utilize it, but dissimilate protocatechuate through the now well-characterized pathway involving the isomeric γ -carboxymuconolactone and its decarboxylation product 3-oxoadipate enol-lactone. Fungi show either no or very low activities with these lactones.

The conversion of β -carboxymuconolactone into 3-oxoadipate thus involves both decarboxylation and hydrolysis, but no intermediate has ever been implicated and this step remains biochemically ill-defined.

We have now purified the enzyme responsible for this conversion in *Aspergillus niger* some 250-fold, at which stage it was homogeneous on polyacrylamide-gel electrophoresis, all the enzyme activity being associated with the single protein band of molecular weight $54\,000 \pm 5000$. The pure enzyme produces stoichiometric amounts of 3-oxoadipate, CO_2 and protons, all at identical rates, from β -carboxymuconolactone with no detectable intermediate formation:

The enzyme displayed classical Michaelis-Menton kinetics with K_m $70\,\mu\text{M}$ at the pH optimum of 8.6; it was inactive with γ -carboxymuconolactone as substrate, but hydrolysed 3-oxoadipate enol-lactone and muconolactone at 9% and 6% respectively of the rate with β -carboxymuconolactone. The purified enzyme had a half-life of less than 24h unless stabilized with glycerol (20%), 2-mercaptoethanol (0.01%) and certain non-substrate analogues, from among which butyrolactone was used as a routine during purification.

These lactones act as competitive inhibitors. Cyanide (K_i 0.1mM), iodoacetate (K_i 0.14mM) and arsenite (K_i 0.8mM) are potent non-competitive inhibitors of the enzyme. A threefold decrease in K_m in the region of pH 8.3–8.7 (Benesch & Benesch, 1955) together with the requirement for 2-mercaptoethanol and the iodoacetate inhibition strongly suggest the presence of an active-site thiol group in this enzyme. Temperatures above 35°C rapidly inactivate the enzyme and Arrhenius plots show an unusual inflexion at 18.5°C . There is also some evidence for the presence of a tightly bound bivalent metal ion.

We conclude that the conversion of β -carboxymuconolactone into 3-oxoadipate is mediated by a single enzyme, 3-carboxy-4-carboxymethyl-4-hydroxycrotonolactone hydrolase (decarboxylating), with both delactonizing and decarboxylase activities.

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Tissue Distribution of Cholecalciferol (Vitamin D₃) in the Rat

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Although cholecalciferol is known to exert a biological effect long after administration of the vitamin has ceased, information on tissue storage sites is sparse.

Reports by Callow, Kodicek & Thompson (1966) and by Neville & DeLuca (1966) indicated that, in the rat, the liver contained much of the radioactivity during the first 2 days after administration of labelled cholecalciferol. We have extended this period of observation and now report changes in the distribution of radioactive cholecalciferol and its metabolites occurring during the first week after administration.

Mature rats that were not vitamin D-deficient were injected intracardially with 5i.u. (125ng) of $[1,2\text{-}^3\text{H}_2]$ cholecalciferol and killed in pairs at intervals from 20min to 1 week after injection. Lipid extracts of tissues were made and chromatographed on silicic acid to separate cholecalciferol and its three principal more-polar metabolites (Mawer & Backhouse, 1969).

The results for distribution of the dose are expressed as the percentage of injected radioactivity that was present in each tissue when extrapolated to the whole body content.

Three groups of tissues were identified: (a) tissues with high early concentrations of radioactivity and rapid decline; liver and serum contained 70% and 11% respectively of the dose at 20min and 1.5% and 4% at 1 week; (b) tissues in which accumulation and decrease of radioactivity were slower; intestinal mucosa, kidney, bone and muscle contained at 1 week 0.05, 0.7, 1.6 and 3.8% respectively of the injected radioactivity; (c) tissues in which the radioactivity rose slowly and showed no decline; adipose tissue contained about 10% of the dose at 1 week. Some 80% of the radioactivity in liver, serum and mucosa, at 1 week, was present as more-polar metabolites, but in kidney, adipose tissue and muscle 70% of the recovered radioactivity was present as cholecalciferol.

These results suggest that, although the liver is

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,