normally detectable until birth, cannot be stimulated by thyroxine *in utero* (Greengard, 1969b). The action of thyroxine may therefore be on the general rate of protein synthesis at this time rather than on specific enzyme induction.

The response of mitochondrial L-alanine-glyoxylate aminotransferase activity to glucagon has disappeared by the second postnatal day (when normal activity exceeds the adult value), but cortisol is still able to enhance the cytosol activity significantly up to the fifth postnatal day. The further increase in the cytosol activity that normally commences at about 15 days (when weaning begins) could not be prematurely induced by cortisol at 11 days; in contrast L-alanine-2-oxoglutarate aminotransferase (which shows a similar natural developmental pattern) could be induced at both 5 and 11 days by cortisol. The cytosol L-alanine-glyoxylate aminotransferase activity was increased precociously at 12 days by cortisol treatment of rats that had been intubated intragastrically with casein (but not by casein alone); casein intubation also enhanced the response of L-alanine-2-oxoglutarate aminotransferase to cortisol. The results indicate the importance of both hormonal and dietary factors in regulating enzyme activity around the time of weaning, as has previously been shown for other enzymes (Walker & Eaton, 1967; Vernon & Walker, 1968; Lockwood, Bailey & Taylor, 1970).

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The Existence of Oxoacyl-Coenzyme A Thiolases of Differing Substrate Specificity and Intracellular Localization in Mammalian Tissues

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Three different oxoacyl-CoA thiolases have been isolated from rat and ox liver. Isoelectric focusing of liver cytoplasm shows that the thiolase present in this fraction has pI values 4.8 (rat) and 5.2 (ox).

This cytoplasmic thiolase is absolutely specific for acetoacetyl-CoA.

Both rat and ox liver mitochondria also contain an acetoacetyl-CoA-specific thiolase. The pI values are 8.4 and 7.8 for the rat and the ox enzyme respectively. The mitochondrial acetoacetyl-CoA thiolase also differs from the cytoplasmic enzyme in having a tenfold-lower K_m for acetoacetyl-CoA and by being inhibited by excess of this substrate. Unlike the cytoplasmic enzyme the mitochondrial acetoacetyl-CoA thiolase is greatly stimulated by K^+ or NH_4^+ ions. Na^+ ions have no effect. The K_a value for K^+ is 2.3 mM for the ox liver mitochondrial enzyme.

Liver mitochondria also contain another oxoacyl-CoA thiolase. This has a wider specificity and works with 3-oxohexanoyl-CoA, 3-oxodecanoyl-CoA and acetoacetyl-CoA. The maximum velocity with 3-oxohexanoyl-CoA is fivefold higher than with acetoacetyl-CoA. The wide specificity of this enzyme and its intracellular location suggest identity with the general oxoacyl-CoA thiolase purified from ox liver by Seubert, Lamberts, Kramer & Ohly (1968). This enzyme may exist as a family of isoenzymes. Thus isoelectric focusing of ox or rat liver mitochondrial extracts consistently gives two peaks with general oxoacyl-CoA thiolase activity. Further purification of the material of these peaks shows that they have identical substrate-specificity patterns. The two major forms of the general oxoacyl-CoA thiolase of ox mitochondria have pI values 5.7 and 6.7.

Chromatography on DEAE-cellulose will separate the cytoplasmic thiolase from the two mitochondrial enzymes. This technique, together with the fact that only the general mitochondrial oxoacyl-CoA thiolase can utilize 3-oxohexanoyl-CoA, has been used to determine the tissue distribution of these enzymes in the rat. All three thiolases are widely distributed. The two mitochondrial enzymes have comparable activity in many tissues, and the cytoplasmic thiolase is usually the least active of the three. These findings show that the use of acetoacetyl-CoA alone in assays for acetoacetyl-CoA thiolase in tissue extracts will give misleading results.

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