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Intestinal Absorption of Ca-45 and Sr-85 as Affected by the Alkaline Earths and pH.* (27489)

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Strontium nuclides are absorbed *via* the digestive tract and are deposited and retained to a high degree by the skeleton(1). Investigators have reported a clear cut reduction of the Sr-90 burden with increased ingestion of calcium(2,3). Harrison *et al.*(4), however, reported that, when dietary strontium increased, absorption of an oral dose of radioactive strontium increased. Wasserman(5) ascertained from everted intestinal sacs that Ca-Sr discrimination under given conditions was dependent upon metabolically active membranes. Dumont *et al.*(6) using an *in vivo* preparation of rat small intestine found that in the concentration range of 0 to 25 mM, strontium flux appears to be passive, though restricted. Calcium transport may not, however, be ascribed to passive independent movement of calcium ions since at higher concentrations (12.5 and 25 mM), the efflux of calcium from blood to intestinal lumen increases more than expected. Earlier studies from this laboratory indicated that the absorption of Sr-85 and Ca-45 from the small intestine was related inversely to the concentration of calcium in the perfusate and that the effect on Ca-45 absorption was greater than the effect on Sr-85 absorption(7). The present study is an extension of this work aimed at determining the effect of other alkaline earths and pH on intestinal absorption of Sr-85 and Ca-45.

Methods. A total of 180 Sprague-Dawley female rats with an average weight of 200 g was perfused and a minimum of 6 rats was used for each treatment. The rats were fed a commercial pelleted diet containing 1.3% Ca, 0.9% P and 5 USP units of vit. D per gram of diet for at least 20 days before being fasted overnight prior to the perfusion. Each rat was anaesthetized with sodium pentobarbital (3.2 mg/100 g body weight), a laparotomy performed, and the small intestine cannulated by insertion of the proximal polyethylene cannula into the mouth, through the stomach and tying it off in the upper 2 cm of the duodenum. The distal cannula was inserted through an incision in the intestinal wall about 2 cm proximal to the ileocecal junction. During the perfusion, the intestine remained inside the body cavity and was kept moist with saline-soaked gauze at a temperature of approximately 37°C. The small intestine of the anaesthetized rat was perfused *in situ*, using the technic described by Schanker *et al.*(8). The perfusate was 5 mM in KCl, 20 mM in dextrose, and 0, 1, 5 or 25 mM in the alkaline earths, calcium, strontium, magnesium, barium or beryllium, all in the chloride form. The total osmolarity of all solutions was adjusted to 300 mOsm. by addition of NaCl. In the alkaline earth series of experiments, pH was maintained at 7, except for the beryllium groups. In the pH series, the zero level of the alkaline earths was used at each pH level from 1 through 12. The total length of the small intestine was

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TABLE I. Absorption of Sr^{85} and Ca^{45} from Perfused Small Intestine of Rats as Affected by the Alkaline Earths.

Alkaline earth in perfusate	mM	% of total dose* Sr^{85}	% of total dose* Ca^{45}	Ratio Sr^{85}/Ca^{45}
Calcium	0	3.4 ± .6	12.3 ± 2.1	.27 ± .01
	1	2.8 ± .8	6.9 ± 2.0	.40 ± .05
	5	1.8 ± .3	3.9 ± .7	.46 ± .05
	25	1.0 ± .4	1.6 ± .6	.65 ± .12
Strontium	0	3.4 ± .6	12.3 ± 2.1	.27 ± .01
	1	2.8 ± .3	11.4 ± 1.7	.24 ± .04
	5	2.3 ± .2	8.9 ± 1.6	.26 ± .07
	25	2.0 ± .3	8.0 ± 1.3	.25 ± .04
	25 + 5 Ca	1.8 ± .4	3.6 ± .7	.49 ± .04
Magnesium	0	3.4 ± .6	12.3 ± 2.1	.27 ± .01
	1	2.6 ± .4	13.0 ± 1.4	.20 ± .03
	5	1.8 ± .4	11.1 ± .8	.16 ± .02
	25	1.4 ± .1	9.7 ± .8	.15 ± .02
	25 + 5 Ca	1.5 ± .2	3.6 ± .9	.42 ± .05
Barium	0	3.4 ± .6	12.3 ± 2.1	.27 ± .01
	1	2.9 ± .3	13.9 ± 3.2	.21 ± .03
	5	1.8 ± .4	10.6 ± 2.6	.17 ± .02
	25	1.3 ± .2	5.5 ± 1.2	.24 ± .03
	25 + 5 Ca	1.1 ± .3	2.5 ± .7	.43 ± .02
Beryllium	0 pH 7	3.4 ± .6	12.3 ± 2.1	.27 ± .01
	0 pH 6	4.5 ± .8	13.9 ± 1.7	.32 ± .05
	0 pH 4	6.3 ±2.4	15.3 ± 4.6	.41 ± .04
	1 pH 6	4.1 ± .8	10.3 ± 2.7	.40 ± .06
	5 pH 6	3.9 ± .9	5.8 ± 1.0	.68 ± .07
	25 pH 4	18.5 ±8.6	21.0 ±12.6	.88 ± .21

* Mean values from 6 rats ± 95% confidence interval.

perfused for 100 minutes at a flow rate of 1 ml/minute (exceptions were the 25 mM level of barium and the pH 1 level, where the perfusion was terminated at 50 minutes to minimize toxic effects). From the 25th to the 75th minute of the perfusion period, 0.16 μc Ca-45 (0.0036 mg) and 0.08 μc Sr-85 (0.0082 mg) were added to each milliliter of the perfusate. At conclusion of the perfusion, the rats were sacrificed, and the whole carcass, after removal of the small intestine, analyzed for Sr-85 and Ca-45.

Results and discussion. In every instance absorption of Sr-85 was less than that of Ca-45 (Table I). Calcium was much more effective than other alkaline earths in reducing absorption of Ca-45. The effect of calcium on Sr-85 absorption was less, and more in line with the effects of other alkaline earths. In confirmation of Palmer and Thompson(7), the ratio of Sr-85 to Ca-45 absorbed increased as the level of calcium in the perfusate was increased. Increased levels of inert strontium reduced Sr-85 and Ca-45 absorption slightly but did not appreciably affect the Sr-85 to Ca-45 ratio. It is interesting that, with the exception of beryllium, strontium was the least effective of the alkaline earths in reducing absorption of Sr-85. As the level of magnesium was increased in the perfusate, absorption of Sr-85 was reduced more rapidly than that of Ca-45. The same behavior was observed with barium, except at the 25 mM level where toxic effects were evident. Thus, the effect of magnesium and barium concentrations on the ratio of Sr-85: Ca-45 absorbed was opposite to that of calcium. This agrees with earlier data obtained with chicks which showed that the ratio of Sr-85 to Ca-45 decreased as the level of magnesium and barium in the diet increased(9). When 5 mM of calcium were added in addition to 25 mM of strontium, magnesium or barium, the ratio of Sr-85 to Ca-45 absorbed was changed to that observed with 5 mM of calcium alone. The results obtained with beryllium added to the perfusate were difficult to interpret in view of the chemical dissimilarity and greater toxi-

TABLE II. Absorption of Sr^{85} and Ca^{45} from Perfused Small Intestine as Affected by pH.

pH of perfusate	% of total dose* Sr^{85}	Ca^{45}	Ratio Sr^{85}/Ca^{45}
1	4.4 ± .7	4.4 ± .7	1.01 ± .09
2	13.4 ± 5.0	13.9 ± 4.6	.97 ± .14
3	10.3 ± 8.1	13.8 ± 4.7	.75 ± .34
4	6.3 ± 2.4	15.3 ± 4.6	.41 ± .04
5	3.7 ± .4	14.0 ± 1.1	.27 ± .03
6	4.5 ± .8	13.9 ± 1.7	.32 ± .05
7	3.4 ± .6	12.3 ± 2.1	.27 ± .01
8	3.8 ± .9	14.5 ± 2.6	.26 ± .10
9	3.2 ± .6	15.0 ± 3.5	.21 ± .04
10	4.6 ± .9	16.3 ± 2.8	.28 ± .02
11	4.7 ± .3	15.0 ± 3.0	.31 ± .05
12	6.0 ± 1.0	8.7 ± 1.2	.68 ± .13

* Mean values from 6 rats ± 95% confidence interval.

city of beryllium as compared to the other alkaline earths.

The results of additional studies to determine the effect of pH are shown in Table II. There was remarkably little effect of pH in the range from pH 5 to pH 11. In the range from pH 4 to pH 2 there was a marked increase in absorption of Sr-85, but not in Ca-45, suggesting that absorption of the two isotopes occurs by different mechanisms. The greater variability of the results in this acid range may be due to animal variation in reserve buffering capacity. The sharp drop in Ca-45 absorption at the extremes of pH 1 and pH 12, and the similarity of the results obtained with Ca-45 and Sr-85 at these extremes, suggests that the mechanisms responsible for the preferential absorption of calcium over strontium are inactivated under these conditions.

The inconstancy in the ability of the small intestine to discriminate between strontium and calcium at varying levels of the alkaline earths and pH of the perfusion fluids, emphasizes the need for caution, expressed by Kornberg(10), in using the ratio concept in hazard assessment.

Summary. Using an ***in vivo*** perfusion technic the absorption of Sr-85 and Ca-45 from the small intestine of the rat was observed to be influenced by the alkaline earths and pH. Addition of calcium or beryllium to the perfusate reduced Ca-45 absorption more than Sr-85 absorption. Magnesium and barium had a greater effect on Sr-85 than on Ca-45, while strontium reduced absorption of Ca-45 and Sr-85 to the same extent. Extremes in pH tended to increase the ratio of Sr-85 to Ca-45 absorption.

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Further Studies on Milk Fat Synthesis.* (27490)

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We have reported evidence(1) for the *de novo* synthesis of milk fat by mammary tissue based on a study of the radioactivity found in milk fat glycerol following intramammary infusion of various C^{14} labeled metabolites. The synthesis of glycerol from glucose(2) and subsequent incorporation of the newly synthesized glycerol into milk fat suggested that

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