Metabolic Basis of Calcium and Strontium Discrimination: Studies with Surviving Intestinal Segments.* (25738)

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Biological membranes, such as those of intestinal tract, kidney, mammary gland, and placenta, apparently can differentiate qualitatively in the same way between calcium and strontium; calcium always moves more rapidly across the membrane system than does strontium(1-3). Recently, Samachson(4) also observed that Ca^{45} moves preferentially to Sr^{85} across membranes involved in cerebrospinal fluid formation. Little attention has been given to the mechanism involved but previous observations that differential movement occurred in passage from dam to fetus and not from fetus to dam suggested that metabolic processes might be involved(2). In the present study, the differential movement of Ca^{45} and Sr^{85} across intestinal membranes was studied directly, using surviving segments of duodenum and ileum of the rat, for the purpose of determining whether the metabolic integrity of the membrane is essential for discrimination to occur.

Methods. The technic was based upon that of Schachter and Rosen(5), who demonstrated that Ca^{45} can be transported against a concentration gradient by surviving everted duodenal sacs from rats and rabbits. Carworth male rats, weighing about 150-170 g, were fasted overnight prior to experiment. Except for first experiment, all rats were maintained on a low calcium diet for about one week. Rats were killed by exsanguination, the duodenum resected at the pylorus distally, and the ileum from ileo-cecal junction proximally. Intestinal segments (5-6 cm long) were washed, everted and rewashed in cold isotonic saline, tied at one end with suture, injected with 0.6 ml of incubating solution, and the other end tied. The intestinal sacs were placed into 25-ml Erlenmeyer flasks, followed by 3 ml of cold incubating medium. A small plastic vial containing filter paper saturated with 6N NaOH for removal of metabolic CO_2 was suspended in the flask. Flasks were gassed 1 minute with O_2, stoppered, and placed in shaking water bath with temperature controlled at 37°C. Maximum incubation was 2 hours. The incubating medium was that of Schachter and Rosen(5) and consisted of NaCl, 0.135M; KCl, 0.011M; $CaCl_2$, 4 x 10^{-3}M; sodium phosphate buffer at pH 7.4, 0.008M; 0.04 μc Ca^{45}; and 0.02 μc Sr^{85}/ml. At end of incubation, residual volume of solution within the sac was determined. Radiocalcium (Ca^{45}) was counted by direct plating on stainless steel planchets with a thin-windowed Geiger tube (self absorption corrections were unnecessary). Radiostrontium (Sr^{85}) was determined in well-type scintillation detector according to usual procedures. Data are presented primarily as ratio of counts/minute/ml on serosal side (inside of sac) to counts/minute/ml on the mucosal side (outside of sac); this ratio will be abbreviated as S/M after Rasmussen(6). Comparative behavior of alkaline earths is given in terms of ratio of Sr^{85}/Ca^{45} on the serosal side to that of Sr^{85}/Ca^{45} on the mucosal side.

Results. Two different procedures were used to obtain complementary information on behavior of Ca^{45} and Sr^{85} in the *in vitro* system. One procedure was to label mucosal and serosal solutions with equal concentrations of radionuclides; the observations then yielded data only on ability of the membrane to transport either alkaline earth against a concentration gradient as indicated by S/M value other than unity. The second procedure was to label only the mucosal solution and observe the movement of Ca^{45} and Sr^{85} across membrane to the unlabeled serosal side; here, relative rates of movement of the 2 ions across the membrane may be due to several processes such as diffusion or "active" transport. If both procedures were carried to equilibrium, one would expect the same final S/M values;

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TABLE I. Effect of Dietary Calcium Levels on Movement of Ca^{45} and Sr^{85} in Surviving Duodenal Sac.*

Dietary history	Ca^{45} (S/M)	Sr^{85} (S/M)	Sr/Ca†
Low calcium diet	3.2 ± .5	.9 ± .1	.28 ± .03
High " "	1.7 ± .2	.9 ± .05	.53 ± .02

* Values represent mean ± stand. error of mean; 6 sacs/value; incubation time, 2 hr; Ca^{45} and Sr^{85} in mucosal and serosal solutions; other conditions given in text.

† Sr/Ca = Sr^{85}/Ca^{45} (serosa) ÷ Sr^{85}/Ca^{45} (mucosa).

most of our studies, however, were not carried this long.

In the first study, influence of dietary calcium level on serosal:mucosal ratio (S/M) of Ca^{45} and Sr^{85} in surviving duodenal intestinal sac was determined. Both groups of rats were on their respective diets for 1 week; low calcium diet contained about 0.2% Ca and high calcium diet about 2.0% Ca. Radionuclides were placed both inside and outside of sac. The data (Table I) show 3 important points: (a) that Ca^{45} was transported against a concentration gradient as shown by S/M > 1; (b) that duodenal segments from rats on low calcium diet were capable of maintaining a higher S/M for Ca^{45} than those on high calcium diet; and (c) that Sr^{85} was not transported against a concentration gradient. The difference in behavior of Ca^{45} and Sr^{85} resulted in Sr^{85}/Ca^{45} ratios of 0.28 and 0.53 in segments from low calcium and high calcium diet, respectively.

The time-course of transfer of Ca^{45} and Sr^{85} across duodenal and ileal segments was then studied; radionuclides were placed only in the outside (mucosal) solution. Fig. 1 shows that both radionuclides moved more rapidly across duodenal sac than across the ileal sac. This agrees with *in vivo* measurements since rate of absorption of both Ca^{45} and Sr^{85} from the duodenum exceeds absorption of these radionuclides from the ileum, and radiocalcium always moved faster than Sr^{85}, in agreement with observations in living animal (Lengemann, F. W., private communication).

The effect of incubating duodenal and ileal segments under nitrogen or at 4°C was determined (Table II). Radionuclides were placed within and without the sacs. The duodenal segment at 37°C and under O_2 gave data similar to that of Table I; the ileal segment

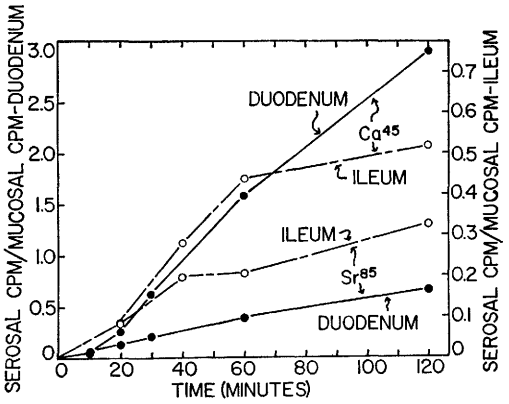

FIG. 1. Rate of transfer of Ca^{45} and Sr^{85} across surviving duodenal and ileal segments; duodenum (—●—), ileum (—○—). Note: S/M ratio for duodenum given on left-hand ordinate; S/M ratio for ileum given on right-hand ordinate.

under same conditions behaved differently. In the ileum, Ca^{45} was not significantly transported against a concentration gradient but an apparent net transfer occurred for Sr^{85} from serosal to mucosal side (S/M = 0.7). This we repeatedly confirmed. Incubation of duodenal and ileal segments under N_2 completely eliminated transport of Ca^{45} in the duodenum (M → S) and eliminated transport of Sr^{85} in the ileum (S → M). At

TABLE II. Effect of Temperature and Gaseous Phase on Metabolism of Ca^{45} and Sr^{85} in Surviving Intestinal Segments.*

Temp. of incubation, °C	Gaseous phase	Duodenum Ca^{45} (S/M)	Duodenum Sr^{85} (S/M)	Duodenum Sr/Ca†	Ileum Ca^{45} (S/M)	Ileum Sr^{85} (S/M)	Ileum Sr/Ca†
37	O_2	3.0 ± .08	1.0 ± .02	.33 ± .02	1.2 ± .01	.7 ± .02	.57 ± .02
37	N_2	1.1 ± .02	1.0 ± .03	.91 ± .03	1.1 ± .01	1.0 ± .02	.91 ± .03
4	O_2	1.0 ± .01	.9 ± .08	.90 ± .03			

* Values represent mean ± stand. error of mean; 6 sacs/value; incubation time, 2 hr; Ca^{45} and Sr^{85} in mucosa and serosa solutions; other conditions as before.

† Sr/Ca = Sr^{85}/Ca^{45} (serosa) ÷ Sr^{85}/Ca^{45} (mucosa).

TABLE III. Influence of Metabolic Inhibitors on Movement of Ca^{45} and Sr^{85} in Surviving Intestinal Segments.*

Treatment‡	Duodenum Ca^{45} (S/M)	Duodenum Sr^{85} (S/M)	Duodenum Sr/Ca†	Ileum Ca^{45} (S/M)	Ileum Sr^{85} (S/M)	Ileum Sr/Ca†
Control	1.9 ± .2	.6 ± .04	.34 ± .03	.8 ± .02	.3 ± .02	.41 ± .05
Sodium cyanide	.7 ± .02	.7 ± .01	1.05 ± .04	.8 ± .04	.8 ± .04	.98 ± .03
Iodoacetic acid	.9 ± .05	.7 ± .02	.71 ± .05	.8 ± .04	.8 ± .03	.99 ± .02
2,4-Dinitrophenol	.7 ± .02	.7 ± .03	1.00 ± .03	.7 ± .01	.7 ± .03	.96 ± .03

* Values represent mean ± stand. error of mean; 6-7 sacs/value; incubation, 2 hr; Ca^{45} and Sr^{85} radioactivity in mucosa solution only; other conditions as before.

† $Sr/Ca = Sr^{85}/Ca^{45}$ (serosa) ÷ Sr^{85}/Ca^{45} (mucosa).

‡ Concentration of inhibitors as follows: NaCN, 5×10^{-4} M; Na iodoacetate, 3×10^{-4} M; 2,4-dinitrophenol, 5×10^{-5} M.

4°C under O_2, there was no transport of Ca^{45} against a concentration gradient. Discrimination between Ca^{45} and Sr^{85} in the duodenum was a result of "active" transport of calcium from M → S, giving a Sr^{85}/Ca^{45} ratio of 0.33. However, discrimination between radionuclides in the ileum resulted from transport of Sr^{85} from S → M with no concomitant transport system for Ca^{45}; Sr^{85}/Ca^{45} ratio for ileum was 0.57.

The influence of sodium cyanide, iodoacetic acid, and 2,4-dinitrophenol on the transfer mechanism and on discrimination in duodenal and ileal sacs also was determined (Table III). Radionuclides were placed only in the outside (mucosal) incubating medium. Since these studies were not run to equilibrium, one would not expect to obtain S/M values for Ca^{45} and Sr^{85} in the duodenum of about 3 and 1, respectively, as before. In duodenal preparations, NaCN, iodoacetic acid, and 2,4-dinitrophenol decreased the rate of Ca^{45} transport but had no observable effect on Sr^{85} movement; in absence of inhibitor, Ca^{45} attained an S/M ratio of about 1.9. Thus, there was elimination of discrimination with NaCN and 2,4-dinitrophenol, and a substantial reduction in discrimination in presence of iodoacetic acid. The effect of inhibitors on the ileal segment was decidedly different: here, inhibitors did not influence Ca^{45} movement but caused a net increase in Sr^{85} movement from mucosa to serosa by blocking the metabolically mediated backflow (S → M) of this radionuclide. Again, the net effect of inhibitor eliminated discrimination between calcium and strontium in the ileum.

Discussion. Extrapolation of *in vitro* studies, like the above, to the intact animal must always be done with caution. However, there are similarities in metabolism of the duodenum and ileum under both situations, as follows: (a) alkaline earth ions move across duodenum more rapidly than across the ileum in both intact animal and *in vitro* situation, (b) Ca^{45} moves across both segments more rapidly than Sr^{85} *in vitro* and *in vivo*, (c) in the ileal segment, Sr^{85} was transferred more rapidly from serosa to mucosa than Ca^{45}, which corresponds to similar observations made by Singer *et al.*(7) in the intact dog. Yet, inconsistencies appear to exist in behavior of alkaline earths in *in vitro* and *in vivo* situations; additional studies may resolve these questions.

Although the term "active" transport is used in the present study, it is realized that existence of such a transport system for calcium has not been conclusively established. That an ion can be transferred against a concentration gradient, and maintenance of concentration gradient by a biological membrane, are not sufficient evidence to postulate existence of transport or carrier system. Regardless of the mechanism, however, the above data demonstrate that calcium-strontium discrimination in surviving intestinal segments is dependent upon a metabolically active membrane.

Summary. (1) The differential movement of Ca^{45} and Sr^{85} across surviving intestinal segments of the rat was studied. (2) In the duodenal segment, Ca^{45} but not Sr^{85} was transported against a concentration gradient from mucosa to serosa. (3) In the ileal segment, Sr^{85} but not Ca^{45} was transferred against a concentration gradient from serosa to mucosa. (4) Incubation of segments at

0°C, under N_2, or in presence of metabolic inhibitors eliminated "active" transport and eliminated strontium-calcium discrimination. (5) Ca-Sr discrimination under given conditions was dependent upon a metabolically active membrane.

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Effect of Guanylic Acid on Plasma NEFA Response to Stress.* (25739)

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A physiological role for the guanine nucleotides in the stress response is suggested by experiments in which pretreatment with these agents significantly extended the swimming time of heavily weighted normal rats(1). We have investigated the effect of guanylic acid on plasma NEFA response to stresses of fasting and tourniquet application in normal and adrenalectomized rats, since this is one metabolic parameter of stress response.

Methods. Male albino rats of the Sprague Dawley strain, weighing 210-230 g were used in most experiments. Male mongrel dogs were used in a few experiments. The rats were maintained on Purina rat chow and water *ad libitum* with the exception of the adrenalectomized animals which were given 0.9% saline in place of tap water. Bilateral adrenalectomy was performed under ether anaesthesia and the animals were not used until at least 7 days postoperative. The fasting period used was 16 hrs. Each experimental and control group contained 8 animals. In the first experiment, normal and adrenalectomized animals were pretreated for 7 days with a daily subc. injection of either 0.3 mg/kg or 1 mg/kg hydrocortisone and then fasted. In the second experiment, normal and adrenalectomized animals were pretreated with only one subc. injection of 1 mg/kg hydrocortisone immediately preceding fasting period. In the third experimental group, normal and adrenalectomized animals were pretreated with 10 mg/kg guanylic acid (racemic sodium guanylate) subc. immediately preceding fasting period. Plasma NEFA levels were determined at end of fasting period. In series 4, tourniquets were applied across both hind limbs just below the inguinal ligament, using a firmly fixed wire clamp. Normal and adrenalectomized animals were used. In these experiments, one group of rats was pretreated with guanylic acid (10 mg/kg) daily, subc., for 7 or 14 days prior to application of tourniquet. A second group received only one injection of the drug on day of experiment. In all cases, the tourniquet was kept in place for 5 hr and then removed. No food was available during this period nor for 10 hr after removal of tourniquet. At the end of this time blood was drawn for analysis and plasma NEFA levels determined. In all experiments blood was taken from the abdominal aorta via a plastic cannula, under ether anaesthesia. NEFA levels were determined by the method of Dole(2). Survival time of the adrenalectomized animals subjected to tourniquet stress was measured after removal of tourniquet. Blood sugar levels were measured in normal dogs at hourly intervals after 10 mg/kg guanylic acid was administered. Simultaneous blood pressure and E.C.G. measurements were made.

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