Contrast, production of the 59,000 M₄ and 70,000 M₄ components was only slightly reduced at higher concentrations (>10 μM) of signal peptide (Fig. 1a, track 1), indicating that these proteins are covalently labelled by non-specific binding or reaction. Comparison of photo-reaction with rat liver microsomes (Fig. 1b, track 1) which were found to yield more stable vesicles, and in which the 45,000 M₄ band is more prominent, or pancreatic microsomes centrifuged either before, or after, irradiation, which also markedly reduced the amount of labelled 70,000 M₄ and 59,000 M₄ bands, and enhanced formation of the 45,000 M₄ band (compare Fig 1a, track 5, with Fig. 1b, track 3), indicates that the higher molecular weight bands result from proteins being released from the vesicles during freezing or storage. In contrast the 45,000 M₄ band is not released from membranes unless high concentrations of detergent are present (octyl glucoside/phospholipid, 10:1 molar ratio) indicating that it is an integral membrane protein. The 45,000 M₄ band is formed by photo-reaction after prior stripping of rat liver microsomes with 0.1 M Na₂CO₃ (pH 11.5), a procedure that removes content and peripheral proteins (Fig. 1b, track 2).

The microsomal membrane protein that gives rise to the 45,000 M₄ peptide–protein adduct acts as a saturable receptor (Kₘ = 1 x 10⁻⁷ M) for signal peptides in keeping with previous binding studies (Austen & Ridd, 1983). This binding protein may act to attach signals in pre-sequences to the membrane as they emerge from the ribosome, after the signal recognition particle and the docking protein have targetted polysomes to the rought ER. Alternatively, it may have other roles in translocating the nascent protein or in cleavage of the signal peptide.

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Comparisons of pancreatic rough microsomal membranes prepared from guinea pigs with and without cholecystokinin stimulation

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Processing of proteins translated from isolated mRNA is conventionally studied by supplementing translation systems in vitro with dog pancreatic rough microsomes, which cleave or glycosylate the segregated products with fidelity (Shields & Blobel, 1977). Although they appear to be important parameters to investigate, the effects of fasting or cholecystokinin (CCK) stimulation on the animal before preparation of pancreatic rough microsomes has not been investigated. In vivo, CCK exerts both short-term stimulatory effects on pancreatic secretion, and long-term effects leading to increased digestive enzyme concentration (Webster et al., 1977).

We have shown previously that a number of major acidic polypeptides associated with pancreatic or liver rough microsomes are strongly conserved in electrical charge and molecular mass in a variety of species (Kaderbhai & Austen, 1984; Kaderbhai et al., 1984). It was proposed that some of these polypeptides may play a role in the translocation or processing of secretory proteins at the rough endoplasmic reticulum membrane. Here, we compare the polypeptide profile of pancreatic rough microsomes prepared from guinea pigs that have either been fasted, or fed and stimulated with exogenous CCK.

Five guinea-pigs were allowed to feed ad libitum and injected subcutaneously with 101 IVY units of CCK-PZ (0.5 ml; Boots, Nottingham, U.K.)/kg 11 times at 8-hourly intervals. A second group of five guinea pigs was fasted for 48 h. Animals were killed by cervical dislocation, the pancreases from each group excised and pooled, and rough microsomes prepared from postmitochondrial supernatants by gel-filtration and centrifugation on discontinuous sucrose gradients as previously described (Kaderbhai & Austen, 1984). For comparison, rough microsomes were also prepared from dog pancreas and rat liver, the animals being allowed to feed ad libitum.

Polypeptides isolated by two-dimensional gel electrophoresis from rough microsomes of guinea-pigs are shown in Fig. 1, and have been numbered as previously (Kaderbhai & Austen, 1984). Polypeptide 18 was previously identified as the digestive protein amylase, trapped in the microsomal preparation, because of its co-migration with the major protein of pancreatic juice, and because the M₄ of guinea-pig amylase has been reported to be the same as that of dog pancreas, whereas its pI is slightly more basic (Scheele, 1975). It is clear that the Coomassie Blue staining of polypeptide 18 is more intense in the profile of rough microsomes prepared after feeding and administration of CCK to the animals (Fig. 1a), than in microsomes prepared after fasting (Fig. 1b), in keeping with the reported increase of digestive enzyme biosynthesis after prolonged administration of low levels of CCK (Webster et al., 1977).

Polypeptide 12 has been identified by comparison of pl and M₄ as the homologue of the enzyme protein disulphide isomerase (EC 5.3.4.1), previously characterized by co-migration with purified enzyme on two-dimensional electrophoresis and mapping of Staphylococcus aureus protease digestion fragments. From the relative amounts of Coomassie Blue staining, it is clear that feeding and CCK stimulation causes an elevation in the amounts of protein disulphide isomerase in the rough microsome. Although this observation awaits confirmation by measuring the specific activities of the enzyme, increased amounts would be in keeping with the postulated role of protein disulphide isomerase in the catalysis of disulphide bond formation in newly synthesized secretory proteins (Freedman, 1984).

A number of other proteins show differences in staining in the two preparations. Of interest is an apparent increase in the amount of polypeptide numbered 21. This protein appears to be of identical M₄ to a canine pancreatic rough
Fig. 1. Comparative profiles of rough microsomal polypeptides analysed by two-dimensional gel electrophoresis.

Membranes (250 µg of protein) were solubilized in 1% NP-40/1% Triton X-100/9.5 M-urea at 10 mg of protein/ml for isoelectric focusing in 4% acrylamide cylindrical gels containing 2% (v/v) pH 3.5–10 Ampholines (LKB, Bromma, Sweden) for 4800 V.h. The second dimension was in a polyacrylamide slab gel containing 7–16% linear gradient of acrylamide in SDS. (a) shows the profile of pancreatic rough microsomes from fed/CCK-stimulated guinea pigs and (b) the profile from fasted animals.
microsomal polypeptide, which has been shown to cross-link to a radioiodinated photoreactive signal peptide under U.V. illumination (Robinson et al., 1985). An increase in the amount of this protein in the fed/CCK-stimulated animals would also be consistent with the proposed function of this protein in processing or translocation of pre-proteins with signal sequences.

Relative activities of rough microsomal membranes in the processing of prolactin biosynthesized from bovine pituitary mRNA in a rabbit reticulocyte lysate translation system was studied as previously described (Kaderbhai et al., 1984). Only nuclease-treated rough microsomes obtained from dog pancreas and rat liver were active, while those obtained from guinea pig inhibited incorporation of $[^35]$S-methionine into protein completely, even in the presence of placental ribonuclease inhibitor. The nature of the inhibitory components present have yet to be elucidated.

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The effect of hydrophobic bis(D-mannose) derivatives on the rat adipocyte sugar-transport system

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Several novel bis(D-mannose) compounds have been synthesized. The position on the sugar chosen for cross-linking was C-4 because specificity investigations of the rat adipocyte sugar-transport system (Holman et al., 1981) have revealed that there would be sufficient space around this position to accommodate the bridge between the sugar moieties. The starting compound for the cross-linked sugars was 1,6-anhydro-2,3-O-isopropylidene-β-D-mannopyranose. This was crosslinked by 2-propylamine, hexane and decane bridges and then the protecting groups were removed. The 2-propylamine compound was further substituted with dinitrophenyl (DNP) or DNP-aminocaproyl groups. The four derivatives, bis(D-mannos-4-yloxy)-hexane, bis(D-mannos-4-yloxy)-decane, N'-DNP-bis(D-mannos-4-yloxy)-2-propylamine and N'-DNP-aminocaproyl-bis(D-mannos-4-yloxy)-2-propylamine, were tested as inhibitors of the uptake of 50 μM-3-O-methyl-D-glucose into rat adipocytes in the presence and in the absence of 10 nM-insulin. The method used for estimating inhibition constants was a modification of the methods described by Gliemann & Rees (1983) and Holman et al. (1981). The $K_i$ values (Table 1) are the concentrations of inhibitor which gave a 50% inhibition of 3-O-methyl-D-glucose uptake.

Bis(D-mannos-4-yloxy)-hexane has a lower $K_i$ than D-mannose. This is probably due in part to the presence of the additional sugar moiety but is likely to be also due to the hydrophobicity of the hexane bridge which may interact with a hydrophobic domain of the transport protein. The $K_i$ value for bis(D-mannos-4-yloxy)-decane is consistent with this proposal. The increase in the bridge length lowers the $K_i$ to a value which is approx. 10-fold less than that which is found for the shorter bridged compound.

The $K_i$ for N'-DNP-bis(D-mannos-4-yloxy)-2-propylamine is very low in insulin-treated cells. The high affinity is likely to be due to the presence of the DNP group, which may interact with the proposed hydrophobic domain in the transport protein. This hydrophobic interaction appears to be quite specific and seems to depend on the DNP group being close to the sugar moiety which is held in the transport site since the introduction of an aminocaproyl spacer between the DNP group and the 2-propylamine bridge increases the $K_i$.

For each of the four derivatives tested the inhibition constants are higher in the non-insulin-treated adipocytes. Previous studies (Holman et al., 1981) have shown that simple sugar analogues have the same $K_i$ in the presence and

**Table 1. Half-maximal inhibition constants for hydrophobic bis(D-mannose) derivatives**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i$ (μM)</th>
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<tbody>
<tr>
<td>D-Mannose</td>
<td>22120 ± 2270</td>
</tr>
<tr>
<td>1.6-Bis(D-mannos-4-yloxy)-hexane</td>
<td>1230 ± 70</td>
</tr>
<tr>
<td>1.10-Bis(D-mannos-4-yloxy)-decane</td>
<td>126 ± 8</td>
</tr>
<tr>
<td>N'-DNP-bis(D-mannos-4-yloxy)-2-propylamine</td>
<td>22 ± 1.6</td>
</tr>
<tr>
<td>N'-DNP-aminocaproyl-bis-(D-mannos-4-yloxy)-2-propylamine</td>
<td>265 ± 38</td>
</tr>
</tbody>
</table>

Results are means ± S.E. ($n = 8$ in all cases).

<table>
<thead>
<tr>
<th>+ 10 nM-Insulin</th>
<th>Basal cells</th>
</tr>
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<tbody>
<tr>
<td>18690 ± 1040</td>
<td>3220 ± 28</td>
</tr>
<tr>
<td>3220 ± 260</td>
<td>315 ± 5.7</td>
</tr>
<tr>
<td>80 ± 8</td>
<td>772 ± 43</td>
</tr>
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