possible that only one transfer channel is needed. A thundercloud can store 10 to 50 coulombs. If the spacecraft is furnished with a number of sharp points, it could distribute electric charge through corona discharges to a vast region around it where charge can be stored. Also a transfer of wave energy along the conducting channel should be considered. The spacecraft must be accelerated up to a height of several hundred kilometers. When it has reached the ionosphere, the transfer problem takes on another aspect. The anisotropy of electric conduction in the earth's magnetic field could be used for energy transfer along the magnetic field lines. It is possible that the spacecraft trajectory should be made parallel to the magnetic field lines. The total power to be transmitted at the launch is comparable to the power of a very large magnetic storm. Our knowledge of the storm processes in the auroral zone will be valuable.

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Intestinal Calcium Transport: The Role of Sodium

Abstract. *The role of sodium in intestinal calcium transport was investigated in everted rat intestine. Ethacrynic acid, but not ouabain, inhibited calcium transport. However, ouabain did inhibit net water transport and, therefore, sodium transport, establishing the dissociation of the two transport processes. In addition to a magnesium-dependent adenosine triphosphatase (activated by sodium and potassium), a phosphatase dependent on sodium and calcium was localized to the lateral and basal membrane fractions of the mucosal cell. Activity of the latter phosphatase, similar to calcium transport in intact tissue, was inhibited by ethacrynic acid and not by ouabain. Sodium, therefore, may participate in the calcium transport process by activating an enzyme complex, dependent on adenosine triphosphate, that mediates calcium transport.*

Although there is controversy about the mechanism of intestinal transport of calcium, the transport process is an energy-dependent, saturable process that moves calcium against concentration and electropotential gradients (*1*). Whereas the movement of calcium across the brush border of the intestinal epithelial cell does not require sodium, the expulsion of calcium from the cell at the basal and lateral membranes is presumably dependent on sodium (*2*, *3*). The nature of this dependency on sodium is not established. This relation between calcium and sodium in the intestinal cell is reminiscent of that in the renal tubule, where inhibition of proximal tubular reabsorption of sodium by saline infusion and volume expansion or by furosamide and ethacrynic acid is also associated with inhibition of calcium reabsorption (*4*). The similarities between renal and intestinal transport mechanisms (*5*) suggest that intestinal calcium transport might also be influenced by inhibitors of renal sodium transport.

Since ethacrynic acid and ouabain have been used to delineate two mechanisms of sodium extrusion from renal tubular cells, that is, sodium exchange and net sodium transport (*6*), these inhibitors may help to delineate the role of sodium in intestinal calcium transport. Accordingly, the influence of ethacrynic acid and ouabain on rat intestinal calcium transport was investigated. Ethacrynic acid inhibited net water and calcium flux across the intestine. Ouabain, however, inhibited water flux without concomitant inhibition of intestinal calcium transport. By fractionation of the mucosal cell, a phosphatase that is dependent on calcium and sodium was identified and localized to the plasma membrane at the base of the mucosal cell. This enzyme activity was also inhibited by ethacrynic acid but not by ouabain. The data suggest that this enzyme may mediate intestinal calcium transport and that sodium is required in the activation of this enzyme.

Calcium transport was studied with the use of everted 3.0-cm duodenal and ileal gut sacs prepared from Wistar rats (4 weeks old), by the method of Wilson and Wiseman (*7*). The animals were maintained on calcium-deficient diets for 5 days before they were killed. The incubation medium contained 140 m*M* NaCl, 0.4 m*M* $CaCl_2$, 2 m*M* glucose, and calcium-45 (0.02 μc/ml) in 4 m*M* phosphate buffer; final *p*H was 7.4. The everted sacs were filled with 0.15 ml of medium that contained the appropriate inhibitor of sodium transport. Ethacrynic acid (*8*) and ouabain (Sigma) were studied at concentrations from 0.1 to 1 m*M*. The intestinal sac preparations were incubated for 50 minutes at 37°C. Water is not actively transported by the intestine, and net water flux is totally dependent on net sodium transport (*9*). Accordingly, net water flux from the mucosal to the serosal side was used as an indicator of net sodium flux. Net water flux was determined gravimetrically as the gain in weight of the intestinal sac during incubation. The accumulation of calcium-45 in the fluid bathing the mucosal surface was also measured. Calcium transport was derived from the difference between the disintegrations per minute in the final sac content and this value in the initial sac content.

Ethacrynic acid inhibited calcium-45 transport and, to a lesser extent, net water flux in the everted duodenal and ileal sacs (Table 1). Ouabain inhibited net water flux in the ileum and, at higher concentrations (1 m*M*), it inhibited net water flux in the duodenum. Although the inhibition of water or sodium flux by ouabain was greater than that by ethacrynic acid, calcium transport was not inhibited by ouabain. Thus, under certain experimental conditions calcium transport may be independent of net water and sodium flux.

Calcium transport across the red cell membrane may be mediated by a calcium-activated, Mg-dependent adenosine triphosphatase localized in the red cell membrane (*10*). We proposed that a similar enzyme, dependent on calcium, exists in the mucosal cell membrane. Two additional constraints were imposed on the proposed enzyme complex: it would be activated by sodium and be localized on the serosal side of the mucosal cell. The movement of calcium across the luminal or brush border surface does not require sodium in the extracellular fluid (*1*) and is not the rate limiting, energy-dependent step in calcium uptake (*2*, *3*). Accordingly, we sought to identify an enzyme activity that is associated with the plasma membrane of lateral and basilar portions of

the intestinal mucosal cell and that requires both sodium and calcium for activity and uses adenosine triphosphate (ATP) as a substrate.

The differentiation of the luminal and basal or serosal surfaces of the mucosal cell with respect to structure and function permits the isolation and separation of the respective plasma membranes. The brush border fraction (BB) of the luminal surface of the cell was prepared by the method of Forstner *et al.* (*11*). Fraction BM, consisting of lateral and basilar plasma membrane fragments that were relatively free of brush border fragments and mitochondria, was prepared by the method of Quigley and Gotterer (*12*) with the use of a discontinuous sucrose gradient. Each preparation was characterized by assay of the marker enzymes β-fructofuranosidase (E.C. 3.2.1.26) (*13*) and Na, K–activated, Mg-dependent adenosine triphosphatase (E.C. 3.6.1.3), which is subsequently referred to as Mg–adenosine triphosphatase (*14*). The former is localized exclusively in the BB fraction, which is devoid of the latter enzyme (*12*, *15*). Mitochondrial contamination was estimated by cytochrome oxidase activity (*16*). By these criteria, the BM fraction was contaminated with brush border fragments and mitochondria. However, the specific activity of β-fructofuranosidase in the BM fraction was less than 27 percent of that in the BB fraction, and the specific activity of cytochrome oxidase in the BM fraction was less than 5 percent of that in the mitochondrial fraction.

In the absence of magnesium, 4 m*M* calcium stimulated ATP hydrolysis in both the BB and BM fractions (Table 2). With 120 m*M* sodium as the only cation, there was very little ATP hydrolysis. Phosphatase activity in the presence of both sodium and calcium was compared to that with calcium alone; activity was enhanced in the BM fraction but inhibited in the BB and mitochondrial fractions. Thus, the major contaminants of this BM fraction —brush border fragments and mitochondria—would not contribute to the activity stimulated by sodium and calcium.

The response of this phosphatase to the inhibitors studied in the intestinal sac preparations was investigated. As for calcium transport in the intact tissue, this enzyme was inhibited by ethacrynic acid but not ouabain. In contrast, the activity of Mg–adenosine triphosphatase was inhibited by ouabain and to a lesser extent by ethacrynic acid, a result consistent with those for different tissues (*6*, *17*). Phosphate esters other than ATP can serve as substrates for the enzyme dependent on sodium and calcium.

The Mg–adenosine triphosphatase enzyme system presumably mediates the pumping of sodium out of the mucosal cell, as it does in other cell systems. This results in an electrochemical gradient across the brush border. This gradient would facilitate uptake of calcium by the cell. However, the failure of ouabain to inhibit net calcium transport at concentrations that inhibit this Mg–adenosine triphosphatase activity and also inhibit net water transport suggests that this downhill electrochemical gradient for calcium ion across the brush border is not the rate-limiting step in calcium transport. Similarly, it has been suggested by others that this same gradient, sustained by the Mg–adenosine triphosphatase system, facilitates the extrusion of calcium in exchange for sodium, which passively reenters the cell along the favorable sodium gradient (*3*). Ouabain inhibition of the Mg–adenosine triphosphatase would also abolish this transport mechanism at the serosal surface of the cell. In addition, ouabain inhibited net flux of sodium from the mucosal to the serosal side, an effect that may be independent of those on the Mg–adenosine triphosphatase (*6*).

Finally, at the sodium concentrations in mucosal medium at which calcium transport is maximal, net sodium transport is reduced to zero (*18*). As mucosal sodium concentration is increased, transport of sodium and water is enhanced and calcium transport is inhibited. These data suggest that calcium transport is independent of both bulk water flow and net sodium transport. This apparent independence of calcium transport from both the sodium-potassium exchange pump and net sodium transport suggests that the transport process for calcium is mediated by a specific enzyme system. The phosphatase dependent on sodium and calcium, which was isolated from the basal membrane, was sensitive to ethacrynic acid but not to ouabain, a result consistent with the response of these inhibitors of calcium transport in

Table 1. Effect of ethacrynic acid and ouabain on calcium and water transport in rat intestine. Fluxes from the mucosal to the serosal side were studied in everted intestinal sacs incubated at 37°C for 90 minutes. The effect of the inhibitor on calcium transport, measured as nanomoles of calcium accumulated by the everted intestinal sac per hour, is expressed both as the difference (Diff.) and as the percentage of change from the control (incubated without inhibitor). The net water flux, measured as the net accumulation of water by the intestinal sac, is expressed similarly. The standard error (S.E.) of the difference is given. Except where indicated, all values differed significantly ($P < .05$) from controls.

Tissue	Inhibitor							
	Ethacrynic acid				Ouabain			
	0.5 m*M*		0.1 m*M*		1.0 m*M*		0.5 m*M*	
	Diff. ± S.E.	Percent	Diff. ± S.E.	Percent	Diff. ± S.E.	Percent	Diff. ± S.E.	Percent
	Calcium transport (nmole/hour)							
Duodenum	−2322 ±869	−51.7	−920 ±623	−20.1	−260* ±521	− 6.8	+314* ±577	+ 5.6
Ileum	−425 ±219	−48.7	−406 ±183	−52.8	+225* ±146	+29.1	− 11* ±116	− 1.2
	Water transport (μl/g)							
Duodenum	−28.9 ±18.2	− 5.2	− 9.3* −12.5	− 0.8	− 73.9 ± 15.0	−23.2	− 20.3* ± 38.8	− 4.6
Ileum	−40.7 ±36.0	−18.1	−27.5 ±12.2	−10.1	−249.3 ± 55.5	−37.8	−146.5 ± 50.3	−32.9

* $P \geq .5$.

Table 2. Adenosine tryphosphatase activity in brush border (BB) and basilar membrane (BM) fractions. Enzyme activity is expressed as micromoles of inorganic phosphate produced per milligram of protein in 10 minutes. Assays were done in 60 m*M* imidazole-histidine buffer, *p*H 7.4. The substrate, the tris(hydroxymethyl)aminomethane salt of ATP, was used at a concentration of 4 m*M*. Other concentrations were ethacrynic acid (EA), 1.0 m*M*; ouabain (Oua.), 1.0 m*M*; Na^+, 120 m*M*; Ca^{2+}, 4 m*M*; Mg^{2+}, 4 m*M*; K^+, 15 m*M*.

Additions	Enzyme activity in:	
	BB (mean ± S.E.)	BM (mean ± S.E.)
None	0.08 ± 0.02	0.05 ± 0.02
Na^+	0.06 ± 0.03	0.08 ± 0.03
Ca^{2+}	0.52 ± 0.02	0.65 ± 0.02
Ca^{2+}, Na^+	0.36 ± 0.02	0.95 ± 0.03
Ca^{2+}, Na^+, EA	0.34 ± 0.04	0.71 ± 0.03
Ca^{2+}, Na^+, Oua.	0.38 ± 0.04	0.97 ± 0.03
Mg^{2+}	0.56 ± 0.03	0.60 ± 0.04
Mg^{2+}, Na^+, K^+	0.82 ± 0.02	1.80 ± 0.02
Mg^{2+}, Na^+, K^+, EA	0.77 ± 0.03	1.50 ± 0.03
Mg^{2+}, Na^+, K^+, Oua.	0.64 ± 0.02	0.82 ± 0.03

the intact cell. Although further characterization of this enzyme is necessary, these observations are consistent with the interpretation that this phosphatase is a part of a specific enzyme system that mediates the energy-dependent translocation of calcium across the plasma membrane at the serosal surface of the mucosal cell. Therefore, the role of sodium in intestinal calcium transport may be the activation of this enzyme system.

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The Immune Reaction as a Stimulator of Tumor Growth

Abstract. *Various numbers of spleen cells from specifically immunized mice were mixed with constant numbers of target tumor cells, and were inoculated subcutaneously into thymectomized, x-irradiated recipients. Small numbers of admixed immune spleen cells produced a statistically significant, and reproducible, acceleration of tumor growth in the inoculum as compared with controls of either nonimmune spleen cells or spleen cells from animals immune to a different, noncross-reacting, tumor. Larger numbers of specifically immune spleen cells, however, produced inhibition of tumor growth. These data imply that the normal immune reaction may have a dual function in relation to neoplasia: (i) stimulation of tumor growth, early in the course of the disease, or whenever the immune reaction is minimal; (ii) inhibition of tumor growth at other times.*

I recently advanced the theory that the effect of immunity on target tumor cells might be biphasic, that is, a mild reaction might stimulate tumor growth although a strong one is cytotoxic (*1*, *2*). This theory is based primarily on the considerable data suggesting that, under some circumstances, a slight degree of immune reactivity may benefit fetal growth and survival (*2*). Since a fetus and a tumor share the quality of being antigenically foreign to their hosts, tumor growth might likewise be stimulated by a mild immune reaction. The mechanisms by which the immune reaction might actually stimulate tumor growth are open to speculation, but a clue is provided by the report that small concentrations of lymphotoxin are stimulatory, rather than cytotoxic, to target cells (*3*).

The theory was tested with Winn procedures (*4*), that is, varying numbers of immune spleen cells were mixed with tumor cells and the effect on the tumor was assayed by inoculating the mixtures subcutaneously into test mice. The tumors were sarcomas that had been induced by treatment of inbred DBA/2 or F_1 hybrid mice [(C_{57}BL × BALB/c) and (C_{57}BL × C_3H)] with 3-methylcholanthrene. The tumor cells were suspended in modified Eagle tissue culture medium by the action of Pronase and deoxyribonuclease. The live cells were counted after they were stained with trypan blue, and a small number (usually 10^4) were mixed with graded numbers of immune or nonimmune, syngeneic, spleen cells. The tumors were in the first to fifth transplant generation when they were used. Because the different tumors varied in their growth rates, the time selected for analysis of the data varied slightly among experiments but it was always when the diameter of the largest tumor most nearly approximated 10 mm.

The syngeneic recipients had been previously thymectomized as adults and then, 24 hours before inoculation of the mixtures of tumor and spleen cells, had been given 450 roentgens of total body x-irradiation. This regimen of thymectomy and x-irradiation crippled the capacity of the mouse to reject a primary skin allograft. Therefore, the effects of the admixed spleen cells on the growth of the inoculated tumors were probably not complicated by host immunity.

The donors of immune spleen cells were syngeneic mice that had grown the particular tumor for 10 to 20 days. Usually the tumors were excised and the spleens were harvested 7 to 12 days after excision. In one experiment, however, spleens were harvested without prior excision of the immunizing tumors.

Initially, there were five experiments with three tumors that were induced separately. Experimental and control recipients were paired for inoculation. Control spleen cells were obtained from nonimmunized donors in four of the five experiments. In the other experiment, the control spleen cells were obtained from mice that had been immunized in the standard manner against a different, and noncross-reacting, tumor.

The results of this first series of experiments are presented in Fig. 1. It became apparent that tumor growth was accelerated when normal syngeneic spleen cells were mixed with the tumor cells as compared with that when no spleen cells were present. A similar finding has been reported by Deckers *et al.* (*5*). In addition, in my experiments, the immune spleen cells produced an even greater acceleration than did the control spleen cells when, and only when, these immune cells were added in amounts of less than 10^5, that is,