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PREPARATION, CHARACTERIZATION AND STABILITY OF RABBIT INTESTINAL BRUSH BORDER MEMBRANE VESICLES

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Brush border membrane vesicles (BBMV) have only recently been used in the study of drug absorption (Iseki et al 1985; Wood et al 1990). There is little available data on the effect of handling/storage conditions on vesicle viability. The present study simultaneously examines the preparation, characterisation, storage and physical properties of rabbit intestinal BBMV and equates this with the transporting capabilities of the vesicles under typical experimental conditions. BBMV were prepared and used as described previously (Wood et al 1989).Incubations were performed at 25°C, pH 7.4 in 310 mOsm buffers. Typically, vesicles were made in <3h yielding $3.20 \times 10^{-3} \pm 0.55 \times 10^{-3}$ mg protein/g fresh tissue (BBMV contain on average 17.13±1.88 mg protein/ml). Transport was quantified using D-glucose (a marker of the biochemical state of the preparation) and L-glucose (a marker of the physical status of the membrane).The tissue could be stored at -70°C for 1 year and vesicles for at least 137 days under liquid nitrogen; the D-glucose overshoot ratio was 19:1 after storage, not significantly different from the 17:1 noted for fresh vesicles. The hydrodynamic diameter of the fresh vesicles was 322±6.89nm, n=115 (11 preparations). After freezing, vesicles were 267±13.01 nm, n=320,(12 preps.). At 25°C the vesicles could actively transport D-glucose for up to 3.5h when fresh and 5h after thawing before maximum uptake and peak:equilibrium ratios fell. L-Glucose uptake was maintained for at least 20h without significant variation. D-Glucose uptake was pH independent (pH 4-8) but temperature dependent. L-Glucose uptake was less temperature dependent reflecting the different natures of the transport processes. Vesicle size decreased with temperature (18% over 10-40°C, n=40, 5 preps).

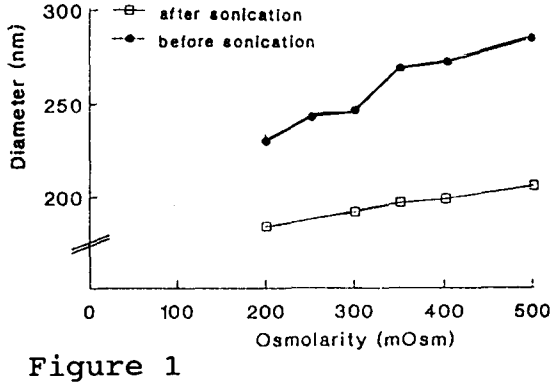

Figure 1

The effect changing buffer osmolarity on vesicle size

Figure 1 shows the dependence of size on medium osmolarity. The size increase corresponded to a 32% decrease in internal volume (calculated from equilibrium uptake values), suggesting that the increase was due to aggregation which was reversible by sonication. D-gluclose uptake was also reduced on sonication but L-glucose uptake was largely unaltered, again reflecting the mechanistic differences between active transport (protein dependent) and passive absorption. The present study shows that frozen tissue produces reproducible vesicles that can be stored successfully for as long as 137 days, and used for 5h after defrosting without loss of protein activity or membrane integrity, thus enabling effective evaluation of their use in drug absorption studies.

Iseki, K. et al (1985) J. Pharm. Pharmacol. 37:374-375
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