UPHILL OUTFLOW OF SUGAR FROM INTESTINAL EPITHELIAL CELLS INDUCED BY REVERSAL OF THE Na^+ GRADIENT: ITS SIGNIFICANCE FOR THE MECHANISM OF Na^+-DEPENDENT ACTIVE TRANSPORT.

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Several years ago, we proposed a mechanism for Na^+-dependent active transport of sugars by the small intestine (Crane, Miller and Bihler, 1961; Crane, 1962). As essential features of this mechanism, we postulated the existence in the brush border membrane of a "mobile carrier" system (Wilbrandt and Rosenberg, 1961) which transported sugars and which required Na^+ for its normal operation. Sodium ions were assumed to move with the sugar into or out of the cell and net movement of sugar into the cell against a concentration gradient was viewed as a consequence of a "downhill" gradient of Na^+ into the cell maintained by the operation of an outwardly-directed, energy-dependent Na^+ pump. We have recently extended the hypothesis to include our observations that the apparent Michaelis constant (Km) of sugar for the transport process increases markedly as the concentration of Na^+ in the incubation medium is decreased (Crane, Forstner, Lyon and Eichholz, 1964). It now seems probable that sugar also accumulates within the cell because the Km for exit from the Na^+-poor intracellular fluid is higher than the Km for entrance from the Na^+-rich medium and equilibrium must thus be established at a tissue/medium concentration ratio greater than one.

However, the essential features of mechanism remain substantially unchanged and appear to be supported by the following observations; namely, (1) the entry of actively transported sugars into the epithelial cells under conditions of limited energy supply is responsive to Na^+ (Bihler, Hawkins and Crane, 1962), (2) accumulation of sugar and entry of Na^+ across the brush border membrane are similarly inhibited by any one of a number of other monovalent cations (Bosackova, 1963) and (3) the addition of an actively transported sugar to the mucosal side of an everted sac of small intestine incubated in vitro increases both the transmural potential (Barry, Dikstein, Matthews and Smyth, 1960; Schachter and Britten, 1961; Schultz and Zalusky, 1963) and the net Na^+ flux from the mucosal to the serosal side of the preparation (Schultz and Zalusky, 1964).

Among the various criteria which may be applied to test a presumed mobile carrier mechanism, the phenomenon of counter-transport appears to be most critical and widely accepted (Wilbrandt and Rosenberg, 1961). Counter-transport is an "uphill" transport, independent of metabolic energy, which is induced by the "downhill" movement of a second substrate using the same carrier. This criterion has been applied to intestinal sugar transport in experiments designed to show that xylose and glucose share the same pathway of absorption (Salomon, Allums and Smith, 1961). It has not previously been applied in terms of the Na^+ requirement of sugar transport as a test of our fundamental assertion that Na^+ interacts with the sugar carrier. In the present situation it is the "downhill" gradient of Na^+ ion into the cell which is presumed to result in the "uphill" movement of sugar in the same direction. The essence of the test, thus, should be to reverse the Na^+ gradient and to observe whether sugar is then moved "uphill" out of the cell into the medium.

The experimental object and the conditions of the experiment were

chosen to provide the ingredients for an appropriate test. The choice of isolated villi was dictated by the fact that the critical and most difficult measurement to make is the concentration of sugar within the epithelial cells. With the more intact preparations, sugar trapped within the underlying layers represents a possibly large and certainly indeterminant background. These underlying layers are removed during the preparation of villi. Although it is likely that not all of the cells of the villi retain normal function throughout the sometimes drastic procedures employed, these preparations exhibit high levels of accumulative activity when appropriately tested, and, most important, it is possible to account for most of the non-epithelial cell volume by measurement of the extracellular space (Crane and Mandelstam, 1960). The experimental conditions (N_2, dinitrocresol) were chosen to suppress energy-dependent transport of Na^+ as completely as possible (Curran and Solomon, 1958; Clarkson and Rothstein, 1960; Schultz and Zalusky, 1964). Under these conditions, accumulation of sugar should be and is limited very closely to that expected from diffusion equilibrium (Bihler and Crane, 1962) and any small gradient established may be attributed to a continued small differential in Na^+ concentration. In recent studies with Dr. Peter Curran, the Na^+ concentration of the epithelial cells of villi incubated under approximately the same conditions as in Table I ranged from 77-116 mEq/L. In experiments A and D the medium contained 120 mEq/L of Na^+ and some small accumulation of sugar was to be expected.

As shown by experiments B and C, when villi previously equilibrated with 6-deoxyglucose, as in A, were transferred to media containing no Na^+, thus reversing the Na^+ gradient, sugar moved "uphill" out of the cells. Experiment D shows that the manipulations of the experiment were not contributory. These results appear to support our thesis that Na^+ interacts directly with the sugar carrier in the brush membrane of the intestinal epithelial cell and to be contrary to the views expressed by others (Csaky, 1963; 1964).

Table 1. 6-Deoxyglucose Outflow Induced by Reversal of the Na^+ Gradient.

Experiment	Duration of Incubation and medium used	Final Concentrations mM 6-Deoxyglucose		Final Ratio
		medium	tissue water	T/M
A	5 min. incubation in Na^+ = 120	1.52	2.06	1.35
B	same as A plus 2 min. incubation in Na^+ = 0	1.61	1.02	0.64
C	same as A plus 5 min. incubation in Na^+ = 0	1.63	0.76	0.47
D	same as A plus 5 min. incubation in Na^+ = 120	1.51	1.72	1.14

Villi from hamster small intestine were prepared, incubated, recovered and assayed as previously described (Crane and Mandelstam, 1960). The media used contained either (in mEq/L) 120 Na^+, 120 Cl^-, 25 tris(hydroxymethyl)aminomethane$^+$ and 25 HCO_3^- or 0 Na^+, 120 Cl^-, 145 tris$^+$ and 25 HCO_3^-. In addition, both contained 1.6 mM 6-deoxyglucose as substrate, 0.05 mM 2,4-dinitro-o-cresol to depress energy yielding processes and approximately 0.002 mM C^{14}-mannitol to serve as a measure of the extracellular space in the pellet of recovered villi. Both media were equilibrated with 95% nitrogen, 5% CO_2. The pH was 7.4. The temperature of incubation was 37°. 6-deoxyglucose was assayed chemically (Dische and Shettles, 1948). C^{14}-mannitol was assayed by its radioactivity as measured with a liquid scintillation spectrometer.

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