rapid elimination from the blood and concomitant uptake by liver and spleen. Immunological studies with liposomes containing [^3H]amyloglucosidase revealed catabolism of the enzyme in the liver.

Our present experiments *in vivo* with liposomes containing invertase (β -D-fructofuranosidase) enabled us for the first time to follow the fate of enzyme activity rather than that of enzyme radio-active label or enzyme immunological identity. Invertase activity 6h after injection of liposomes containing invertase (220 units and 17.5mg of lipid) is recovered in the liver (45%) and spleen (10% of the dose) and it slowly declines to low values within 100h. Subcellular fractionation of such livers indicates that the mitochondrial-lysosomal fraction accounts for most of the invertase activity. Additional evidence for the lysosomal uptake and catabolism of the liposomal protein was sought by two approaches. (i) Fractionation on Ficoll-mannitol gradient of the liver lysosome-rich fraction from rats injected with liposomes containing invertase coupled with deliberate prior alteration of the sedimentation properties of the lysosomes by Triton WR-1339 and dextran, known respectively to decrease (Wattiaux *et al.*, 1963) and to increase (Baudhuin *et al.*, 1965) the specific gravity of lysosomes. The results obtained show association of invertase activity with lysosomes, since the changing patterns of *N*-acetyl- β -D-glucosaminidase activity on the gradient after the specific-gravity manipulations are paralleled by invertase distribution. (ii) Incubation of the lysosome-rich liver fraction obtained after injection of entrapped ^{131}I -labelled albumin and measurement of trichloroacetic acid-soluble ^{131}I -labelled tyrosine radioactivity (Mego *et al.*, 1967) showed a doubling within 2h.

These experiments increase our confidence that liposomes may be used as carriers of entrapped material (e.g. enzymes, drugs) to counteract disorders of lysosomal activity, especially in view of the possibility of liposomal membrane manipulations that could induce endocytosis of liposomes in cell types and tissues other than liver and spleen. The possibility of utilizing liposomes to introduce segments of DNA into defective cells coupled with modification of catabolic activity of lysosomes is worth considering.

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Ornithine Metabolism in the Developing Sheep

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It has been found that the ability of sheep liver slices to synthesize urea is constant throughout development from 50 days gestational age to adulthood, but there are marked changes in arginase (L-arginine amidinohydrolase, EC 3.5.3.1) activity in developing sheep liver as measured by the method of Folley & Greenbaum (1948). An increase in foetal liver arginase activity occurred from 260 ± 80 units (mean $\pm$ S.E.M.) (1 unit of enzyme activity being defined as $1 \mu\text{mol}$ of urea formed/min per g wet wt. at 37°C) at 50 days to 3010 ± 110 units at 140 days gestational age. Activity decreased rapidly after birth and at 19 days of age was 630 ± 80 units, thereafter declining to the adult value of 240 ± 20 units. Ornithine carbamoyltransferase (carbamoyl phosphate-L-ornithine carbamoyltransferase, EC 2.1.3.3) activity was measured by the method of Brown & Cohen (1959) (1 unit of activity being defined as $1 \mu\text{mol}$ of citrulline formed/min per g wet wt. at 37°C). The activity of this enzyme in foetal liver was constant from 50 days gestational age to term, being 65.0 ± 4.2 units, but after birth the activity increased gradually to the adult value of 89.5 ± 10.8 units. Ornithine-keto acid aminotransferase (L-ornithine-2-oxo acid aminotransferase, EC 2.6.1.13) activity was measured by the method of Peraino & Pitot (1963) (1 unit of activity being defined as $1 \mu\text{mol}$ of Δ^1 -pyrroline-5-carboxylate formed/min per g wet wt. at 37°C). The activity in livers of 65-day-old foetuses was 0.73 ± 0.07 unit; during further foetal and postnatal development the activity decreased to the adult value of 0.10 ± 0.03 unit.

Arginase activities of sheep kidney and placenta were less variable during development than those observed in liver and averaged 0.5% and 1% of adult liver values respectively. Ornithine carbamoyltransferase was not detectable in these tissues. Ornithine-keto acid aminotransferase activities in foetal kidney were 25% of those in foetal liver but higher in adult kidney than in adult liver. Placental ornithine keto acid aminotransferase activity was 1.07 ± 0.17 units throughout gestation, and this high activity may contribute to the failure of raised plasma ornithine concentrations to be reflected in foetal plasma (Hopkins *et al.*, 1971b). The high

hepatic arginase activity observed in near-term foetuses would enable arginine that is released into the foetal circulation (Hopkins *et al.*, 1971a) to be metabolized in the liver.

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The Molecular-Weight Factor in the Biliary Excretion of Quaternary Ammonium Cations

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There appears to be a molecular-weight threshold, which varies with species, for the significant biliary excretion (i.e. >5–10% of dose) of organic anions. This threshold appears to be about 325 ± 50 for the rat, 400 ± 25 for the guinea pig and 500 ± 50 for the rabbit (Millburn *et al.*, 1967; Abdel Aziz *et al.*, 1971). A study is now in progress on the biliary excretion of organic cations, and from the results already obtained it would appear that for mono-quaternary cations the molecular-weight threshold for significant biliary excretion is less than for anions and that species variation may not occur to any marked extent.

The cations used contained ^{14}C -labelled methyl groups and the animals were bile-duct-cannulated as described by Abou-El-Makarem *et al.* (1967). In the rat the biliary excretion of the cations *N*-methylpyridinium (mol.wt. 94), trimethylphenylammonium (mol.wt. 136), diethylmethylphenylammonium (mol.wt. 164), dibenzyltrimethylammonium (mol.wt. 226), cetyltrimethylammonium (mol.wt. 284) and tribenzylmethylammonium (mol.wt. 302) was 0.6, 0.5, 6, 27, 15 and 39% of the dose in 3h respectively. These cations were not extensively metabolized and were excreted in the bile mainly unchanged. These results and others in the literature would suggest a threshold value in the region of 225 ± 25 for these cations in the rat.

Preliminary studies with three of the above-mentioned compounds in rats, guinea pigs and rabbits showed no significant species differences in the extent of biliary excretion. Thus the biliary excretion of

trimethylphenylammonium was 0.5% in the three species, whereas for dibenzyltrimethylammonium and tribenzylmethylammonium all three species excreted 30–40% of the dose in the bile in 3h.

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A Kinetic Analysis of Pinocytosis in Rat Yolk-Sac

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Rat visceral yolk-sac, from 17.5-day pregnant animals, was suspended in medium TC 199 containing 10% (v/v) of calf serum and incubated at 37°C. ^{125}I -labelled polyvinylpyrrolidone added to the culture medium (3 µg/ml) was accumulated at a linear rate, up to 7h. In four separate experiments the endocytic index, defined as the volume of medium processed by the tissue in unit time, was $1.92 \pm \text{s.d. } 0.15 \mu\text{l/h}$ per mg of yolk-sac protein. Accumulated polyvinylpyrrolidone was not released from the tissue, and the endocytic index was found to be independent of polyvinylpyrrolidone concentration at least within the concentration range 0.15–15.0 µg/ml.

Similar experiments with as substrate ^{125}I -labelled bovine serum albumin (3 µg/ml), which is digested intracellularly after pinocytosis by rat yolk-sac (Williams *et al.*, 1971), showed a steady tissue concentration of substrate once the rate of accumulation was balanced by the rate of digestion. When this steady state was reached (after about 2h) the rate of release of trichloroacetic acid-soluble radioactivity was linear for at least 5h and was used to calculate the endocytic index. In four separate experiments this value was $15.80 \pm \text{s.d. } 1.29 \mu\text{l/h}$ per mg of yolk-sac protein. Further experiments showed this endocytic index to hold over the concentration range 1.5–52.0 µg of ^{125}I -labelled albumin/ml.

The discrepancy between the two values obtained for the endocytic index could reflect a stimulation by ^{125}I -labelled albumin of the rate of internalization of culture medium, or alternatively a depression by polyvinylpyrrolidone. Such explanations are made unlikely by the concentration-independence of the