PN 1306

Biosynthesis of butyric and longer-chain fatty acids in the lactating rabbit mammary gland

In the last few years overwhelming evidence has accumulated demonstrating malonyl-CoA as an obligatory intermediate in the synthesis of long-chain fatty acids from acetyl-CoA by enzyme preparations from various animal tissues and microorganisms[1,2]. The purified fatty acid-synthesizing enzyme preparations were found to be ineffective in utilizing substituted acyl-CoA derivatives such as β-keto-, β-hydroxy-, and α,β-unsaturated acyl-CoA, for the synthesis of long-chain fatty acids[1,2]. However, recently partially purified enzyme preparations from rat brain and adipose tissue were shown to reduce acetoacetyl-CoA, β-hydroxybutyryl-CoA and crotonyl-CoA to butyrate, which was further elongated to palmitate by subsequent condensation with malonyl-CoA[3,4].

Synthesis of fatty acids from acetate by cell-free preparations from lactating mammary glands has been reported[5,6] and there are data to suggest the implication of malonyl-CoA in this process[7,8]. However, the utilization of acetoacetate, β-hydroxybutyrate or butyrate for the synthesis of fatty acids by this tissue preparation has not yet been demonstrated.

In the present study incorporation of [1-^{14}C]acetate, [3-^{14}C]acetoacetate, DL-β-hydroxy-[3-^{14}C]butyrate and [1-^{14}C]butyrate into fatty acids by particle-free supernatant (hereafter referred to as supernatant) from lactating rabbit mammary gland has been demonstrated. The supernatant fraction was found to be more active than either total homogenate or mitochondria and microsomes singly or in combination. Hence, only the supernatant was used in all these experiments.

The synthesis of the fatty acids of six or more carbon atoms from β-hydroxybutyrate was significantly stimulated by the preincubation of the supernatant with citrate (Table I). Also, avidin was found to suppress markedly the synthesis of these acids from β-hydroxybutyrate as well as acetate, acetoacetate and butyrate (Table II). However, neither of these treatments had any significant effect on the synthesis of butyric acid.

TABLE I

EFFECT OF PREINCUBATION OF LACTATING RABBIT MAMMARY GLAND SUPERNATANT WITH CITRATE ON THE INCORPORATION OF DL-β-HYDROXY-[3-^{14}C]BUTYRATE INTO BUTYRIC AND LONGER-CHAIN FATTY ACIDS

The tissue was homogenized in 0.25 M sucrose solution containing 0.01 M β-mercaptoethanol (1 g tissue: 3 ml of homogenizing medium). 1 ml of particle-free 80000 × *g* supernatant preincubated with 50 μmoles of potassium citrate was incubated for 2 h at 37.5° with 240 μmoles glycylglycine buffer (pH 7.2), 70 μmoles $MgCl_2$, 2 μmoles $MnCl_2$, 10 μmoles $KHCO_3$, 0.13 μmole CoA, 0.25 μmole NADP, 0.25 μmole NAD^+, 50 μmoles nicotinamide, 10 μmoles ATP, 0.1 μmole β-mercaptoethanol and 7.25 μmoles DL-β-hydroxy-[3-^{14}C]butyrate ($6.23 \cdot 10^4$ counts/sec) in a total volume of 3.5 ml. The total fatty acids were isolated and fractionated into butyric and longer-chain acids by silica gel chromatography[9].

Period of preincubation of supernatant with citrate (min)	*Per cent incorporation*	
	Butyric acid	*C_6 and longer acids*
—	0.86	1.79
15	0.79	2.84
30	0.80	2.82
45	0.76	2.80

The stimulation and inhibition of the synthesis of long-chain fatty acids by citrate and avidin, respectively, are mediated through their effect on acetyl-CoA carboxylase, the enzyme which catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, a rate-limiting step in the biosynthesis of long-chain fatty acids[7,10]. Citrate is known to activate[11], and avidin to inhibit[12] acetyl-CoA carboxylase reaction and hence, malonyl-CoA formation.

TABLE II

EFFECT OF AVIDIN ON THE SYNTHESIS OF BUTYRIC AND LONGER-CHAIN FATTY ACIDS BY LACTATING RABBIT MAMMARY GLAND SUPERNATANT

The experimental conditions were the same as described in Table I. The supernatant was pre-incubated with citrate for 30 min. The substrate was 5 μmoles [1-^{14}C]acetate ($8.50 \cdot 10^4$ counts/sec), or 29 μmoles [3-^{14}C]acetoacetate ($4.10 \cdot 10^4$ counts/sec), or 7.25 μmoles DL-β-hydroxy-[3-^{14}C]-butyrate ($6.23 \cdot 10^4$ counts/sec), or 2.70 μmoles [1-^{14}C]butyrate ($3.97 \cdot 10^4$ counts/sec).

Supernatant preparation	*Substrate*	*Additions*		*Per cent incorporation*	
		Avidin (mg)	*Biotin (mg)*	*Butyric acid*	*Longer-chain (C_4 and above) fatty acids*
Fresh	DL-β-Hydroxy-[3-^{14}C]butyrate	—	—	0.79	2.84
Fresh	DL-β-Hydroxy-[3-^{14}C]butyrate	1.6	—	0.80	0.08
Fresh	DL-β-Hydroxy-[3-^{14}C]butyrate	1.6	1.2	0.74	2.11
Stored*	[1-^{14}C]Acetate	—	—	3.11	11.42
Stored*	[1-^{14}C]Acetate	1.6	—	2.25	1.35
Stored*	[1-^{14}C]Acetate	1.6	1.2	2.83	14.23
Stored*	[3-^{14}C]Acetoacetate	—	—	0.68	2.62
Stored*	[3-^{14}C]Acetoacetate	1.6	—	0.62	0.40
Stored*	[3-^{14}C]Acetoacetate	1.6	1.2	0.80	5.58
Stored*	[1-^{14}C]Butyrate	—	—	—	4.18
Stored*	[1-^{14}C]Butyrate	1.6	—	—	1.05
Stored*	[1-^{14}C]Butyrate	1.6	1.2	—	5.18

* These preparations had been stored at $-10°$ for 3 days. It has been invariably observed that the addition of biotin to the stored preparation resulted in considerable stimulation of long-chain fatty acid synthesis, whereas it had no effect on the fresh preparation.

It seems reasonable to conclude, therefore, that the syntheses of butyric and longer-chain fatty acids in the lactating mammary gland of rabbits follow different biosynthetic pathways. The fatty acids other than butyric are, perhaps, synthesized by a pathway where malonyl-CoA is an obligatory intermediate. Butyric acid, on the other hand, appears to be synthesized by the direct condensation of acetyl-CoA units, which is independent of the acetyl-CoA carboxylase reaction. A similar scheme for the synthesis of butyric acid in *Clostridium kluyveri* has been suggested[13].

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Changes in the occurrence of different lipid classes during postnatal development of the rat

It has been pointed out previously (HAHN *et al.*[1], KOLDOVSKÝ *et al.*[2]) that milk is a high-fat diet. It appears that the suckling mammal is well adjusted to such a diet, since fat utilization is high (HAHN AND KOLDOVSKÝ[3]) and fat absorption and transport also differ from the same processes in adult animals (KOLDOVSKÝ *et al.*[4]). There are some indications that the lipid composition of some organs also changes during development (YARBRO AND ANDERSON[5], NOVÁK *et al.*[6]).

In the present work the lipid composition of the livers, lungs, brown interscapular fat and small intestine of rats aged 1 and 10 days postnatally and in adult animals was studied. Thin-layer chromatography on silica gel G (PEIFER[7]) was used. Infant rats were taken directly from the mother rat and adult animals were in the fed state.

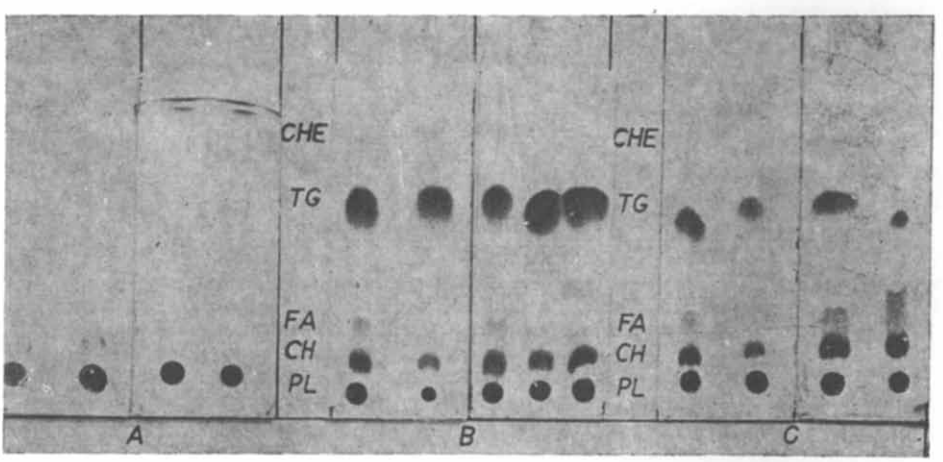

Fig. 1. Thin-layer chromatograms showing the distribution of lipids in the rat intestine. A, 1-day-old rats; B, 10-day-old rats; C, adult rats (200 g females). For 1-day-old rats 3–4 animals and for 10-day-old rats 2 animals were pooled for 1 determination. Abbreviations: PL, phospholipids; Ch, cholesterol; FA, free fatty acids; TG, triglyceride; ChE, cholesterol esters.