and other investigators[6] collect nerve-end membranes from the leading edge of this band.

Detailed studies on the use of combined rate and isopycnic zonal separation techniques[9] to the fractionation of brain will be presented in detail elsewhere.

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Spatial relationship between intestinal disaccharidases and the active transport system for sugars

With the present great increase in the number of proposals for the biochemical secret of membrane transport, it may be a truism, but nonetheless worthwhile to note (1) that there is a vast array of thermodynamically feasible mechanisms from which to choose and (2) the fact that one is feasible does not mean that it is actually used by the cell. Temporal or spatial juxtaposition of events as evidence for a mechanism are especially suspect and proposals based on such evidence should be subjected to rigorous tests at the earliest moment. The recent suggestion[1] that membrane-bound trehalase is the terminal catalyst in a reaction sequence leading to the membrane active transport of glucose and other sugars is one of this sort. This particular speculation was directed primarily to the proximal convoluted tubule of the kidney but

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has been extended to the gut owing to the prevalence of trehalase activity in both tissues[1,2]. On this account, it appears to be open to direct test in experiments based upon much previous work[3–5]. The question can be asked and answered whether trehalase lies inside or outside the tissue barrier to the free diffusion of sugars. According to the reaction sequence proposed, trehalase should lie inside.

The active transport process for sugars in the intestine is characterized by its strong dependence on Na^+ and energy-yielding cell processes[6–8]. It is also sensitive to phlorizin which is an extremely potent competitive inhibitor[9]. The active transport process has been shown to be internal to the loci of those brush border enzymes already examined. Sucrose, maltose and phosphate esters were shown to be hydrolyzed at the brush borders in a location from which the products of hydrolysis are prevented entry into the cell by the presence of phlorizin or the absence of Na^+ (refs. 3, 4). Identical experiments using substrates for lactase and trehalase were carried out to establish the spatial relationships of these enzymes to the transport process.

TABLE I

TISSUE ACCUMULATION OF GLUCOSE DERIVED FROM LACTOSE AND TREHALOSE

Segments of everted intestine (200–400 mg) from hamster starved for 16 h were incubated for 10 min in 5 ml of media containing lactose or trehalose at 25 mM according to our usual methods[8]. The control medium was Krebs–Henseleit phosphate buffer[10] and the effects of phlorizin were studied in the same medium. When Na^+ was excluded from the medium, 125 mM choline$^+$ and 16 mM K^+ were used as the substituting cations. Glucose was measured enzymically using glucose oxidase[11]. The results shown are the averages of at least three experiments in each group.

Medium	*Lactose*			*Trehalose*		
	Tissue glucose (mM)	*Medium glucose (mM)*	*Total lactose hydrolyzed (μmoles)*	*Tissue glucose (mM)*	*Medium glucose (mM)*	*Total trehalose hydrolyzed (μmoles)*
Control	12.7	0.13	3.13	16.3	0.19	2.26
+1 mM Phlorizin	0.81	0.745	3.00	0.92	0.908	1.92
Na^+ substituted with 125 mM choline$^+$ and 16 mM K^+	0.625	0.593	3.08	0.922	0.863	2.25

The results assembled in Table I clearly indicate that while addition of phlorizin or the absence of Na^+ have no detectable effect on the activity of either hydrolase, these conditions do prevent the entry and cellular accumulation of the products of hydrolysis. Thus it may be concluded that lactase and trehalase are also localized in a region of the brush border external to the active transport process for sugars. Consequently, we find it difficult to accept an intestinal transport mechanism involving trehalase as proposed[1]. Not only is an α,α-glycosyl glycoside an inappropriate intermediate from considerations of the demonstrated specificity of the process[12] but also the terminal enzyme in the postulated sequence is external to the barrier across which the transport of sugar is said to be catalyzed.

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Observations on the permeability of thymocytes to non-electrolytes

In the course of experiments with calf thymus nuclei isolated in sucrose solutions a [^{14}C]sucrose-impermeable fraction of the particles was found. This fraction corresponds to intact cells since Ficoll isolation of nuclei substantially reduces the sucrose-impermeable fraction and the cellular fraction of these preparations has a much higher sucrose-impermeable space[1]. The present experiments further support the contention that the sucrose-impermeable fraction corresponds to intact cells since the permeability of this fraction to malonamide is identical to that of intact thymocytes isolated by passing the tissue through a screen by the method of BLECHER AND WHITE[2].

The present communication reports on this validation and in addition furnishes the permeability constants for several non-electrolytes suspended in a sucrose medium containing Ca^{2+} and Mg^{2+}.

Techniques. The suspensions of nuclei and thymocytes were isolated by a method where the tissue is homogenized in a Waring blendor and centrifuged as previously described[3] and detailed in another communication[4]. These preparations are likely to contain 30 % intact cells. The substances used were labeled with ^{14}C and were purchased from New England Nuclear Corp. (Boston, Mass.), except for [^{14}C]erythritol which was obtained from Nuclear-Chicago Corp. (Chicago, Ill.).

Aliquots of 2 or 3 ml were incubated in 12-ml tubes of cellulose nitrate. All samples were incubated at 0° and shaken in a Dubnoff shaker (130 cycles/min). At zero time, 50 μl of the isotope solutions were added. The incubation medium consisted of 0.4 osmolal sucrose, 0.02 M Tris buffer, 5 mM $CaCl_2$ and 5 mM $MgCl_2$ at a pH of 7.4. In addition to the ^{14}C-labeled compounds, the medium contained 50- to 200-fold excesses of non-radioactive penetrant intended to saturate possible binding sites. The ^{14}C-labeled penetrant was added in the order of decreasing period of exposure. In this fashion all tubes were shaken for the same length of time, but the incubation time in the presence of the penetrant was varied. At the end of the incubation period all tubes were centrifuged at 12800 $\times$ g for 5 min in a Lourdes LRA refrigerated centrifuge. The supernatant was immediately poured off and saved for counting. The