matters arising

Intestinal transport protein

We would like to take issue with the recent letter of Faust and Shearin[1] reporting on a "sugar and amino acid transport protein . . . from hamster jejunum".

The questionable significance of the observed D-glucose binding proteins from the intestine to intestinal Na^+-dependent monosaccharide transport has been discussed in detail by Eichholz and Howell[2] and Garcia-Castineiras *et al.*[3]. In addition, we would like to point out that it is highly misleading to give a binding protein the adjunct transport when it does not fulfil one of the fundamental requirements for transport, namely high rates of binding and debinding. That the debinding of D-glucose is extremely slow has been previously noted by Eichholz *et al.*[4], and can be deduced from the gel chromatography data of Faust and Shearin[1]. In fact, D-^{14}C-glucose remains tightly bound to a macromolecule during passage through Sephadex G-75[1]. If anything, this tight binding suggests that the "protein" plays a role in storage rather than transport of D-glucose.

That the bulk of the observed D-glucose binding may be unrelated to transport is also indicated by one of our observations. We have investigated D-glucose transport in isolated and highly purified vesicles of brush border membrane from rat small intestine[5,6]. Previously, D-glucose uptake by the isolated membranes had been measured mainly at *p*H 7.5. When investigating D-glucose uptake as a function of *p*H we noticed that with lower *p*H values the equilibrium uptake increased above the *p*H 7.5 level. This 'extra' D-glucose uptake exhibited a pronounced maximum around *p*H 6.0. The time curve of uptake did not seem to be altered by *p*H, that is, constant levels of D-glucose were always reached by 5 min incubation at 25° C. The absolute amounts of 'extra' uptake varied between 0.3 and 1.0 nmol per mg membrane protein (measured at 1 mM D-glucose) in different preparations. Bacterial contamination is not responsible for our observation.

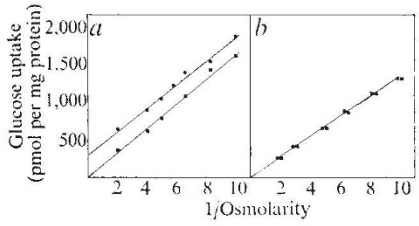

Fig. 1 Effect of medium osmolarity on D-glucose uptake in isolated intestinal brush border membranes. Preparation of membranes (in 100 mM cellobiose, 1 mM HEPES, adjusted to *p*H 7.5 with Tris, and 0.1 mM $MgSO_4$) and the uptake procedure have been previously described[5]. Uptake was measured from a medium with the following final composition: NaSCN (25 mM), Tris-HEPES (1 mM), $MgSO_4$ (0.1 mM), D-1-^{3}H-glucose (1 mM) and L-1-^{14}C-glucose (1 mM) and enough cellobiose to give the indicated osmolarity (shown as inverse osmolarity). Time of incubation: D-glucose, 10 min; L-glucose, 20 min; temperature, 25° C. The lines were calculated by regression analysis (correlation coefficient in all cases: 0.99). *a*, D-glucose; *b*, L-glucose. ■, *p*H 7.5; ●, *p*H 6.0.

To determine whether the 'extra' D-glucose uptake represented binding to the brush border membrane or transport into the intravesicular membrane space we measured the dependence of D- and L-glucose uptake on the osmolarity of the suspension medium at *p*H 7.5 and *p*H 6.0 (Fig. 1). D- and L-glucose uptake at *p*H 7.5 and also L-glucose uptake at *p*H 6.0 can be accounted for totally by transport into an osmotically active space (line goes through the origin). The 'extra' D-glucose uptake at *p*H 6.0, however, is independent of the medium osmolarity suggesting binding (300 pmol per mg protein in Fig. 1; intersection of the line with the ordinate).

The rate of D-glucose transport was found to be equally fast at all *p*H values between *p*H 8.0 and 5.0. That transport can proceed without measurable binding, at least on the scale indicated in Fig. 1, suggests to us that transport and measured binding are unrelated. At the moment, we have no hint as to function or origin of the D-glucose binding.

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1 Faust, R. G., and Shearin, S. J., *Nature*, **248**, 60 (1974).
2 Eichholz, A., and Howell, K. E., *Gastroenterology*, **62**, 647 (1972).
3 Garcia-Castineiras, S., Torres-Pinedo, R., and Alvarado, F., *FEBS Lett.*, **30**, 115 (1973).
4 Eichholz, A., Howell, K. E., and Crane, R. K., *Biochim. biophys. Acta*, **193**, 179 (1969).
5 Hopfer, U., Nelson, K., Perrotto, J., and Isselbacher, K. J., *J. biol. Chem.*, **248**, 25 (1973).
6 Murer, H., and Hopfer, U., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 484 (1974).

Dr Faust replies—Since the brush border region of the intestinal mucosal cell is a digestive-absorptive organelle, the pumping of monosaccharides and amino acids across this entire organelle must be considered in the mechanism by which these substrates are accumulated against their concentration gradients within the cytoplasm.

The phenomenon that Hopfer and Sigrist-Nelson have observed concerns the movement of D-glucose across one section of the structurally complex brush border, the plasma membrane. We agree that their observed D-glucose binding is not related to transport and we feel that it could be caused by binding to disaccharidases in the plasma membrane. Our binding protein, however, is located in the core fraction of disrupted brush borders which does not contain hydrolytic enzymes. It possesses sites for the Na^+-dependent 'trapping' of specific monosaccharides and amino acids that are derived either indirectly from the hydrolysis of dietary oligosaccharides and polypeptides or directly from dietary monomers in the intestinal lumen. We have shown that the specificity for this binding is similar to that for the active transport of hese substances by the small intestine. Furthermore, these observations could not be attributed to bacterial contamination.

We visualise that our isolated binding protein is involved in the Na^+-dependent active transport of sugars and amino acids from the core region of the brush border into the cytoplasm. This occurs after the initial penetration of these monomers through the plasma membrane.

We concede, however, that this protein can only be regarded provisionally as a transport protein until debinding is adequately studied. Debinding of the sugar and amino acid ternary complexes could be triggered by a conformational change in the binding protein caused by the low intracellular Na^+ concentration, the replacement of Na^+ in the ternary complex by K^+ from the high K^+ intracellular region, and by a combination of these phenomena with the aid of energy derived directly from cellular metabolism. None of these conditions was prevalent in our Sephadex G-75 fractionation studies, therefore D-^{14}C-glucose and L-^{14}C-histidine remained tightly bound to the isolated protein subunits.

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