Fig. 1. T.l.c. of neutral oligosaccharides in swainsonine-induced mannosidosis

T.l.c. was carried out on silica-gel plates using propan-1-ol/water (8:3, v/v) as the solvent and carbohydrates were detected with orcinol [0.2% (w/v) in H$_2$SO$_4$, 5% (w/v) in methanol]. M$_2$G, Man($\alpha$1-3)Man($\beta$1-4)GlcNAc; M$_3$G, Man($\alpha$1-2)Man($\alpha$1-3)Man($\beta$1-4)GlcNAc; M$_5$G, Man($\alpha$1-2)Man($\alpha$1-3)Man($\beta$1-4)GlcNAc.

ities, subcellular locations, turnover and the significance of the multiple forms of $\alpha$-D-mannosidase remain to be studied.

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Exchange of lysosomal enzymes between cells

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If transplanted cells are to be effective for replacement therapy, the enzymes they produce must be capable of reaching the lysosomes within a wide range of deficient target cells. This exchange of enzymes can take place by two different mechanisms: secretion followed by receptor-mediated endocytosis and direct cell-to-cell transfer.

Lysosomal enzymes, in common with other glycoproteins, have the capacity to bind to specific receptors present on the plasma membranes of many types of cell (Neufeld & Ashwell, 1980) and can thus be taken up rapidly. Different types of cell express different surface receptors. Hepatocytes, for example, have receptors for galactose

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(Pricer & Ashwell, 1971) as well as fucose (Prieels et al., 1978), fibroblasts recognize mannose 6-phosphate (Kaplan et al., 1977), while alveolar macrophages selectively bind mannose and N-acetylglucosamine (Stahl et al., 1978). Further heterogeneity is introduced by the observation that the same enzyme isolated from different tissues may be taken up into cells at greatly differing rates. For example, the β-glucuronidase from human platelets is taken up into deficient human fibroblasts at a rate more than 10 times that of similar preparations from human urine, placenta or liver (Brot et al., 1974). Again, β-glucuronidase and N-acetylhexosaminidase isolated from rat-liver lysosomes are cleared from the circulation much more rapidly than the corresponding enzymes prepared from serum (Stahl et al., 1976). In order, therefore, to determine the potential usefulness of cells such as fibroblasts and lymphocytes as transplanted enzyme donors, their ability to transfer a typical lysosomal enzyme, β-glucuronidase, to other types of cell was investigated.

Initial experiments showed that cultured mouse 3T3 fibroblasts spontaneously secreted large amounts of lysosomal enzymes (Diment & Dean, 1983) and that β-glucuronidase purified from medium in which these cells had been cultured was rapidly taken up into mouse A9 fibroblasts. Uptake was not, however, species-specific, since β-glucuronidase deficient human fibroblasts at a rate more than 10 times that of similar preparations from human urine, placenta or liver (Brot et al., 1974). Again, β-glucuronidase and N-acetylhexosaminidase isolated from rat-liver lysosomes are cleared from the circulation much more rapidly than the corresponding enzymes prepared from serum (Stahl et al., 1976). In order, therefore, to determine the potential usefulness of cells such as fibroblasts and lymphocytes as transplanted enzyme donors, their ability to transfer a typical lysosomal enzyme, β-glucuronidase, to other types of cell was investigated.

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Table 2. The rates of uptake of 125I-labelled ligands into different types of cell

Cells were incubated for 1 h with 2 μg/ml β-glucuronidase or 10 μg/ml polyvinylpyrrolidone. Values represent the means of two separate experiments.

<table>
<thead>
<tr>
<th>Type of cell</th>
<th>125I-β-glucuronidase (A)</th>
<th>125I-polyvinylpyrrolidone (B)</th>
<th>A/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse peritoneal macrophages</td>
<td>2.2</td>
<td>0.004</td>
<td>550</td>
</tr>
<tr>
<td>Human-skin fibroblasts</td>
<td>1.1</td>
<td>0.002</td>
<td>550</td>
</tr>
<tr>
<td>Mouse neuroblastoma</td>
<td>0.5</td>
<td>0.002</td>
<td>250</td>
</tr>
<tr>
<td>Rat glioma</td>
<td>0.2</td>
<td>0.004</td>
<td>50</td>
</tr>
<tr>
<td>Rabbit chondrocytes</td>
<td>0.7</td>
<td>0.015</td>
<td>47</td>
</tr>
<tr>
<td>Pig chondrocytes</td>
<td>0.7</td>
<td>0.021</td>
<td>33</td>
</tr>
<tr>
<td>Mouse lymphocytes</td>
<td>Not detectable</td>
<td>Not detectable</td>
<td></td>
</tr>
</tbody>
</table>

The precise mechanism by which fibroblasts acquire β-glucuronidase during direct contact is still uncertain. It is possible that factors produced by lymphocytes might have stimulated synthesis of endogenous p-glucuronidase or that a messenger RNA or even DNA might be transferred from the lymphocytes. β-Glucuronidase is a tetramer of four identical subunits (Tomino et al., 1975) and somatic cell hybrids resulting from fusion of human and mouse fibroblasts produced a series of heteropolymers following association between subunits of mouse and human β-glucuronidases (Chern, 1977). If either of the above explanations were correct, the enzyme acquired by GM151 fibroblasts following interaction would have the characteristics either of human β-glucuronidase or of mouse/human hybrids. Chromatography of the acquired enzyme on DEAE-cellulose or electrophoresis on polyacrylamide gel, however, showed that there were no heteropolymers and that all of the acquired enzyme activity had a mobility characteristic of the donor cell enzyme (Dean et al., 1982). Furthermore, when donor lymphocytes were pre-labelled with thymidine or uridine there was little or no transfer of polymeric DNA or RNA to fibroblasts during co-culture (M. F. Dean, & B. Jenne, unpublished work).

The most probable explanation, therefore, is that complete molecules of lymphocyte β-glucuronidase were transferred directly to recipient fibroblasts. Support for this supposition was provided by the fact that when lymphocytes from strains of mice that synthesize either heat-stable or heat-labile forms of β-glucuronidase (Paigen, 1961) were used for co-culture experiments, the enzyme acquired by recipient fibroblasts displayed thermal stability characteristics identical to those of the donor lymphocytes (Dean et al., 1982). Furthermore, when rabbit lymphocytes were used as donor cells, the β-glucuronidase extracted from the recipient fibroblasts was precipitated by a specific anti-rabbit β-glucuronidase antiserum (Dean et al., 1982), showing that the transferred enzyme retained the antigenic determinants characteristic of the donor cells. None of the β-glucuronidase in extracts made from normal human fibroblasts were precipitated with this anti-rabbit antiserum although it was precipitated by a second anti-human β-glucuronidase antiserum (Dean et al., 1982).

Thus, although lymphocytes do not take up fibroblast β-glucuronidase by receptor-mediated endocytosis, nor secrete enzyme which is recognized as a high-uptake form by fibroblasts, they can donate enzyme directly during cell-to-cell contact. Lymphocytes do therefore have a potential value as donor cells for replacement therapy. Fibroblasts in contrast are able to secrete a lysosomal enzyme which is taken up by receptor-mediated endocytosis into a wide variety of cell types, including other fibroblasts, chondrocytes, reticuloendothelial cells and cells of the central nervous system. They thus have a wide potential as donor cells for the treatment of systemic lysosomal deficiency diseases.

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