The Pattern of Fatty Acid Synthesis in Lactating Rabbit Mammary Gland Studied *in vivo*

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The biosynthesis of fatty acids has been studied in lactating rabbits at 6h after intravenous injection of sodium [1-¹⁴C]acetate. The specific radioactivities of the individual fatty acids (C_{6:0} to C_{14:0}) and the proportions of these fatty acids synthesized were similar in mammary tissue and milk. Hexanoic acid had the highest specific radioactivity, and the C_{8:0}–C_{14:0} fatty acids had similar specific radioactivities, which were about five times those of C₁₆ and C₁₈ acids. No radioactivity was detected in fatty acids of chain length <C₁₄ in the liver, blood or adipose tissue and the specific radioactivities of fatty acids of chain length >C₁₄ in these tissues were similar to those of the long-chain fatty acids in the milk and mammary gland. The results show that the C_{4:0}–C_{14:0} fatty acids are synthesized within the mammary gland rather than by fatty acid uptake from circulating blood or by oxidation of long-chain fatty acids within the gland. We conclude that *de novo* synthesis of esterified fatty acids *in vivo* by this tissue has a high degree of chain-length specificity.

Fatty acids of medium chain length (C_{8:0} and C_{10:0}) account for about 70% of the fatty acids in rabbit milk triglycerides (Smith *et al.*, 1968). Popják *et al.* (1953) found that after intravenous injection of sodium [2-¹⁴C]acetate into lactating rabbits, the steam-volatile fatty acids of the milk glycerides had a higher specific radioactivity than the non-volatile fatty acids of the milk or liver glycerides. The synthesis *in vivo* of esterified fatty acids within the lactating rabbit mammary gland appears therefore to be highly specific with respect to fatty acid chain length.

We have investigated in more detail this specificity by using radio-g.l.c. to determine the specific radioactivities of the individual fatty acids of chain lengths C₄–C₁₈ in milk and tissue glycerides after injection of sodium [1-¹⁴C]acetate.

Materials and Methods

New Zealand White Rabbits (albinos) were obtained from the Joint Animal Breeding Unit, Sutton Bonington, Leics., U.K. Sodium [1-¹⁴C]acetate was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Radioactivity was determined by liquid-scintillation counting (Smith *et al.*, 1966).

Sodium [1-¹⁴C]acetate (200 µCi, as a 20mm soln.) was injected into the ear vein of a lactating rabbit at 10 days *post partum* (rabbit 1). A second lactating rabbit was similarly injected with sodium [1-¹⁴C]acetate (500 µCi, as a 20mm soln.) at 14 days *post*

partum (rabbit 2). Offspring were kept with the mother and both rabbits were killed after 6h by cervical fracture. Blood was removed from the jugular vein and plasma prepared. Mammary tissue with attached skin was rapidly excised and milk (about 25ml) obtained after removal of the nipples. The skin was then removed and the mammary tissue finely chopped, filtered through gauze and excess of milk removed by thorough washing. The livers and portions of white subcutaneous adipose tissue from the dorsal area were quickly removed. Lipids were extracted from all samples by the method of Folch *et al.* (1957). A portion of each lipid extract was saponified, acidified and the fatty acids were extracted into light petroleum (b.p. 40–60°C). Samples of whole blood were saponified directly (i.e. without chloroform–methanol extraction), acidified and the fatty acids extracted similarly. The specific radioactivity (nCi/mg dry wt.) of each total fatty acid extract was measured and the specific radioactivities of the individual fatty acids were determined by radio-g.l.c. of either the fatty acids themselves (Carey & Dils, 1970) or of their methyl esters (Smith & Dils, 1965). Since the specific radioactivities of individual unsaturated fatty acids were too low to be determined with accuracy, the overall specific radioactivities of the C₁₆ and C₁₈ fatty acids are quoted (Table 1).

Results

The incorporation *in vivo* of sodium [1-¹⁴C]acetate into the individual fatty acids of tissues of lactating rabbits is shown in Table 1. At 6h after the injection

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Table 1. *Synthesis of fatty acids from sodium [1-¹⁴C]acetate by the lactating rabbit*

Sodium [1-¹⁴C]acetate (200 μ Ci, as a 20 mM soln.) was injected into the ear vein of a lactating rabbit at 10 days *post partum* (rabbit 1). A second lactating rabbit was injected with sodium [1-¹⁴C]acetate (500 μ Ci, as a 20 mM soln.) at 14 days *post partum* (rabbit 2). Both rabbits were killed after 6 h and lipid was immediately extracted from tissue samples. The molar and radioactivity percentages of individual fatty acids are the mean values for the two rabbits $\pm$ half the range between the two values. To compare the values for the specific radioactivities of individual fatty acids obtained from the same tissues of rabbits 1 and 2, the overall specific radioactivities of the total fatty acid extracts from rabbit 1 were multiplied by 2.5 (see the Results section). The mean values $\pm$ half the range between the two values for the specific radioactivities (nCi/mg) of individual fatty acids are presented. Only the adipose tissue of rabbit 2 was analysed.

Tissue	Sp. radioactivity of total fatty acid extract (nCi/mg)			Individual fatty acids													
	Rabbit 1	Rabbit 2	Fatty acid	C _{4:0}	C _{6:0}	C _{8:0}	C _{10:0}	C _{12:0}	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}		
Milk	0.5	1.4	Mole %	—	9 $\pm$ 5	44 $\pm$ 6	30 $\pm$ 2	2 $\pm$ 1	1 $\pm$ 0	8 $\pm$ 1	1 $\pm$ 0	1 $\pm$ 1	6 $\pm$ 3	5 $\pm$ 2	1 $\pm$ 1		
			Radioactivity (%)	1 $\pm$ 1	6 $\pm$ 3	51 $\pm$ 4	34 $\pm$ 4	3 $\pm$ 2	2 $\pm$ 1	3 $\pm$ 2	—	3 $\pm$ 0	—	—	—		
Mammary gland	0.4	1.3	Sp. radioactivity	—	4.2 $\pm$ 0.8	1.7 $\pm$ 0.1	1.5 $\pm$ 0.1	1.3 $\pm$ 0.1	1.2 $\pm$ 0.4	0.3 $\pm$ 0.1	—	—	0.2 $\pm$ 0.1	—	—		
			Mole %	—	1 $\pm$ 0	40 $\pm$ 6	26 $\pm$ 2	2 $\pm$ 1	2 $\pm$ 1	11 $\pm$ 1	2 $\pm$ 1	1 $\pm$ 0	9 $\pm$ 3	7 $\pm$ 1	2 $\pm$ 1		
Plasma	0.05	0.10	Radioactivity (%)	1 $\pm$ 1	3 $\pm$ 1	51 $\pm$ 3	36 $\pm$ 3	2 $\pm$ 1	2 $\pm$ 1	2 $\pm$ 0	—	3 $\pm$ 1	—	—	—		
			Sp. radioactivity	—	4.6 $\pm$ 1.0	1.9 $\pm$ 0.1	1.8 $\pm$ 0.1	1.4 $\pm$ 0.4	1.0 $\pm$ 1.3	0.2 $\pm$ 0.1	—	—	0.2 $\pm$ 0.1	—	—		
Liver	0.05	0.10	Mole %	—	—	—	—	—	4 $\pm$ 1	30 $\pm$ 3	5 $\pm$ 1	8 $\pm$ 4	26 $\pm$ 4	23 $\pm$ 4	4 $\pm$ 1		
			Radioactivity (%)	—	—	—	—	—	10 $\pm$ 1	56 $\pm$ 11	—	34 $\pm$ 9	—	—	—		
Adipose tissue	0	0	Sp. radioactivity	—	—	—	—	—	0.2 $\pm$ 0.1	0.3 $\pm$ 0.2	—	9 $\pm$ 1	15 $\pm$ 5	14 $\pm$ 9	4 $\pm$ 1		
			Mole %	—	—	—	—	—	1 $\pm$ 1	46 $\pm$ 8	12 $\pm$ 3	41 $\pm$ 11	—	—	—		
Adipose tissue	0	0	Radioactivity (%)	—	—	—	—	—	1 $\pm$ 0	58 $\pm$ 12	—	—	—	—	—		
			Sp. radioactivity	—	—	—	—	—	—	0.2 $\pm$ 0.1	—	—	0.1 $\pm$ 0	—	—		
Adipose tissue	0	0	Mole %	—	—	—	—	—	3	35	4	7	24	26	1		
			Radioactivity (%)	—	—	—	—	—	—	—	—	—	—	—	—		
Adipose tissue	0	0	Sp. radioactivity	—	—	—	—	—	—	—	—	—	—	—	—		
			Sp. radioactivity	—	—	—	—	—	—	—	—	—	—	—	—		

of the label, the specific radioactivities of the total fatty acid extracts of milk and mammary glands from the two rabbits were approximately proportional to the radioactivity of the acetate injected. This indicates that the amount of injected acetate was negligible compared with the body pool of acetate and that the pool size was similar in the two animals. Since $200\mu\text{Ci}$ of sodium $[1-^{14}\text{C}]$ acetate was injected into rabbit 1 and $500\mu\text{Ci}$ into rabbit 2, by multiplying the overall specific radioactivities of the total fatty acid extracts from rabbit 1 by 2.5, the specific radioactivities of the individual fatty acids in these extracts were compared with those from the corresponding tissues of rabbit 2.

With both animals, the molar proportions of fatty acids in the mammary tissue and milk were similar; the percentage of acetate incorporated into the individual fatty acids and therefore the specific radioactivities of the individual fatty acids were also similar in mammary tissue and milk. No radioactivity or mass peak corresponding to butyric acid was detected by g.l.c. analysis of the milk or mammary tissue from one of the rabbits, and only a very small peak of radioactivity (but no corresponding mass peak) was observed during analysis of these tissues from the second rabbit. Some 85–87% of the acetate incorporated into milk or mammary-gland fatty acids was recovered in $\text{C}_{8:0}$ plus $\text{C}_{10:0}$ fatty acids and only a very small proportion was recovered in saturated or unsaturated long-chain fatty acids. Hexanoic acid had the highest specific radioactivity of the fatty acids synthesized. The $\text{C}_{8:0}$ – $\text{C}_{14:0}$ fatty acids had similar specific radioactivities, which were about five times those of C_{16} and C_{18} acids. In contrast, no radioactivity and less than 1 mole % of mass was detected in the short- and medium-chain ($<\text{C}_{14}$) fatty acids of the liver, whole blood, plasma or adipose tissue samples analysed. The specific radioactivities of the long-chain ($>\text{C}_{14}$) fatty acids in these samples were similar to those of the long-chain fatty acids in the milk and mammary-gland tissue.

Discussion

Our finding that the medium-chain fatty acids had higher specific radioactivities than the long-chain fatty acids (Table 1) supports the earlier findings of Popják *et al.* (1953) that the steam-volatile fatty acids of rabbit milk triglycerides are synthesized within the mammary gland from acetate rather than by oxidation of long-chain fatty acids within the gland

or by fatty acid uptake from circulating blood. In addition, radio-g.l.c. has enabled us to show that the pattern of individual $\text{C}_{4:0}$ to $\text{C}_{14:0}$ fatty acids synthesized within the gland corresponds closely to the molar percentage found in rabbit milk. Bu'Lock & Smith (1965) administered ^{14}C -labelled stearate, palmitate and decanoate orally to lactating rabbits and compared the incorporation of the label into the carbon atoms of the mammary-gland triglyceride fatty acids. They concluded that a proportion of the administered fatty acids was oxidized to C_2 units and reutilized for fatty acid synthesis, and that fatty acids of chain length $\text{C}_{4:0}$ – $\text{C}_{10:0}$ were exclusively synthesized *de novo*, whereas chain elongation of fatty acids of chain lengths greater than $\text{C}_{10:0}$ was observed. The striking decrease we found between $\text{C}_{10:0}$ and $\text{C}_{12:0}$ fatty acids in both the molar proportions of these fatty acids in the mammary tissue and milk and in the percentage of radioactivity incorporated into these fatty acids (Table 1) corresponds to the break in chain-length specificity of the two biosynthetic pathways observed by Bu'Lock & Smith (1965). Since $\text{C}_{8:0}$ plus $\text{C}_{10:0}$ fatty acids comprise 88 ± 2 mol/100 mol of the fatty acids synthesized within the gland (Table 1), the synthesis of these medium-chain fatty acids within the gland must be a highly specific process. This specificity for the chain length of fatty acids synthesized was not observed with cell-free extracts (Smith & Dils, 1964, 1966) or purified fatty acid synthetase (Carey & Dils, 1970) from this tissue. This implies that a degree of cellular organization is required for the specific synthesis of esterified medium-chain fatty acids.

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