erythrocyte membrane corresponded to cytochrome b₅₆. This evidence suggests that specific association of cytochrome b₅₆ with erythrocyte membranes occurs (George et al., 1988). To investigate whether the spontaneous membrane associative properties of cytochrome b₅₆ are transferable, we have constructed hybrid proteins locating the hydrophobic domain of cytochrome b₅₆ at the C-terminus. On expressing these β-galactosidase:HP fusion proteins in E. coli, the bulk of the hybrid protein (90%) is associated with the membrane fraction. In contrast, normal β-galactosidase coded for by the parent plasmid is found largely in the cytoplasm.

On incubating the hybrid proteins with either liposomes or erythrocytes spontaneous association was observed in each case. Under identical conditions only minimal binding occurred for normal β-galactosidase synthesized from the parent plasmid. These experiments suggest that transposition of the cytochrome b₅₆ hydrophobic domain on to other proteins enables these proteins to acquire membrane-associative properties. This suggests the possibility of developing a variety of proteins for liposome- and erythrocyte-based therapeutic carrier systems.

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Activity of hexokinase in mouse oocytes and preimplantation embryos

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Unfertilized and fertilized mouse oocytes and preimplantation embryos up to the 4-cell stage have a requirement for pyruvic acid to support their development in culture. Glucose as sole energy substrate is unable to support development until the 8-cell stage. Barbechenn et al. (1974, 1978) proposed that this block to glucose utilization resided in glycolysis, probably at the level of 6-phosphofructokinase (EC 2.7.1.11), but possibly at the level of hexokinase (EC 2.7.1.1). Gardner & Leese (1988) have suggested that glucose transport across the plasma membrane may also be involved in limiting glucose uptake.

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We have investigated the possible role of hexokinase by measuring its activity in groups of five or ten embryos or in single embryos using a modification of an ultramicrofluorometric technique (Leese & Barton, 1984). Hexokinase has previously been measured on groups of 10–25 preimplantation mouse embryos by Brinster (1968).

Unfertilized ova and fertilized ova and preimplantation embryos were obtained from superovulated (CBA/Ca × C57BL/6) female mice mated with F1 males. The oocytes and embryos were flushed from the oviduct or uterus with 0.9% (w/v) NaCl/4% (w/v) bovine serum albumin and frozen once at −70°C in 1 µl of extraction medium containing 1 mM-EDTA, 5 mM-mercaptoethanol, bovine serum albumin (2 mg/ml) and phenylmethylsulfonyl fluoride (0.17 mg/ml) in 100 mM-K2HPO4 buffer, pH 7.5. Thawed extracts were added to 0.5 µl of reaction mixture containing 5 mM-MgCl2, 5 mM-ATP, 1.5 mM-NADP+, 1 mM-glucose, glucose-6-phosphate dehydrogenase (EC 1.1.1.49; 5 units/ml) in 100 mM-triethanolamine buffer, pH 7.6.

The assay method was a modification of that described by Hooper & Leese (1986) and Leese (1987). The reaction mixture (0.5 µl) plus extract (1 µl) were placed in a small reaction chamber (capacity 10 µl) formed by drilling a 3 mm diameter hole in a siliconized microscope slide and gluing the slide on top of a second slide. The chamber was covered with a cover slip. Reactions were carried out at 20°C. The formation of NADPH was monitored using a Diavert MPV Compact inverted fluorescence microscope (Leitz Instruments Ltd).

The profile of hexokinase activity throughout development is shown in Fig. 1. The mean activities (pmol/h per embryo) for each stage of development were as follows, with the values obtained by Brinster (1968) in parentheses: oocyte; 2.3 (1.2); 2-cell: 4.3 (1.7); 8-cell: 6.4 (2.2); morula: 8.8 (5.6); blastocyst: 26.1 (7.9). There was no increase in hexokinase activity following sonication or the inclusion of 0.1% or 0.5% (v/v) Triton X-100 in the extraction medium.

Brush-border membrane vesicles (BBMV) were prepared from the frozen intestine of male white New Zealand rabbits according to the method of Barton (1984). Typically, 1 g of fresh tissue yielded approximately 3 mg of BBMV (three times the yield of Semenza et al., 1984). Aliquots of the vesicles were adjusted to contain about 25 mg of protein/ml, then stored frozen under liquid nitrogen before use. Glucose uptake was measured using an inhibitor-stopped rapid filtration technique (based on that of Hopfer et al., 1973). The reaction was initiated by mixing 40 µl of 0.1 mM-D- or -L-glucose (containing ±2H]glucose or ±14C]glucose) in 100 mM buffered NaSCN with 20 µl of BBMV. After incubation at 25°C, the reaction was stopped with 1 ml of ice-cold buffered 200