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(75–80\% of the control values for up to 4h) was also observed with glucose oxidase associated with phosphatidylinositol liposomes. It appears that association of insulin and of glucose oxidase with phosphatidylinositol offers some protection to these agents from inactivation in the gut, and, at the same time, facilitates their transport into the blood. Whether this is mediated via intact liposomes (as is probably the case for the small proportion of polyvinylpyrrolidone) or by the lipid moiety of disrupted liposomes, with which insulin and glucose oxidase seem to interact, is not as yet clear.

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**Preparation of Glucocerebroside β-Glucosidase for Entrapment in Liposomes and Treatment of Patients with Adult Gaucher's Disease**

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Gaucher's disease is an inherited glycosphingolipidosis, in which there is an accumulation of ceramide glucoside, mostly in the lysosomes of spleen and liver, caused by a deficiency of glucocerebroside β-glucosidase (Brady et al., 1965). Attempts have been made to alleviate the clinical symptoms of the adult form of Gaucher's disease by the direct administration of partially purified placental enzyme. Although liver and erythrocyte concentrations of glucocerebroside decreased, no improvement in the clinical condition of the patients was reported (Brady et al., 1974). Disadvantages of this form of enzyme-replacement therapy include premature inactivation (immunological or otherwise) of the enzyme and its inability to selectively reach diseased areas. One possible way of circumventing these problems is by entrapment of the enzyme in a carrier, which would afford protection from inactivation and also enhance its rate of uptake by the liver and spleen. Liposomes appear to be suitable candidates as carriers of enzymes and other therapeutic agents (Gregoriadis, 1974), and their role in the treatment of lysosomal storage diseases is indicated by results obtained with a model system, in which liposome-entrapped invertase was able to hydrolyse sucrose stored in the lysosomes of cultured cells (Gregoriadis & Buckland, 1973). It was therefore proposed to use liposomes as carriers of glucocerebroside β-glucosidase, in the treatment of adult Gaucher's disease.

Glucocerebroside β-glucosidase is considered to be firmly membrane bound and is usually solubilized by the action of a detergent, for example Triton X-100 (Ho, 1973). However, since the presence of such a detergent is incompatible with liposome formation, it was necessary to devise a method of enzyme preparation that could not only process a large amount of tissue but also produce the enzyme in a form suitable for entrapment.

Human placenta (300–600g) received in ice usually immediately after delivery, was freed of membranes, cut into small pieces, minced through a meat mincer, homogenized in a Waring Blender, in 10mm-sodium phosphate buffer, pH 7.0, (30\%, w/v) at 4°C and centrifuged in an MSE 18 refrigerated centrifuge for 90min at 23,000g. The precipitate was rehomogenized in the above buffer supplemented with 0.15\%(v/v) Triton WR1339
Table 1. Purification of glucocerebroside β-glucosidase from human placenta

Activity is expressed as nmol of 4-methylumbelliferone released/min. Enzyme activity was measured in 0.1 M-sodium acetate buffer (pH4.5) containing 0.1% Triton X-100 and 0.1% (w/v) sodium taurocholate, as described by Ho (1973).

<table>
<thead>
<tr>
<th>Step of procedure</th>
<th>Total activity units</th>
<th>Yield %</th>
<th>Protein mg</th>
<th>Specific activity units/mg protein</th>
<th>Fold purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenization</td>
<td>214000</td>
<td>100</td>
<td>75000</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Solubilization with Triton WR1339</td>
<td>25200</td>
<td>12.5</td>
<td>2000</td>
<td>12.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Concanavalin A–Sepharose</td>
<td>20300</td>
<td>10</td>
<td>170</td>
<td>120</td>
<td>34</td>
</tr>
<tr>
<td>Precipitation with ethanol/ chloroform followed by solubilization with 10 mm-sodium phosphate buffer</td>
<td>2170</td>
<td>1.0</td>
<td>2.4</td>
<td>910</td>
<td>260</td>
</tr>
</tbody>
</table>

(Winthrop Laboratories, Newcastle upon Tyne, U.K.) and centrifuged as before. The supernatant was applied on 150–200 ml of concanavalin A-Sepharose [Pharmacia (G.B.) Ltd., London W.5, U.K.] held in a sintered glass funnel (9 cm height × 15 cm diameter) and equilibrated with 10 mM-sodium phosphate buffer, pH7.0, containing 0.15% (v/v) Triton WR1339. Non-absorbed material was washed exhaustively with the equilibration buffer. Bound material was eluted with the buffer containing the detergent together with 1M-a-methyl-D-mannoside (Sigma Chemical Co., London S.W.6, U.K.) and 2.5% (w/v) EDTA. The concanavalin A-Sepharose was regenerated by elution with 10 mM-sodium phosphate buffer, pH7.0, to remove the detergent and also 0.1 mM-MnCl₂, 0.1 mM-CaCl₂ and 0.5% (w/v) concanavalin A. The eluate was dialysed extensively against 10 mM-sodium phosphate buffer, pH7.0, to remove the sugar and then concentrated by dialysis against polyethylene glycol 6000 (BDH, Poole, Dorset, U.K.). The enzyme, together with other proteins, was subsequently precipitated by the addition of 4–5 vol. of ethanol/chloroform (9:1, v/v). The supernatant was removed by centrifugation as above at 38000g for 30 min, and the precipitate washed 2–3 times with the solvent to remove any detergent still present. After the final centrifugation and evaporation of the solvents under N₂, the enzyme was dissolved in 5–7 ml of 10 mM-sodium phosphate buffer, pH7.0, and separated from insoluble proteins by centrifugation as before.

The duration of the procedure was 3 days, and after the final stage the enzyme, which constituted 1% of the original activity, had been purified 260-fold (Table 1) when measured (Ho, 1973) with 4-methylumbelliferone β-glucosidase as substrate. Electrophoresis on polyacrylamide gel showed the enzyme preparation still to be heterogeneous, and the β-glucosidase could be identified as one of six or seven protein bands. When assayed (Ho, 1973) with the natural substrate (human spleen glucocerebroside) the specific activity was 1100 nmol/min per mg, similar to that obtained with the synthetic substrate. In pH-activity-profile studies, optimal activity could be observed between pH4.5 and 5.0.

Glucocerebroside β-glucosidase, the activity of which remained unaltered at −20°C for at least 2 weeks, was entrapped (Gregoriadis & Buckland, 1973) in liposomes composed of egg phosphatidylcholine, cholesterol and phosphatidic acid (molar ratio 7:2:1). The entrapped enzyme was separated from the non-entrapped material by centrifugation of the unsonicated liposomes at 100000g for 1 h in an MSE 65 centrifuge, and the pellet suspended in 10 mM-sodium phosphate buffer, pH7.0. This suspension contained 40–60% of the enzyme activity used, which is well above the entrapment values obtained.
for other proteins (Gregoriadis, 1974), and it suggests an interaction of the enzyme with the liposomal lipids. Entrapment of proteins in liposomes that can be tolerated by man can be achieved under sterile conditions (Gregoriadis et al., 1974).

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**Precocious Development of Uridine Diphosphate Glucuronyltransferase Activity in Chick-Embryo Liver after Administration of 11β-Hydroxy Steroids with and without Thyroxine**

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UDP-Glucuronyltransferase (EC 2.4.1.17) activity is low in or absent from fresh homogenates or microsomal preparations of chick-embryo liver until hatching at 21 days, when it rapidly increases to adult values (Dutton & Ko. 1966). Precocious development of adult activity can occur in embryo liver of 15 days or later, after grafting, 3–4 days earlier, of the cephalic region of the anterior-pituitary gland from a hatching or hatched bird on to the chorioallantoic membrane (Wishart & Dutton, 1974, 1975a). Injection of adrenocorticotrophic, follicle-stimulating or thyrotrophic hormone, known to be secreted by this region of the gland (Brasch & Betz, 1971), had no reproducible effect on the transferase activity (Leakey & Dutton, 1975).

To mimic natural release of hormone from endogenous or grafted tissue we developed a new technique of hormone administration, involving continuous flow down a paper strip from a reservoir on to the chorioallantoic membrane. Rate of flow is controllable. By this means we have demonstrated that adrenocorticotrophic hormone and corticosterone (11β,21-dihydroxy-4-pregnene-3,20-dione) provoke precocious appearance of the transferase on day 17 when applied from day 13 (Leakey & Dutton, 1975).

We summarize here further effects on the enzyme after treatment with corticosteroids of the embryo in ovo and of liver in organ culture.

Corticosterone, cortisol (11β,17,21-trihydroxy-4-pregnene-3,20-dione) and aldosterone (11β,21-dihydroxy-3,20-dioxo-4-pregnene-18-al) all stimulated precocious activity, but 11-deoxycorticosterone, progesterone (4-pregnene-3,20-dione), pregnenolone (3β-hydroxy-5-pregnen-20-one), tetracortisol (3a,17a,21-trihydroxy-5β-pregnane-11,20-dione), testosterone (17β-hydroxy-4-androsten-3-one) and oestradiol [1,3,5(10)-estratriene-13,16α,17β-triol] had no effect. Ability to stimulate appeared to require the 11β-hydroxy group; cortisone (17α,21-dihydroxy-4-pregnene-3,11,20-trione) was much less effective than cortisol, requiring 5 times its concentration for stimulation.

Although lower doses occasionally stimulated, the minimal amount of corticosterone or cortisol for reproducible effect was 0.18 μmol per egg administered over the 4 days. Maximal stimulation of transferase was 30-fold, i.e. to some 3–4 times adult activity.

Minimal time of administration required for evident enzyme stimulation 4 days after onset of administration of high doses of corticosteroid was 2–4 h. The minimal time for