important in the metabolism of cholecalciferol, it is less important than skeletal muscle and adipose tissue as a storage site for unchanged cholecalciferol.

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Formation and Interconversion of Ketone Bodies in Tissues of Developing Lambs

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The rates of acetoacetate production by homogenates of adult sheep liver were compared with those found in livers obtained from foetal lambs by using the method described by Williamson, Bates & Krebs (1968), in which acetyl phosphate, CoA and phosphotransacetylase (EC 2.3.1.8) are used as an acetyl-CoA-generating system. The rate of acetoacetate production in adult sheep liver was 1.37 ± 0.56 (mean $\pm$ S.E.M.) $\mu\text{mol/min per g wet wt. of tissue at } 37^\circ\text{C}$, whereas it was barely measurable in foetal livers at 113 days gestational age and at 140 days it was only 15% of the adult rate. Acetoacetate production was also measured in placental cotyledons and in some cases it was found to be greater in this tissue than in maternal liver.

The activity of 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30), which mediates the interconversion of acetoacetate and 3-hydroxybutyrate, was determined in the liver, heart and cerebral cortex of foetal and 15-week-old lambs by the method of Lehninger, Sudduth & Wise (1960). In the immature foetus (100–110 days) the activities of this enzyme were 0.51 ± 0.27 unit, 1.20 ± 0.18 units and 0.07 ($0.00, 0.14$) unit in liver, heart and brain respectively (1 unit of activity is defined as $1 \mu\text{mol of NADH formed/min per g wet wt. of tissue at } 37^\circ\text{C}$). In the mature foetus (140 days) the activities had risen to values of 0.88 ± 0.16 unit, 3.86 ± 0.53 units and 0.72 ($0.64, 0.79$) unit, and in the 15-week-old lambs the activities were even higher, being 1.45 ± 0.19 units, 20.48 ± 1.36 units and 1.30 ($0.90, 1.70$) units. In placental cotyledons 3-hydroxybutyrate dehydrogenase activity was similar to that of mature lamb liver.

Thus it would appear that the immature foetal lamb liver is incapable of synthesizing acetoacetate but can interconvert ketone bodies, whereas the

term foetus is capable of both the synthesis of acetoacetate and its conversion into 3-hydroxybutyrate, although at lower rates than those found in the adult sheep. These results support the findings of Alexander, Britton, Cohen & Nixon (1969). Their foetal perfusion studies indicated that there is little endogenous acetoacetate production in foetal lambs of 113 and 132 days, and they also showed that foetal sheep can convert infused acetoacetate into 3-hydroxybutyrate and can oxidize acetoacetate. However, these authors considered that ketone bodies contribute little to the nutrition of the foetus because of the low permeability of the placenta to these substrates.

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The Solution of Erythrocyte Membranes in Organic Solvents

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The low solubility of many of the proteins of cell membranes causes great difficulties in their isolation and study. Detergents are widely used to disrupt membranes (Bakerman & Wasemiller, 1967; Schneiderman & Junga, 1968; Rosenberg & Guidotti, 1969), but the proteins solubilized thereby may be micellar and irreversibly associated with detergent. Some organic solvents have been reported to solubilize membranes (Blumenfeld, 1968; Rega, Weed, Reed, Berg & Rothstein, 1967; Zwaal & van Deenen, 1968; Zahler, 1968) though the conditions used have often been such as might degrade glycoproteins present.

Here a search has been made for some organic solvents of human erythrocyte membranes in order to determine the necessary structural features of good solvents (miscible with water) and the extent to which they dissociate and degrade the proteins and glycoproteins of the membrane.

Human erythrocyte 'ghosts' (ORh+, water-washed, at 2%, w/w) were used as a standard membrane preparation. Solvents were studied at 90%, 60% and 30% (v/v) concentrations, at pH 9,

7 and 4, and at 18°C and 0°C. Solution was measured spectrophotometrically at 530nm. The stability of the solutions was tested over 12h.

Degradation of the glycoproteins of the membrane was studied by exposing samples of purified membrane glycoprotein (Stoddart & Metcalfe, 1969) to the solvents and examining the changes in their electrophoretic patterns (Takayama, MacLennan, Tzagoloff & Stoner, 1966) and composition. The extent of the dissociation of both membranes and purified protein was studied by analytical ultracentrifugation.

It was found that pyridine, the picolines and 2,6-lutidine were good solvents at 30% (v/v) above pH 8.7 (as confirmed by ultracentrifugation) but not below this pH. At 18°C there was degradation of sialoglycoprotein, but this was stable up to pH 9.7 at 0°C. Piperidine and pyrrolidine were effective solvents only at pH values far above 10. *N*-Methylpyrrolidone (90%, v/v) was a good solvent at pH 7.

No esters or glycols were found to be good solvents. 2-Mercaptoethanol (90%, v/v) dissolved membranes at pH 7, and 2-chloroethanol was fairly effective at 90% (v/v), at 60% (v/v) at pH 4 and at 30% (v/v) at pH 9. 2,2-Dichloroethanol was a good solvent above 50% (v/v) at all pH values and dissociated the protein very extensively.

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Reversible Dissociation of Fatty Acid Synthetase from Lactating Rat Mammary Gland

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The native fatty acid synthetase isolated from lactating rat mammary gland and maintained at 21°C exists as a 13S species. Aging at 0°C for 1 week, in either the presence or the absence of dithiothreitol, results in complete dissociation into half-molecular-weight 9S subunits (Smith & Abraham, 1970). Synthetase activity could be demonstrated with the 9S species formed by aging in the presence of dithiothreitol even after removal of the dithiothreitol. However, no activity was observed with the 9S species formed by aging in the absence of dithiothreitol.

The question whether both native and subunit species possess synthetase activity or whether reassociation occurs under conditions of the enzyme assay was investigated by studying the effect of temperature on enzyme activity and structural integrity of the 9S and 13S species. The temperature-dependence of fatty acid synthetase activity was markedly different when 9S and 13S species were added to the assay medium. Enzyme activity was observed with the 13S species at temperatures as low as 0°C, but no significant activity could be detected below 20°C with the 9S species. Between 20°C and 30°C the activity observed with the 9S species was very much more temperature-dependent than that with the 13S species. The 9S species was incubated in the absence of dithiothreitol for 3 min at 20°C and at 30°C and analysed by sucrose-density-gradient centrifugation at 2°C. Whereas the original 9S preparation contained only 6% of the 13S species, the samples incubated at 20°C and 30°C contained 22% and 41% respectively of the 13S species. Thus, whereas the cold-induced dissociation of the native enzyme is a slow process occurring over a period of days, the heat-induced reassociation is extremely rapid, taking place in a matter of minutes. These findings indicate that the native 13S species is the only enzymically active form of fatty acid synthetase and that the activity observed with the 9S species results from heat-induced reassociation to the active 13S form during assay.

When the 13S species was titrated with *p*-chloromercuri[¹⁴C]benzoate at 20°C, 30% of the enzyme dissociated into the 9S species when about six of the enzyme's 57 thiol groups were blocked. The *p*-chloromercuri[¹⁴C]benzoate-labelled 13S species retained approx. 70% of its enzyme activity, whereas no enzyme activity could be demonstrated