PATHWAYS OF FATTY ACID BIOSYNTHESIS IN THE RABBIT MAMMARY GLAND*

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It is now generally agreed that long-chain fatty acid biosynthesis in the organism is being achieved predominantly through the malonyl-CoA pathway (Brady, 1958; Wakil, 1959). Whether short-chain representatives are synthesized by the same process has not yet been clearly established. It would rather seem more probable, according to Goldman et al. (1963) and to Singh and Kumar (1963) that they could be formed by a mechanism which does not involve malonyl-CoA as an obligatory intermediate. The results obtained in the series of experiments to be briefly described thereafter seem to support the view that short and long-chain fatty acids are more likely to be synthesized by distinct enzymatic pathways.

EXPERIMENTAL WORK AND RESULTS

Mammary gland tissue extracts obtained from lactating rabbits have been prepared by a technique similar to the one described by Hele et al. (1957) and incubated in the presence of various cofactors and labelled substrates. Details pertaining to the incubation procedure appear in the tables summarizing the results. Fatty acids were isolated from the incubation medium by a technique derived from the one used by Hele et al. (1957) and analysed by gas-liquid radiochromatography.

The first series of experiments aimed at establishing the nature of biosynthesis products from acetate-1-C^{14} by fresh and aged preparations of mammary gland tissue. In the experiments done with freshly prepared extracts,

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the order of appearance of the various fatty acids formed was determined by taking samples from the incubation medium at the time intervals indicated in Table I.

TABLE I

FATTY ACID BIOSYNTHESIS FROM 1-C^{14}-ACETATE BY CELL-FREE EXTRACTS OF LACTATING RABBIT MAMMARY GLAND: NATURE OF THE REACTION PRODUCTS ACCORDING TO:

	a) The incubation time				b) The age of the extract	
Age of preparation, days	< 1	< 1	< 1	< 1	5	21
Time, minutes..	15	30	60	120	120	120
C^{14} incorporation, mμmoles..	58	90	239	444	280	204
Distribution						
C^{4}	26	25	47	127	32	20
$C^{4=}$	9	8	36	81	129	141
C^{6}	3	7	19	45	11	20
C^{8}	3	2	8	16	3	2
C^{10}	4	4	8	17	8	2
C^{12}	5	16	43	75	22	9
C^{14}	3	18	43	51	38	6
C^{16}	5	10	35	32	37	4

Incubation: 38°C, in air.
Medium: phosphate buffer 0.1M, pH 8.0: 100μM; $MgCl_2$: 20μM; cystein: 30μM; ATP: 30μM; nicotinamide: 3μM; CoASH: 2μM; NADH: 0.6μM; NADPH: 0.6μM; glucose-6-phosphate: 0.4μM; acetate-1-C^{14}: 10μM = 10μC; S-104,000xg: 1.0cc.
Final volume: 4.5 ml. Extract kept at -18°C during aging.

The results obtained with a freshly prepared extract indicate that butyric acid, which is the first component to accumulate in the incubation medium, is by far the predominant product of fatty acid biosynthesis from acetate. Higher saturated homologues with the exception of octanoic and decanoic acids, are also being synthesized in appreciable amount. Whether short-chain fatty acids appear to be the precursors of longer chain representatives cannot be stated from the above data.

Aging of the enzymatic preparation in the frozen state causes a gradual loss of its ability to synthesize fatty acids from acetate. The extract still retains 50% of its original activity at 21 days, but cannot anymore achieve to a very significant extent the synthesis of short- and long-chain saturated fatty acids. Crotonic acid appears then to be term of the lipogenetic processes. The same trend is already observable as early as five days

after the preparation of the extract; the synthesis of long-chain fatty acids is not however so drastically impaired at that time.

Such a failure of the extract to achieve the synthesis of saturated derivatives could be possibly attributed to the deterioration of the reducing enzyme of the fatty acid cycle (Seubert et al., 1957; Lachance et al., 1958). This hypothesis was checked by adding crotonyl reductase to a deteriorated fatty acid synthesizing system. The results obtained appear in Table II.

TABLE II

EFFECT OF CROTONYL REDUCTASE UPON FATTY ACID BIOSYNTHESIS FROM 1-C^{14}-ACETATE BY A PARTIALLY PURIFIED LIPOGENETIC SYSTEM (LS)

	LS alone		LS + crotonyl-reductase	
	NADH	NADPH	NADH	NADPH
C^4	1,742 cpm	1,927 cpm	25,940 cpm	64,130 cpm
$C^{4=}$	4,704 "	5,504 "	2,945 "	597* "
C^6	1,494 "	1,791 "	4,528 "	9,405 "
C^8	1,498 "	1,461 "	1,355 "	835 "
Total activity, cpm**				
Found	9,168	10,183	34,768	74,967
Expected	9,100	10,526	49,742	94,149

Incubation: 90 minutes in air at 37°C
Medium: tris buffer pH 8.6 , 100μM; $MgCl_2$: 10μM; cystein: 30μM; ATP: 30μM; NADH: 1μM; $NADP^+$: 1.0μM; G-6-P: 2μM; glucose-6-P-dehydrogenase(containing some crotonyl-reductase activity): 0.1ml; LS: 0.20ml ($AmSO_4$: 35→60% saturation); H_2O: ad 3.0 ml.

* Sample partially lost when transferred onto a planchet for counting
** cpm at infinite thinness.

The addition of glucose-6-phosphate dehydrogenase and of crotonyl-reductase to the fatty acid synthesizing system obtained from lactating rabbit mammary gland stimulates markedly the incorporation of acetate into fatty acids, and mainly into butyric acid. The synthesis of hexanoic acid is also significantly increased, whereas that of octanoic acid remains about the same. It is worth pointing out that the radioactivity recovery in the various short-chain fatty acids isolated from the medium when the lipogenetic system had been fortified with crotonyl reductase is far from being

complete. These missing counts were later on found to be associated with octenoic acid.

Since the enoyl reductase preparation which has been used in the latter experiments was found, on the basis of spectrophotometric assays, to be unable to catalyse the reduction of octenoyl- and dodecenoyl-CoAs into their corresponding saturated derivatives, its inability to restore fully the lipogenetic activity of the aged extract to its original level becomes quite easily understandable.

Those results would anyhow suggest that enoyl reductase might play a major role in the processes of short-chain saturated fatty acids. Whether this enzyme is normally present in freshly prepared extracts of rabbit mammary gland tissue was next investigated. This was done by studying the conversion of 1-C^{14}-crotonyl-CoA into saturated fatty acids by various cytoplasmic fractions obtained from homogenates of this tissue (Table III).

TABLE III

CONVERSION OF C^{14}-CROTONYL-CoA INTO SATURATED FATTY ACIDS BY VARIOUS CYTOPLASMIC FRACTIONS OF RABBIT MAMMARY GLAND

Cytoplasmic fraction:	S-10,000xg		Mitochondria		Microsomes	
Electron donor:	NADH	NADPH	NADH	NADPH	NADH	NADPH
C^{14} distribution, %: C^4	2.8	19.6	1.0	6.0	0.6	7.4
$C^{4=}$	89.0	69.4	89.2	85.6	86.5	88.0
C^6	2.3	2.4	1.6	1.6	2.3	1.0
$C^8 \rightarrow C^{16}$	5.9	5.9	8.2	6.8	10.6	3.6

Incubation medium: phosphate buffer, pH 7.4, 100μM; $MgCl_2$: 20μM; Na-thioglycholate: 30μM; ATP: 30μM; CoASH: 2μM; NADH or NADPH: 2μM; G-6-P: 4μM; S-10,000xg: 1.0 ml; washed and resuspended particles: 0.1 ml; C^{14}-crotonyl-CoA: $\simeq$ 2μM.
Incubation: 2 hours at 37°C under nitrogen.

The results obtained in one of these experiments, as shown in Table III, indicate that some crotonyl reductase activity can indeed be detected in all of the cytoplasmic fractions assayed, particularly in the 10,000xg supernatant. It is also worth mentioning that the reducing enzyme of the rabbit mammary gland, such as the one found in rat microsomes (Lachance et al., 1958) and

yeast, appears to be more specific towards crotonyl-CoA and requires NADPH as the electron donor. The requirement for NADPH is quite obvious in the experiments done with mitochondria or microsomes, these particles being apparently devoid of NADPH-NAD oxydoreductase activity. It should be finally pointed out that, even though the same extract incorporated very actively 1-C^{14}-acetate into saturated short- and long-chain fatty acids, crotonyl-CoA was not incorporated to any significant extent in fatty acids other than butyric and, possibly, hexanoic acid. The slight activity found in higher analogues is more likely to be due to transesterification and column bleeding phenomena.

CONCLUSION

The results obtained from this series of experiments would indicate that short- and long-chain fatty acid synthesis in the rabbit mammary gland tissue follows somewhat different enzymatic pathways. Butyric and hexanoic acid appear to be synthesized by a process similar to the one already described by Seubert et al. (1957) whereas intermediate- and long-chain fatty acids could be formed via the malonyl-CoA (Wakil, 1959) and, possibly, the chain-elongation pathway (Wakil, 1960).

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