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Development of *in Vivo* Cecal and Colonic Calcium Fluxes in Growing Rats[1] (40381)

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In growing rats, intestinal calcium transport in balance and isotopic studies (1–3) as well as *in vitro* studies of duodenum (4–6) declines and excretion of endogenous calcium increases as a function of increasing age (1, 3). Since the large bowel has been shown to be the major excretory route for endogenous calcium in the intact rat (7), adaptation of this organ to increasing age is of particular interest with regard to calcium homeostasis. Previous in vivo studies examined net cecal and colonic calcium transport in rats taking low calcium (8), low magnesium (9) or normal (10) diets, as well as in vitamin D deficient and repleted rats (11). In these studies, net absorption was consistently found to be higher in cecum than in colon, but effects of age were not investigated. A brief report on in vivo calcium transport by the entire large intestine showed greater permeability and net secretion in suckling than in weanling or adolescent rats (12), but contributions of individual segments and of unidirectional fluxes were not evaluated. The current study examines net cecal as well as colonic calcium transport in three age groups of young rats and assesses unidirectional fluxes by separately measuring lumen-to-plasma and plasma-to-lumen flux.

Materials and methods. Three age groups of young male albino rats (Table I) obtained from Charles River Breeding Laboratories, Wilmington, MA (CD strain) were studied after an adjustment period of several days, while consuming regular laboratory chow and tap water *ad libitum.* Calcium transport in cecum and colon was studied in anesthetized rats by an *in situ* perfusion technique described in detail previously (8, 9). Perfusates consisted of 1.6 m*M* $CaCl \cdot 2H_2O$ and phenol red 50 mg/liter as marker for water movements and were isotonic with saline. On each day of study, net calcium movements and lumen-to-plasma flux was studied in two rats by using a perfusate containing additional tracer ^{45}Ca (initial specific activity, 20–22 μCi/mg, New England Nuclear Corporation, Boston, MA); in two other animals net calcium movement and plasma-to-lumen flux was studied by perfusing animals with ^{45}Ca free perfusate 24 hr after intramuscular injection of 100 μCi of ^{45}Ca per 100 g body wt. After 2 hr of perfusion at 4 ml/min, luminal samples and test solution were analyzed for ^{40}Ca, ^{45}Ca, and phenol red; blood obtained from the aorta at the end of the perfusion was analyzed for ^{40}Ca and—in ^{45}Ca injected animals—also for ^{45}Ca as described (8, 9, 13); all samples were done in duplicate. Perfused segments were excised and processed as described (8, 9) to obtain surface area, full thickness wet and dry weight.

The following equations adapted from Wasserman and Comar (14) were used to calculate net calcium movements, lumen-to-plasma (LP) flux and plasma-to-lumen (PL) flux (in μmoles/g full thickness dry weight of segment per hr):

$$\text{Net Ca movements} = \frac{V[^{40}Ca_i - (^{40}Ca_f)(PRR)]}{W \times t}$$

$$\text{LP flux} = \frac{V[^{45}Ca_i - (^{45}Ca_f)(PRR)]}{\bar{x}\ SA_L \times W \times t}$$

$$\text{PL flux} = \frac{V \times {}^{45}Ca_f \times PRR}{SA_S \times W \times t}$$

where V refers to volume of perfusates (in ml); subscripts i and f = initial (test solution) and final luminal values; ^{40}Ca = the chemical calcium content of the luminal fluid (in μmol/ml); ^{45}Ca = the radioactive calcium content of the fluid (in counts/min per ml); PRR = the ratio of initial to final phenol red

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TABLE I. ANIMAL AND INTESTINAL DATA.[a]

Age group, weeks	4	7	10
Number of animals	15	27	31
Body weight, unfasted (g)	92 ± 3	187 ± 7	267 ± 5
Cecum			
surface area (cm^2)	12.8 ± 0.4	22.2 ± 0.7	26.2 ± 0.6
dry weight (mg)	74 ± 3	143 ± 4	189 ± 4
dry weight (mg/cm^2)	5.7 ± 0.1	6.4 ± 0.1	7.2 ± 0.1
Colon			
surface area (cm^2)	11.3 ± 0.4[b]	17.7 ± 0.6[b]	19.5 ± 0.4[b]
dry weight (mg)	75 ± 3	130 ± 4[b]	159 ± 4[b]
dry weight (mg/cm^2)	6.7 ± 0.2[b]	7.4 ± 0.3[b]	8.2 ± 0.1[b]

[a] Mean values ± SE in three age groups of rats.

[b] $P < 0.005$ compared with cecum by paired Student t test. All corresponding data differ significantly between the three age groups by modified Tukey's test (see Methods).

concentrations; $\bar{x}SA_L$ = the mean specific activity of luminal fluid (in counts/min per μmol ^{40}Ca); SA_S = the specific activity of serum (in counts/min per μmol ^{40}Ca); W = segment dry weight (in g); t = perfusion time (in hours). Representative values are 45,000 cpm/ml (LP flux studies) for $^{45}Ca_i$, 40,000 cpm/ml (LP flux studies) and 2000 cpm/ml (PL flux studies) for $^{45}Ca_f$, 1.06 (cecum) and 0.98 (colon) for PRR, and 26, 29, and 28/1.3 for SA_S, SA_i (lumen: LP flux studies) and SA_f (lumen: LP flux/PL flux studies) respectively.

This method for estimating *in vivo* unidirectional fluxes of ions is similar to that used by other groups of investigators (10, 14–17) and that employed repeatedly in our laboratory and its assumptions can be expected to apply in the current studies. Back-flux of marker into lumen during lumen-to-plasma flux studies and into plasma during plasma-to-lumen flux studies is assumed to be negligible. In the latter case this is likely to be correct because of the large pool of cold calcium perfused intraluminally and in the former case, because of the large plasma pool of cold calcium and the ready exchange (18) with bone calcium, although plasma and bone pools increase with growth. The assumption that the arithmetic mean of the luminal specific activity closely reflects the correct mean finds support in experiments with labeled sodium showing little difference between arithmetic and correct means if the difference between final and initial specific activity is less than 40% (19); in the current studies, final specific activities were on the average 6.2% below initial ones, the greatest difference being 21.4%. It is further assumed that serum specific activity after perfusion closely reflects mean values during the two hour perfusion. In previous experiments, serum ^{45}Ca counts were shown to be stable within 1–2% during a 2-hour perfusion period 24 hours after injection of label (20); to avoid excessive blood loss in the youngest animals, serum values were therefore based on blood samples obtained after perfusion in all groups.

The paired Student t test was used to compare corresponding values from cecum and colon (21); groups were compared by a modified Tukey's test for multiple comparisons with unequal sample sizes (22).

Results and discussion. Body weight and intestine (Table I). Three groups of rats were studied at mean ages of 4, 7, and 10 weeks. Compared with the 4-week group, body weight doubled at 7 weeks and tripled at 10 weeks. Gut dry weight and surface area increased sharply between 4 and 7 weeks; rate of growth declined between 7 and 10 weeks and mean values and rate of growth tended to be higher in cecum than in colon. Gut dry weight per unit surface area was higher in colon than cecum and increased in both segments in linear fashion. No effect of growth on mean cecal (83.6%) or colonic (80%) water content was observed (data not shown). Since growth affected intestinal parameters, we present rates for *in vivo* cecal and colonic calcium transport both per g dry tissue (Table II) and total segment (Table III).

Cecal transport. Rates for cecal net calcium absorption, lumen-to-plasma flux, and plasma-to-lumen flux declined sharply between 4 and 7 weeks, and minimally between 7 and 10 weeks; the difference between 4 and

10 weeks was significant (Table II). However, due to intestinal growth, transport rates per total segment were maintained (Table III); their apparent increase with age did not reach statistical significance when analyzed by multiple comparison of means, but was significant ($P < 0.05$) for net calcium absorption and lumen-to-plasma flux when correlating transport rate and body weight for all data (correlation coefficient, $r = +0.30$ and $+0.42$ respectively). In contrast to these *in vivo* findings in cecum, total calcium transport by everted sacs of rat duodenum decreases when growth rates decline. Thus, Adams *et al.* (6) documented a high correlation between rate of body growth and serosal/mucosal ^{45}Ca ratios; calcium transport measured in this way declined as a logarithmic function of age ($r = -0.85$). A similar study of everted sacs of duodenum showed serosal/mucosal ^{45}Ca ratios of 4.8, 3.7, and 1.9 for rats weighing 50–90, 150–250, and 400–500 g respectively (4). Furthermore, in Ussing-chamber like but not short-circuited preparations of duodenum net calcium transport was found in rats weighing 150–200 g, but not in adult rats weighing 350–400 g (5). Since balance and isotopic studies indicate a decline of overall calcium absorption from the alimentary tract with age (1–3), depression of calcium transport in duodenum and presumably other parts of small intestine appears to be prevalent adaptation in the intact animal. However, our finding of maintained or even increased cecal net absorption and lumen-to-plasma flux suggests that the proximal large bowel may play a role for calcium homeostasis in the adaptation to declining body growth, and furthermore indicates that regulation of intestinal calcium transport may greatly differ in various parts of the alimentary tract.

Colonic transport. Colonic calcium transport/g dry tissue differed in the age groups, although the pattern did not follow the cecum: lumen-to-plasma flux declined significantly between 4 and 10 weeks, but plasma-to-lumen flux did not change; net calcium absorption fell sharply and significantly between 4 and 7 weeks (Table II). Rates/segment are shown in Table III. As the colonic segment grew (Table I), lumen-to-plasma flux/segment increased slightly ($r = +0.38$, $P < 0.05$), when rates and body weight are correlated), but plasma-to-lumen flux/segment increased sharply and significantly between 4 and 7 weeks, and doubled by 10 weeks. Net colonic calcium transport did not change; however, since net colonic calcium transport reflects the relatively small difference between large lumen-to-plasma and plasma-to-lumen fluxes, the effects of age on net calcium movements in colon cannot be fully defined by the present study. The slight increase of total colonic lumen-to-plasma flux appears to have little impact on total intestinal calcium absorption (see discussion above) and is likely to be offset by the dramatic enhancement of colonic plasma-to-lumen flux. The latter finding suggests that

TABLE II. CALCIUM TRANSPORT PER G DRY TISSUE.[a]

	Net absorption (μmoles/hr)		Lumen-to-plasma flux (μmoles/hr)		Plasma-to-lumen flux (μmoles/hr)	
Age, weeks	Cecum	Colon	Cecum	Colon	Cecum	Colon
4	(15)[b] 12.1 ± 2.1	4.1 ± 0.7[c]	(8) 14.9 ± 2.8	11.7 ± 2.9	(7) 2.9 ± 0.6	7.1 ± 1.6[c]
7	(27) 7.8 ± 0.9	1.5 ± 0.6[c, d]	(14) 9.8 ± 1.0	8.4 ± 0.8	(13) 1.8 ± 0.3	5.1 ± 0.3[c]
10	(31) 7.3 ± 0.8[d]	0.9 ± 0.3[c, d]	(17) 8.7 ± 0.9[d]	7.1 ± 0.5[c, d]	(14) 1.4 ± 0.2[d]	4.8 ± 0.2[c]

[a] Cecal and colonic calcium transport (mean ± SE) in three age groups of rats studied by *in situ* perfusion with 1.6 m*M* Ca, with tracer ^{45}Ca added to perfusate to measure lumen-to-plasma flux or injected i.m. to measure plasma-to-lumen flux.

[b] Number of animals in each group.

[c] $P < 0.05$ compared with cecum by paired t test.

[d] Differs significantly from 4-week groups by modified Tukey's test (see Methods).

TABLE III. CALCIUM TRANSPORT PER TOTAL SEGMENT.[a]

	Net absorption (μmoles/hr)		Lumen-to-plasma flux (μmoles/hr)		Plasma-to-lumen flux (μmoles/g)	
Age, weeks	Cecum	Colon	Cecum	Colon	Cecum	Colon
4	(15)[b] 0.90 ± 0.15	0.29 ± 0.04[c]	(8) 1.02 ± 0.19	0.83 ± 0.15	(7) 0.22 ± 0.05	0.38 ± 0.05[c]
7	(27) 1.14 ± 0.14	0.18 ± 0.08[c]	(14) 1.44 ± 0.16	1.11 ± 0.12	(13) 0.24 ± 0.04	0.66 ± 0.06[c, d]
10	(31) 1.37 ± 0.15	0.15 ± 0.05[c]	(17) 1.66 ± 0.18	1.14 ± 0.09[c]	(14) 0.16 ± 0.03	0.76 ± 0.04[c, d]

[a] Symbols same as in Table 2.

the colon is the major site of the reported age-related fecal loss of endogenous calcium: thus, in rats studied at mean body weights of 36, 176, and 306 g, the total percentage of intraperitoneally injected ^{45}Ca excreted in feces during a 96 h balance period was 0.75, 3, and 12 respectively (1). Our view also finds support in previous observations suggesting that the large bowel is the major excretory organ for endogenous calcium, at least in the rat (7).

Cecum versus colon. Rates of net calcium absorption are consistently and significantly higher in cecum than colon for all age groups, confirming previous *in vivo* observations in rats taking normal laboratory chow (10) or semisynthetic diets (8, 9); the current study shows this difference to be greater for adolescent than for younger rats (Tables I and II). Lumen-to-plasma flux tends to be higher in cecum than in colon, but this difference is significant only in the oldest age group (Table I and II). Plasma-to-lumen flux is consistently and significantly higher in colon than in cecum in all age groups. This previously unreported feature of *in vivo* colonic calcium transport and the anatomical location of the colon make the latter a logical candidate for governing the excretion of endogenous calcium from the alimentary tract.

Summary. Small intestinal calcium transport correlates with body growth rates and declines with increasing age, but effects of age on calcium transport by large intestine have not been examined. We studied groups of rats at mean ages of 4, 7, and 10 weeks. Cecal and colonic net calcium absorption and unidirectional fluxes were measured by in situ luminal perfusion of 1.6 m*M* Ca. Rates/g dry tissue declined with age in both segments, except for colonic plasma-to-lumen flux. Rates per segment reflected progressive tissue growth; lumen-to-plasma fluxes and cecal net absorption increased slightly, colonic net absorption was maintained, and colonic plasma-to-lumen flux doubled. Thus, in contrast to small intestine, total contribution of cecum to calcium homeostasis increases slightly with age; the colon may be responsible for the increasing loss of fecal endogenous calcium with decreasing body growth reported by isotopic studies.

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