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THE MECHANISM OF CALCIUM TRANSPORT BY RAT INTESTINE

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SUMMARY

Short-circuit techniques for the study of ion transport by isolated membrane systems have been applied to defining the mechanism for calcium ion transport in the small intestine of the rat. In the absence of phosphate, the movement of calcium is passive. There is no evidence for a membrane-bound carrier to facilitate its transport. The calcium flux is a linear function of its concentration. However, phosphate ion is actively transported from the mucosal to the serosal surface. In the presence of phosphate, calcium appears to be actively transported, possibly as a counter-ion to the phosphate. The role of chelation in the transport of calcium has also been clarified.

INTRODUCTION

There has been widespread interest in calcium** transport and metabolism by investigators with diverse concerns including nutrition, bone metabolism, effects of various hormones and vitamins, the metal's role in enzyme action, its movement during muscle contraction, and its accumulation by mitochondria, to cite but a few. A comprehensive review of the dynamics and function of calcium has been given by BRONNER[1]. The research presented here is directed to a single aspect of calcium transport—the mechanism by which the metal ion is moved across the small intestine of the rat. There have been two excellent reviews on this subject which should be consulted for perspective[2,3].

Our own interest in calcium metabolism began as a consequence of the studies carried out in this laboratory on the role of chelation in the passive transport of Fe^{3+} (ref. 4) and other trace metals into a wide variety of tissues and across many different cellular membranes. One particular class of metal chelates was both novel and of nutritional significance, the metal–sugar complexes[5]. We were intrigued particularly by the concept that the nutritional stimulation of calcium metabolism by lactose and other sugars could be attributed to the formation of a calcium–lactose

Abbreviations: P_i refers to the multiple species of inorganic phosphate present. Flux from mucosal to serosal surface, $(M \rightarrow S)$; from serosal to mucosal, $(S \rightarrow M)$.

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** Calcium refers to all of the charged and uncharged species of calcium that are present under the conditions described.

chelate[6]. We therefore began a series of experiments specifically to determine the mechanisms by which this metal is transported across the intestinal membrane.

A systematic attempt to demonstrate active transport *in vitro* using rat small intestine was made by SCHACTER and his colleagues[7,8]. Fundamentally their technique consisted of everting a section of the small intestine, suspending the everted sac in a physiological buffer solution and studying the distribution of radioactive ^{45}Ca introduced in both the serosal and mucosal compartments. Since the ratio of ^{45}Ca within the sac compared to that of the bathing medium exceeded 1.0, they proposed that active transport of calcium, *per se*, was involved. This asymmetric distribution was inhibited by metabolic poisons. Confirmation of the active transport hypothesis was presented by WASSERMAN *et al.* who studied calcium movement against a concentration and electropotential gradient *in vivo* using rat[9,10] and chicken intestines. In these experiments, all calcium removed from the duodenal lumen was considered to be transported actively.

A careful analysis of data from WASSERMAN's group[12] indicated that there was a linear relationship between transport rate and the concentration of calcium in the lumen. This would suggest a mechanism controlled fundamentally by diffusion, involving no membrane-bound carrier to facilitate ion transport, or a carrier system far from saturation. Evidence for a passive mechanism has been presented by MCKENNEY[13].

One of the most direct, sensitive, and unambiguous methods currently available for the study of membrane transport has been provided by USSING and his coworkers[14,15]. This method, known as the short-circuit technique, permits continuous measurement of trans-epithelial ion flux in the absence of a membrane potential. Using this technique, we have demonstrated that movement of calcium ion, *per se*, is a passive process and is not mediated by an active carrier system in the rat intestine. Further, chelating agents which stabilize the calcium as a soluble low-molecular-weight complex in the presence of competing ligands enhance the rate of movement of the ion. In the absence of P_i, we find no evidence for a facilitating carrier in the membrane and must conclude that the calcium moves either as the divalent cation, or, more likely, complexed to one of many endogenous or exogenous ligands. We have been able to demonstrate that P_i is actively transported by the intestinal mucosa. This active movement of P_i can result in an apparent active transport of calcium.

METHODS

In all experiments *in vitro* where buffer solutions were required, a modified Krebs–Henseleit–Ringer solution was employed[16]. The modifications consisted of the removal of Mg^{2+} from the buffer as well as the addition of 2 mg/ml glucose to provide a metabolic substrate. As indicated in the specific experiments, the concentration of calcium and/or P_i was altered as needed to investigate specific aspects of the transport process. The pH of all solutions was 7.4.

The ^{45}Ca used as a tracer in the studies *in vitro* was obtained from New England Nuclear Laboratories and diluted to a specific activity of 1 mC/ml and further diluted as needed with carrier $CaCl_2$. Approx. $2 \cdot 10^6$ counts/min per ml were initially present in the compartment to which isotope was added. For whole animal experiments, the gamma-emitting, short-lived isotope ^{47}Ca was obtained from Abbott Laboratories as

a $CaCl_2$ solution and diluted with appropriate amounts of carrier $CaCl_2$ before administration to an animal. Radioactivity of the ^{45}Ca was determined using a Packard liquid scintillation counter. ^{47}Ca was measured using a whole animal counter employing two 3-inch NaI crystals and a pulse-height analyzer peaked at 1.29 meV. In experiments designed to measure active transport of sodium, ^{22}Na (obtained from New England Nuclear Corporation) was diluted with appropriate amounts of carrier NaCl. The radioactivity of ^{22}Na was determined in a scintillation well counter peaked to the 1.3 meV signal. The radioactive compartment of the experiments *in vitro* with ^{22}Na contained initially approx. 80 000 counts/min per ml. In some flux experiments, it was necessary to measure both ^{45}Ca and ^{22}Na in the same sample by determining the radioactivity of the ^{22}Na in the scintillation well counter and measuring the ^{45}Ca by liquid scintillation techniques. Appropriate corrections were made for the extraneous counts introduced in the ^{45}Ca determination by the ^{22}Na which was in all cases less than 3% of the ^{45}Ca activity. P_i was obtained as $H_3{}^{32}PO_4$ from New England Nuclear Corporation, specific activity 100 mC/ml. It was freed of pyrophosphate by acid reflux prior to use. Activity was determined by liquid scintillation techniques.

Techniques in vitro for two-way flux determinations

A modification of the Ussing short-circuit apparatus was designed and built as shown in Fig. 1. The shape of the opening was an elongated oval to comply with the physical limitations of the tissue. The total area of the orifice was 0.87 cm^2. The electrical potential difference was monitored using a Keithley electrometer (Model 600-A) *via* a pair of calomel electrodes and agar–saline bridges on each side of the preparation. Before each experiment, electrical potential between the bridges was measured without the intestinal preparation and a correction was applied for any deviation from zero. At the same time the resistance of the Ringers between the bridges was measured and a correction was subsequently applied to the short-circuit experiments on the intestinal mucosa. The short-circuit current was supplied from a 1.5-V dry cell using Ag–AgCl electrodes and agar–saline bridges. Circulation and aeration was achieved using 100% O_2 or 95% O_2–5% CO_2. Where anaerobiosis was

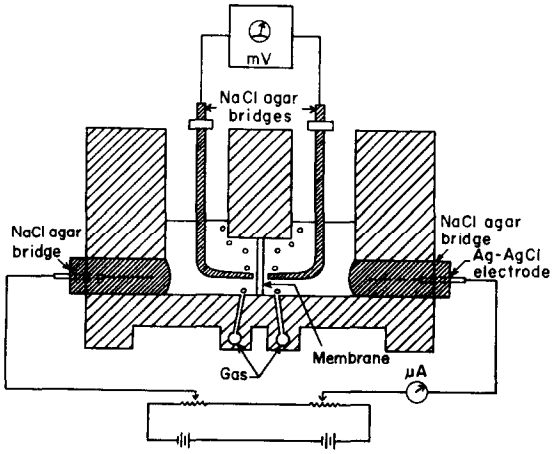

Fig. 1. Schematic representation of short-circuit membrane system *in vitro*.

desired, 95% N_2–5% CO_2 was substituted. At the times indicated, aliquots were withdrawn from both compartments and the radioactivity determined as indicated above.

All experiments reported in this paper were carried out using Sprague–Dawley male rats, approx. 400 g, which had been deprived of food 24 h prior to killing. Animals were killed with a blow on the head and the small intestine immediately extirpated. A 5-cm section of the small intestine lying distal to the pyloric musculature was utilized. It was washed several times in cold 0.9% saline solution, opened along the mesentery and laid out as a flat sheet. This membrane was then mounted in the apparatus which was immediately filled with Ringers solution; the isotope was added and the experiments begun. Throughout the course of the experiments, short-circuit current was recorded. Periodically, the current was removed in order to monitor the transmucosal potential difference.

Large-scale apparatus for measurements in vitro

An alternate technique for flux measurements *in vitro* was developed which permits a large number of experiments to be carried out simultaneously with great ease. The apparatus is shown in Fig. 2. A segment of intestine extending from about 2 cm to 14 cm below the pyloric valve was taken, washed with cold saline, everted, and mounted as indicated. Ringer solution was added to both compartments. Circu-

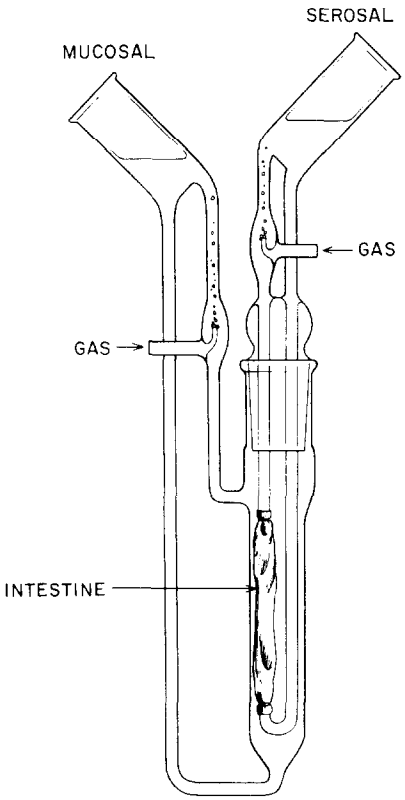

Fig. 2. Apparatus used for flux measurements *in vitro* where no short circuit was applied.

lation was maintained and aeration kept constant using the bubbling devices as illustrated. The serosal compartment contained 20 ml of buffer, the mucosal compartment contained 60 ml. Aliquots were removed at desired time intervals from both sides to determine radioactivity. The temperature was maintained by emersing the apparatus in a circulating water bath at 37°.

Calcium flux in vivo

The animals were anaesthetized with 15 mg sodium pentobarbital, the abdomen incised, and a segment of intestine 25 cm distal to the pyloric valve isolated by two ligatures. At all times, care was taken to maintain unimpaired blood supply to the ligated segment. The experimental solution, 2.0 ml, containing ^{47}Ca at the desired concentration and in the desired medium was injected with a 27-gauge needle just below the proximal ligation. Immediately following removal of the needle, a third ligature was placed distal to the point of injection to avoid back leakage of the radioactive solution. Precautions were taken to prevent leakage from the site of injection. The segment of intestine was returned to the abdominal cavity and the rat maintained for 1 h under a warming lamp. At the conclusion of this period, the whole animal was counted in the animal counter. The isolated intestinal segment was then excised, and the animal was again counted. The amount of radioactivity within the animal with the intestinal segment removed divided by the total radioactivity (*i.e.* the animal *plus* intestinal segment) represented the fractional uptake of calcium during the 1-h period.

RESULTS

Flux of ^{45}Ca under short-circuit conditions

In experiments using short-circuit conditions, the initial concentration of calcium on both sides of the membrane was 10^{-5} M. The membrane potential measured in a series of over 20 intestinal segments ranged from −3.0 to −4.5 mV (mucosa negative with respect to serosa in an external circuit). The current required to reduce

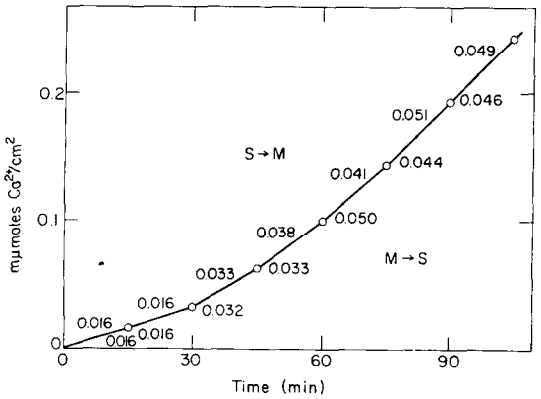

Fig. 3. Bi-directional calcium flux measurements for short-circuited system. The points represent time markers. The slope of the curve is the flux for that interval. The average numerical values for 10 experiments in each direction are presented for the S → M direction above the curve, and the M → S below. Initial calcium concentration, 10^{-5} M; no P_i present.

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