Acetyl-Coenzyme A Formation in the Tissues of the Developing Lamb

By R. V. BRIERLEY, MARJORIE K. JEACOCK and D. A. L. SHEPHERD. (*Department of Physiology and Biochemistry, University of Reading*)

Hanson & Ballard (1968) have shown that, in liver slices from the ox, the utilization of acetate and glucose for the production of fatty acids occurs at higher rates in the foetus than in the adult animal. As the production of extramitochondrial acetyl-CoA for fatty acid biosynthesis from either acetate or carbohydrate involves the action of acetyl-CoA synthetase (EC 6.2.1.1) or ATP citrate lyase (EC 4.1.3.8) respectively (Kornacker & Lowenstein, 1965), the activities of these enzymes have been measured in the liver, kidney cortex and perirenal adipose tissues of the lamb at three stages of development: 140 days' gestational age and 1 day and 7 days after birth.

The activities of acetyl-CoA synthetase in liver, kidney and adipose tissue of mature foetal lambs were 1.76 ± 0.38 (mean $\pm$ S.E.M.), 2.60 ± 0.71 and 3.25 ± 0.48 units respectively (1 unit of activity is defined as 1 nmole of acetyl-CoA formed/mg. of protein/min. at 37°). At 1 day after birth there was a significant decline in the activity of this enzyme in all three tissues to values of 1.02 ± 0.12 , 1.44 ± 0.16 and 0.81 ± 0.05 units respectively. Activities of the enzyme measured at 7 days of age did not differ significantly from those measured immediately after birth.

The activities of ATP citrate lyase in liver, kidney and adipose tissue also decreased after birth. Values observed before birth were 2.79 ± 0.69 , 3.24 ± 0.60 and 2.26 ± 0.46 units respectively in the three tissues studied (1 unit of activity is defined as 1 nmole of acetyl Co-A formed/mg. of protein/min. at 37°). These fell to values of 0.95 ± 0.19 , 1.49 ± 0.19 and 0.42 ± 0.05 units respectively at 1 day of age. Again, no further changes were observed after 7 days.

These findings indicate that both acetate and citrate are possible precursors of long-chain fatty acids in the foetal lamb, and that the capacity of the tissues investigated to convert these substrates into acetyl-CoA is lower in the newborn lamb than in the mature foetus.

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Catalysis of Transamination by Acetate

By S. VAN HEYNINGEN* and H. B. F. DIXON (*Department of Biochemistry, University of Cambridge, CB2 1QW*)

Banks, Diamantis & Vernon (1961) found that the rate of transamination between pyridoxal and amino acids increased with buffer concentration; Bruce & Topping (1963*a,b*) showed that there was general acid- and base-catalysed transamination between amino and oxo acids. They suggested that the base removed the proton shown in the structure

Braunstein & Shemyakin, 1953; Metzler, Ikawa & Snell, 1954). They did not extend their conclusions to transaminations catalysed by metal ions because aluminium salts did not accelerate their reaction. Dixon (1968), found, however, that transamination between alanine and pyridoxal was catalysed by pyridine or imidazole and cupric salt much more than by either base or salt alone. The effect of pyridine (Mix & Wilcke, 1960) on transamination with copper salts between amino and oxo acids may therefore be another example of such a basic catalysis, and acetate similarly catalyses transamination between glyoxylate and proteins (Dixon, 1967). It was therefore decided to test whether acetate would accelerate the metal-ion-catalysed transamination between pyridoxal and amino acids.

Alanine (10mM) was incubated with 10mM-pyridoxal and 1mM-CuSO₄ at 25° in 2M-sodium acetate-0.4M-acetic acid medium, pH 5.4. Samples (0.1ml.) were withdrawn at intervals and assayed for oxo acids by reaction with 2,4-dinitrophenylhydrazine (Dixon, 1964).

The reaction was slow and exhibited a lag phase, not surprising in view of the complex series of reactions involved in forming the chelate of the aldimine. 5-Deoxy-pyridoxal, which exists as a free aldehyde rather than a hemiacetal, formed pyruvate without a lag at 1.4mmhr.⁻¹. In an attempt to form the aldimine of pyridoxal before the start of the reaction, CuSO₄ was added after pyridoxal, alanine and buffer, but the lag persisted. Dr D. E. Metzler then pointed out that in the absence of metal ions much aldimine would form only at alkaline pH. Alanine (0.1M) was therefore incubated with 0.1M-pyridoxal hydrochloride and 0.2M-NaOH, pH 9, after 5 min. an equal volume of 10mM-CuSO₄ was added and after a further 5 min. the mixture was diluted (five-fold) with buffer to the final composition. The initial rates of pyruvate production (mmhr.⁻¹) were: complete system, 0.60; buffer

* Present address: Department of Chemistry, Northwestern University, Evanston, Ill. 60201, U.S.A.