

medium nor by the liver. The experiments with amyloglucosidase showed no difference in glycogen concentration in the liver nor in glucose release into the medium when compared with control perfusions.

Nevertheless there was penetration of liver tissue by circulating amyloglucosidase during perfusion corresponding to 0.03–0.4 mg of enzyme/g wet wt. of liver (corrected for endogenous glucosidase activity). The concentration in liver was never greater than that in the medium.

When amyloglucosidase was administered intravenously to intact rats, a major proportion of the enzyme found in the liver was identified in a mitochondrial-lysosomal fraction prepared by the procedure of Lloyd (1969a), confirming the results of Lloyd (1969b). After perfusion with amyloglucosidase increased glucosidase activity was also associated with a mitochondrial-lysosomal fraction. Although penetration of liver lysosomes by amyloglucosidase apparently occurred during perfusion under the present conditions, the extent of this process was less than *in vivo*. This may be due to the absence of cofactors or to lysosomal damage in the isolated liver.

Attempts to separate hepatocytes from Kupffer cells in an effort to identify the involvement of each in enzyme uptake *in vivo* have been made.

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### Liposomes as Carriers of Enzymes or Drugs: a New Approach to the Treatment of Storage Diseases

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Patients with disorders in which a specific enzyme activity is absent from one or more tissues have in a few cases been treated by direct administration of an enzyme designed to remove undesirable accumulation products (Baudhuin, Hers & Loeb, 1964;

Fernandes & Huijing, 1968; Kissel, Lamazche & Royer, 1968). Such a therapeutic approach, however, involves possible immunological response arising from the foreign protein and problems related to undesirability of the presence of some enzymes in the circulation and in directing the given protein to a particular tissue.

It seemed possible that some of the difficulties mentioned could be avoided by entrapping relevant enzymes within liposomes. Liposomes (Bangham, 1968) consist of a series of concentric bilayers of lipids alternating with aqueous compartments within which soluble substances can be entrapped (Sessa & Weissman, 1968).

The present experiments describe conditions for the entrapment of *Aspergillus niger* amyloglucosidase (EC 3.2.1.3) [which splits (1→4)- and (1→6)-bonds of glycogen] and <sup>131</sup>I-labelled albumin into liposomes composed of phosphatidylcholine, cholesterol and dicetyl phosphate. An entrapment of 4–10% of the proteins used was achieved (Gregoriadis, Leathwood & Ryman, 1971). More than 60% of protein-containing liposomes (with a lipid content of 2.5 mg) are eliminated within 10 min after their intravenous administration to rats. Experiments with <sup>131</sup>I-labelled albumin entrapped into [<sup>3</sup>H]cholesterol liposomes suggest that liposomes remain intact during their circulation and indicate that there is no measurable leakage of <sup>131</sup>I-labelled albumin. Most of the radioactivity removed from plasma is recovered in liver (and to a smaller extent in spleen) and it reaches a maximum (56% of that injected) during the first 15 min. There is a rapid decrease in liver radioactivity thereafter. After subcellular fractionation of the liver it appears that the mitochondrial-lysosomal fraction and cytosol are the major sites of liposomal recovery. Further preliminary fractionations on sucrose density gradients suggest that the liposomes may be associated with lysosomes of decreased density. Radioautography of the liver after injection of [<sup>3</sup>H]cholesterol liposomes reveals that both parenchymal and Kupffer cells participate in the uptake of liposomes. Liposomes appear to be a promising vehicle for the direction of enzymes and drugs to the liver and spleen.

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