Short Communications

Fatty Acid Synthesis in the Brown Fat and Liver of Foetal and Newborn Rabbits

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(Received 7 February 1973)

The postnatal fall in fatty acid synthesis in the liver and brown fat of newborn rabbits is accompanied in both tissues by a decrease in the capacities of the enzymes of fatty acid synthesis and an apparent increase in the degree of inhibition of one or more of these enzymes.

Although foetal rat liver has a high capacity for synthesizing fatty acids from glucose (Ballard & Hanson, 1967), the livers of newborn rats (Ballard & Hanson, 1967) and the brown fat of newborn rabbits (Knight & Myant, 1970) incorporate only small amounts of glucose into fatty acids. Acetate is also incorporated only to a small extent in the brown fat (Hahn *et al.*, 1968) and liver (Ballard & Hanson, 1967) of newborn rats and in the liver of newborn mice (Smith & Abraham, 1970). These observations raise the question as to how far the developmental changes that occur in fatty acid synthesis after birth are caused by changes in the capacities of the relevant enzymes. Hahn & Drahota (1966) have shown that the activity of citrate lyase (EC 4.1.3.6) in liver and in white fat and brown fat of rats falls markedly after birth. However, in the livers of newborn rats the incorporation of glucose and acetate into fatty acids falls more rapidly than does the activity of citrate lyase (Ballard & Hanson, 1967). Hence it is difficult to explain the postnatal fall in fatty acid synthesis by changes in citrate lyase activity. In the present work the activities of citrate lyase, acetyl-CoA synthetase (EC 6.2.1.1), acetyl-CoA carboxylase (EC 6.4.1.2) and fatty acid synthetase have been measured in the brown fat and liver of foetal and newborn rabbits. The incorporation of glucose into fatty acids by both tissues was measured in parallel with the determinations of enzyme activity.

Methods

Rabbits of the Dutch strain, maintained on Dixon's diet no. 18, were used for all experiments. Foetuses were removed from pregnant rabbits on the 27th day after mating. Newborn rabbits were used when 2–4 days old. For experiments on whole tissue, incubation flasks contained 3 ml of Krebs–Ringer bicarbonate buffer, pH 7.4 (Umbreit *et al.*, 1964) (with half the recommended concentration of Ca^{2+}), containing 10 mM-glucose (a concentration sufficient for maximal

incorporation of glucose into fatty acids), [$\text{U-}^{14}\text{C}$]-glucose (1 $\mu\text{Ci}/\text{flask}$) and between 100 and 300 mg of liver slices or of pieces of brown fat (3–5 mg each). Flasks were gassed with $\text{O}_2 + \text{CO}_2$ (95:5) and then incubated for 2 h at 37°C with continuous shaking. Under these conditions incorporation was linear for 2 h and results from duplicate incubations agreed to within 5% of the mean. Fatty acids were extracted at the end of the incubation and prepared for assay of radioactivity as described previously (Iliffe & Myant, 1964). For the enzyme assays, a soluble tissue fraction was prepared from a mince of each tissue suspended in homogenizing fluid (250 mM-sucrose–1 mM-EDTA, pH 7.4) in the ratio 1:5 (w/v). The suspension was homogenized in a Potter–Elvehjem homogenizer and the homogenate was spun at 700g for 10 min. The supernatant was withdrawn and spun at 100000g for 1 h. The supernatant from this centrifugation was the soluble fraction used for the enzyme assays. Acetyl-CoA carboxylase activity was assayed by measuring the incorporation of ^{14}C from [^{14}C]bicarbonate into acid-stable products (Smith *et al.*, 1966). Fatty acid synthetase was assayed by measuring the incorporation of ^{14}C from [^{14}C]malonyl-CoA into fatty acids (Lynen, 1962). In each assay, cofactor concentrations were optimal for the particular enzyme. The time-course of each reaction was linear and enzyme activity was proportional to protein concentration. Protein was determined by the method of Lowry *et al.* (1951), with crystalline bovine albumin as standard. Crude homogenate samples were hydrolysed and extracted with light petroleum (b.p. 40–60°C) before the protein was determined.

Results and discussion

The incorporation of ^{14}C from [^{14}C]glucose into fatty acids was determined in whole tissue obtained from the pooled tissues of a single litter of three to six animals. Incorporation into liver fatty acids, expressed in nmol/h per 100 mg of mince protein

Table 1. *Activities of enzymes of fatty acid synthesis in brown fat and liver of foetal and newborn rabbits*

Enzyme activities are expressed as mean values $\pm$ S.E.M. of three or four determinations. Each determination was carried out on the soluble fraction obtained from the combined tissues from a single litter of three to six animals. Experimental details are given in the text. For each enzyme and for each tissue the difference between the mean values for foetal and newborn animals was statistically significant ($P < 0.05$ by Student's *t* test).

	Activity (nmol of substrate converted into product/h per mg of soluble protein)			
	Brown fat		Liver	
	Foetal	Newborn	Foetal	Newborn
Citrate lyase	3546 $\pm$ 399	1690 $\pm$ 327	918 $\pm$ 76	231 $\pm$ 30
Acetyl-CoA synthetase	1173 $\pm$ 190	279 $\pm$ 48	935 $\pm$ 34	135 $\pm$ 19
Acetyl-CoA carboxylase	161 $\pm$ 27	43.5 $\pm$ 0.8	62 $\pm$ 13	28.6 $\pm$ 2.9
Fatty acid synthetase	837 $\pm$ 19	93 $\pm$ 19	301 $\pm$ 36	65 $\pm$ 19

(means $\pm$ S.E.M. of determinations on three pools of tissue) fell from 16.4 ± 1.0 in foetuses to 2.8 ± 1.3 in newborn animals. Incorporation into fatty acids of brown fat fell from 479 ± 73 to 38.4 ± 3.5 during the same period of development. Thus the conversion of glucose into fatty acids in brown fat was much greater than that in liver, in both foetuses and newborn animals.

Table 1 shows the activities of the enzymes of fatty acid synthesis in brown fat and liver of foetal and newborn rabbits. At both stages of development the activity of each enzyme was greater in brown fat than in liver, and in both tissues the activity of acetyl-CoA carboxylase was lower than those of the other three enzymes. In both tissues the activities of all four enzymes fell after birth, the percentage fall being greatest for fatty acid synthetase in brown fat and least for citrate lyase in brown fat. Thus, as in the adaptive response to weaning from a milk diet, when there is an increase in the activities of several hepatic enzymes related to fatty acid synthesis (Smith & Abraham, 1970), decreased fatty acid synthesis in the early postnatal period is accompanied by a fall in the activities of at least four of the relevant enzymes in brown fat and liver.

Kornacker & Lowenstein (1965) have suggested that in some instances the adaptive behaviour of citrate lyase alone is sufficient to account for changes in the rate of fatty acid synthesis. However, except in foetal liver, the activity of citrate lyase was greater than those of the other enzymes in both tissues at both stages of development. Hence the postnatal fall in the activity of this enzyme is unlikely to be the cause of the fall in fatty acid synthesis.

Although there was a substantial fall in the activities of acetyl-CoA synthetase and fatty acid synthetase after birth, the activity of each of these enzymes was higher than that of acetyl-CoA carboxylase in both tissues of foetal and newborn animals. It

is therefore unlikely that a fall in activity of either enzyme is responsible for the very low rates of fatty acid synthesis from acetate and from glucose that are observed in the tissues of newborn animals.

Acetyl-CoA carboxylase had the lowest activity of the four enzymes in both tissues at both stages of development. Enzyme activities were measured *in vitro* in the presence of cofactors, including citrate, at optimal concentrations. Each assay therefore provides an estimate of the maximal capacity of the enzyme when fully activated. It follows that, if cofactor concentrations were optimal and if acetyl-CoA carboxylase were fully activated in the intact preparations used in the present work, the postnatal fall in the maximal capacity of this enzyme would be sufficient to explain the fall in fatty acid synthesis. However, our observations cannot be explained solely in terms of changes in enzyme capacity. Thus in brown fat the activity of acetyl-CoA carboxylase (expressed in μ g-atoms of substrate C converted/h per 100mg of mince protein) was 7.26 in foetuses and 2.38 in newborn animals, whereas the corresponding values for incorporation of ^{14}C from [^{14}C]glucose into fatty acids (expressed in μ g-atoms of C/h per 100mg of mince protein) were only 2.87 and 0.23. Fatty acid synthesis in whole tissues may be underestimated if it is calculated from the incorporation of label from a radioactive precursor, since radioactive intermediates may be diluted with carbon from endogenous sources. Nevertheless the marked discrepancy between enzyme capacities and incorporation of ^{14}C from [^{14}C]glucose into fatty acids suggests that, in intact cells, some or all of the fatty acid-synthesizing enzymes utilize only a proportion of their total capacity and that the proportion utilized decreases immediately after birth. Hence, although the postnatal fall in fatty acid synthesis may be due partly to decreased capacities of the relevant enzymes, particularly of acetyl-CoA carboxylase, other

factors may be of equal importance. These factors might include, for example, increasing limitation of cofactors and an increase in the local concentrations of inhibitors, such as long-chain acyl-CoA.

We thank Mrs. Rosemary A. Fordham for technical help.

Ballard, F. J. & Hanson, R. W. (1967) *Biochem. J.* **102**, 952–958

Hahn, P. & Drahota, Z. (1966) *Experientia* **22**, 706–958

Hahn, P., Greenberg, R., Dobiášová, H. & Drahota, Z. (1968) *Can. J. Biochem.* **46**, 735–741

Illiffe, J. & Myant, N. B. (1964) *Biochem. J.* **91**, 369–377

Knight, B. L. & Myant, N. B. (1970) *Biochem. J.* **119**, 103–111

Kornacker, M. S. & Lowenstein, J. M. (1965) *Biochem. J.* **94**, 209–215

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Lynen, F. (1962) *Methods Enzymol.* **5**, 443–451

Smith, S. & Abraham, S. (1970) *Arch. Biochem. Biophys.* **136**, 112–121

Smith, S., Easter, D. J. & Dils, R. (1966) *Biochim. Biophys. Acta* **125**, 445–455

Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1964) *Manometric Techniques*, 4th edn., p. 132, Burgess Publishing Co., Minneapolis