not affect the absorption rate of the highly water-soluble carbamates. This is in agreement with previous work (Levy & Perala, 1970). It was shown that the Tween 80 maintained all carbamates in solution throughout the experiment. The amount of carbamate remaining in the tissue after the incubation was also determined and a similar trend was observed.

The same pattern was established for the absorption of these carbamates through skin.

In the gut-sac system butyl carbamate showed optimal lipophilicity for absorption. Contrary to the hypothesis of previous investigators (Schanker, 1960), any further increase in compound lipophilicity resulted in a decrease in absorption. The validity of these findings *in vivo* remains to be elucidated.

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Levy, G. & Perala, A. (1970) *J. Pharm. Sci.* **59**, 874

Schanker, L. S. (1960) *J. Med. Pharm. Chem.* **2**, 343

Wilson, T. H. & Wiseman, G. (1954) *J. Physiol. (London)* **123**, 116

The Effect of Increasing Concentrations of Potassium Chloride on Rat Liver Lysosomes

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In muscle, and possibly in other tissues, phosphorylated compounds provide the bulk of the negative charge that balances intracellular cations (Harris, 1960) and, during freezing, these salts will increase in concentration within the cell irrespective of whether ice forms intracellularly or extracellularly. As lysosomes are known to be damaged by freezing and thawing (Sellinger *et al.*, 1960), and we decided to study the effect of increasing concentrations of salt on these organelles. However, the intracellular anionic composition of most cells is unknown, and since determinations of the composition are likely to be affected by the time required to arrest metabolic reactions (Wollenberger *et al.*, 1960; Lowry *et al.*, 1964) we opted to study the effect of increasing concentrations of KCl.

The latency of acid phosphatase and β -glucuronidase in homogenates of rat liver was found to decrease progressively on exposure to concentrations of KCl between 0.13 and 1.5M in spite of the fact that the concentration of sucrose was maintained at 0.25M. These latencies were determined solely with Triton X-100, and the response could have been due to disruption of the lysosomes or to an alteration in the permeability of the lysosomal membrane that would allow access of substrate to enzymes still within the lysosomal membrane. However, we were also able to demonstrate the decrease in latency when centrifuga-

tion (300000g-min) was used to determine free acid phosphatase, and it appears that lysosomes are actually disrupted by exposure to high concentrations of salt.

The early work on rat liver lysosomes showed that they were freely permeable to salts such as NaCl and KCl (Berthet *et al.*, 1951), but our own studies and those of Lloyd (1971) indicate that these salts penetrate the rat liver lysosomal membrane relatively slowly. Consequently the disruptive effect of high concentrations of KCl on rat liver lysosomes could be due to transient osmotic pressure gradients that will arise when the concentration of KCl is lowered, but we cannot exclude a direct effect on the lysosomal membrane.

Cryoprotective agents lower the concentration of salt that is in equilibrium with ice at any given temperature (see Farrant & Woolgar, 1970), and they could therefore protect cells against damage during freezing by preventing the disruption of lysosomes. However, compounds such as glycerol and dimethyl sulphoxide have also been shown to have a deleterious effect on lysosomes (Lee, 1971, 1972), and this could be one of the mechanisms of toxicity of cryoprotective compounds.

Berthet, J., Berthet, L., Appelmans, F. & de Duve, C. (1951) *Biochem. J.* **50**, 182

Farrant, J. & Woolgar, A. E. (1970) *Frozen Cell Symp.* **1969** 97

Harris, E. J. (1960) *Transport and Accumulation in Biological Systems*, 2nd. edn., Butterworths, London

Lee, D. (1971) *Biochim. Biophys. Acta* **233**, 619

Lee, D. (1972) *Biochim. Biophys. Acta* **266**, 50

Lloyd, J. B. (1971) *Biochem. J.* **121**, 245

Lowry, O. H., Passonneau, J. V., Hasselberger, F. X. & Schulz, D. W. (1964) *J. Biol. Chem.* **239**, 18

Sellinger, O. Z., Beaufay, H., Jacques, P., Doyen, A. & de Duve, C. (1960) *Biochem. J.* **74**, 450

Wollenberger, A., Ristau, O. & Schoffa, G. (1960) *Pflügers Arch. Gesande Physiol.* **270**, 399

Lysosomal Localization of Enzyme-Containing Liposomes Injected into Rats

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Liposomes with entrapped material have been proposed as carriers for enzyme or drug administration (Gregoriadis *et al.*, 1971; Gregoriadis & Ryman, 1971, 1972). Our experiments with liposomes containing ^{131}I -labelled albumin or *Aspergillus niger* [^3H]amyloglucosidase have shown that injection of such liposomes into rats results in their

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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- Baudhuin, P., Beaufay, H. & de Duve, C. (1965) *J. Cell Biol.* **26**, 219
Gregoriadis, G. & Ryman, B. E. (1971) *Biochem. J.* **124**, 58P
Gregoriadis, G. & Ryman, B. E. (1972) *Eur. J. Biochem.* **24**, 485
Gregoriadis, G., Leathwood, P. D. & Ryman, B. E. (1971) *FEBS Lett.* **14**, 95

- Mego, J. L., Bertini, F. & McQueen, J. D. (1967) *J. Cell Biol.* **32**, 699
Wattiaux, R., Wibo, M. & Baudhuin, P. (1963) *Arch. Int. Physiol. Biochem.* **71**, 140

Ornithine Metabolism in the Developing Sheep

By J. M. RATTENBURY, MARJORIE K. JEACOCK and D. A. L. SHEPHERD (*Department of Physiology and Biochemistry, University of Reading, Whiteknights, Reading RG6 2AJ, U.K.*)

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