student's t test ($p < .025$). The duodenal and ileal segments were about 7.5 cm in length.

[d] Mean ± standard error of the mean.

At the end of a 0.5 hr period, the animals were killed in an ether chamber. The gut segment was removed and immediately counted for total ^{47}Ca activity in a well-type scintillation counter, along with suitable counting standards. After counting, each gut segment was slit open lengthwise and placed mucosa side up on a glass plate. After the mucosa was rinsed with cold Tris buffer (1.37×10^{-2} *M* Tris HCl, 0.119 *M* NaCl, 4.74×10^{-3} *M* KCl, pH 7.4) and blotted, the mucosa was stripped from each segment with a clean microscope slide, weighed, and placed in a tube with 4 vol of ice-cold Tris buffer. The mucosal sample from each gut was then homogenized in a Potter-Elvehjem homogenizer with a Teflon pestle. After the sample was spun at 38,000*g* in a refrigerated centrifuge for 20 min, the supernatants were recovered. The supernatants were analyzed for total protein by the Lowry and co-workers' method (10) and for calcium-binding activity by an ion-exchange method, using ^{45}Ca and Chelex 100 resin (11). After determining the binding activity and protein concentration of each supernatant, the specific activity of protein binding (% dose ^{45}Ca bound/mg of protein) was calculated. The femur was also recovered and counted for ^{47}Ca, as above.

Results and Discussion. The data for intestinal absorption of ^{47}Ca, intestinal mucosal calcium-binding activity, and femoral deposition of radiocalcium for each of the two intestinal sites are summarized in Table I. Radiocalcium absorption from the duodenum averaged 66% of the dose, while that from the ileum averaged only 36%. This was a highly significant difference ($p < .005$). Femoral deposition of radiocalcium reflected the difference in absorption: the femurs after duodenal absorption contained an average of 0.60% of the dose while those after ileal absorption contained about 0.30% of the dose. Again this was a significant difference ($p < .025$).

Net calcium-binding activity was significantly greater ($p < .005$) in the supernatant fraction of duodenal mucosal homogenates than in similar homogenates from the ileum. The increase in duodenal binding activity over that of ileum was about 33%. The specific activity of protein binding of calcium was approximately 50% greater in duodenal supernatants than in those from the ileum. The difference between net calcium binding and specific activity was due to the presence of a slightly higher protein level in the ileal, compared to the duodenal, mucosal supernatant preparations.

The present results differ from the *in vitro* studies of Schachter *et al.* (5, 9) in that calcium absorption was found to be significantly greater in the duodenum than in the ileum. Both studies yielded a similar sequence regarding calcium-binding activity, *i.e.*, duodenum>ileum. Thus, in our hands, the level of calcium-binding activity was found to be directly related to the degree of calcium transport in the intestine of the golden hamster.

The discrepancy between the present results and those of Schachter is probably due to the difference in technique for determining Ca absorption, and it is more likely that the *in vivo* preparation would yield less

artifactual results than the everted sac technique. In the *in vivo* situation, blood vessels penetrate to the level of the lamina propria, thus calcium has only to move across the mucosal cell, the basement membrane and elements of the lamina propria before it is available for removal from the intestinal region by blood. With the *in vitro* preparation, on the other hand, the calcium must move across the mucosa, submucosa, muscularis, and serosa before it enters the serosal fluid. These secondary morphological and/or physiological differences of the nonepithelial layers of duodenum and ileum could account for the reversal in relative transport of calcium as determined by *in vitro* and *in vivo* methods. Other investigators have also noted that intestinal absorption measured *in vivo* may well be different from that determined *in vitro* (12–14).

Summary. In the golden hamster, the absorption of ^{47}Ca from ligated duodenal segments *in situ* was significantly greater (66% of dose) than that of the ileum (36% of dose). The calcium-binding activity of supernatant fluid prepared from intestinal mucosa was also significantly greater in the duodenum than in the ileum. There is, thus, a direct correlation between ^{47}Ca absorption and intestinal calcium-binding activity in the two intestinal segments of this species, in contrast to previous findings by others based on *in vitro* everted gut procedures for measuring absorption.

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1. Wasserman, R. H., Taylor, A. N., Science **152**, 791 (1966).

2. Kallfelz, F. A., Taylor, A. N., and Wasserman, R. H., Proc. Soc. Exp. Biol. Med. **125**, 54 (1968).

3. Wasserman, R. H., and Taylor, A. N., Proc. Soc. Exp. Biol. Med. **136**, 26 (1971).

4. Taylor, A. N., Wasserman, R. H., and Jowsey, J., Fed. Proc. **27**, 675 (1968).

5. Schachter, D., Kowarski, S., and Reid, P., J. Clin. Invest. **46**, 1113 (1967).

6. Taylor, A. N., and Wasserman, R. H., Arch. Biochem. Biophys. **119**, 536 (1967).

7. Corradino, R. A., Wasserman, R. H., Pubols, M. H., and Chang, S. I., Arch. Biochem. Biophys. **125**, 378 (1968).

8. Ebel, J. G., Taylor, A. N., and Wasserman, R. H., Amer. J. Clin. Nutr. **22**, 431 (1969).

9. Schachter, D., Kowarski, S., and Reid, P. A., Symp. Calcium Cell. Funct. (A. W. Cuthbert, ed.), p. 108. St. Martin's Press, New York (1970).

10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, P. J., J. Biol. Chem. **193**, 265 (1951).

11. Corradino, R. A., Ebel, J. G., Craig, P. H., Taylor, A. N., and Wasserman, R. H., Calcif. Tissue Res. **7**, 81 (1971).

12. Williams, G. A., Bowser, E. N., Henderson, W. J., and Uzgiris, V., Proc. Soc. Exp. Biol. Med. **110**, 889 (1962).

13. Urban, E., and Schedl, H. P., Amer. J. Physiol. **217**, 126 (1969).

14. Clark, I., *in* "Cellular Mechanisms for Calcium Transport & Homeostasis" (G. Nichols and R. H. Wasserman, eds.). Academic Press, New York, in press.

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