BBA 3894

RELATIVE INCORPORATION OF THE VARIOUS PROPIONATE CARBONS INTO FATTY ACIDS BY LACTATING RAT MAMMARY GLAND

P. CADY, S. ABRAHAM AND I. L. CHAIKOFF
Department of Physiology, University of California, Berkeley, Calif. (U.S.A.)

(Received August 17th, 1962)

SUMMARY

1. Propionate labeled with ^{14}C in each of its carbons was incubated with slices prepared from lactating rat mammary glands. In the absence of added glucose, ^{14}C of [2-^{14}C]propionate was more rapidly incorporated into fatty acids than was ^{14}C of either [1-^{14}C]propionate or [3-^{14}C]propionate.

2. The addition of glucose to the medium increased fatty acid synthesis from all three carbons of propionate. The ^{14}C of [2-^{14}C]propionate and [3-^{14}C]propionate was incorporated at identical rates and exceeded that from [1-^{14}C]propionate.

3. Unlabeled acetate, at low concentrations in the presence of glucose, depressed conversion of ^{14}C of [1-^{14}C]propionate into fatty acids. At higher acetate concentrations the conversion from all propionate carbons was depressed.

4. The [^{14}C]fatty acids synthesized from [1-^{14}C]-,[2-^{14}C]- and [3-^{14}C]propionate were subjected to gas-chromatographic analysis, and the presence of both even-chain [^{14}C]fatty acids and odd-chain [^{14}C]fatty acids was demonstrated.

5. The results suggest that there are at least three pathways for conversion of propionate carbon to fatty acids by lactating rat mammary gland. Direct condensation with malonyl-CoA to form odd-chain fatty acids is by far the most predominant pathway. Glucose stimulated incorporation of propionate carbon into fatty acids by all three pathways equally.

INTRODUCTION

The metabolism of propionic acid has been studied mainly in ruminants because this acid forms naturally in the rumen, as a result of bacterial fermentation. KLEIBER *et al.*[1] demonstrated that, while propionate carbon can serve as a minor precursor of milk fat in the intact cow, the major end product of this three-carbon acid is lactose. LAURYSSENS *et al.*[2] perfused cow udders with ^{14}C-labeled propionate and found radioactivity in the udder's fatty acids. JAMES *et al.*[3] fractionated milk fatty acids isolated after cow udders were perfused with [1-^{14}C]propionate, and found that

Abbreviation: PPO, 2,5-diphenyloxazole.

most of the radioactivity resided in odd-chain fatty acids. These workers suggested that the odd-chain fatty acids were derived from propionate by successive condensation of active two-carbon units with propionyl-CoA. In these same experiments [1-^{14}C]propionate contributed ^{14}C, to a small extent, to the formation of even-chain fatty acids. Of particular interest was the isolation of [^{14}C]acetate arising from the perfused [1-^{14}C]propionate. This was taken as evidence that acetate could be formed by removal of the methyl carbon of propionate.

HORNING *et al.*[4] have shown that, in the presence of propionyl-CoA, [2-^{14}C]-malonyl-CoA was converted to fatty acids of odd-numbered carbon chain lengths (predominantly pentadecanoic acid) by a particle-free fraction obtained from rat epididymal adipose tissue. FAVARGER AND GERLACH[5], in their studies on the conversion of [^{14}C]propionate to fatty acids by adipose tissue, found that most of the ^{14}C-activity resided in the pentadecanoic and heptadecanoic acid fractions. These investigators[5] have also provided evidence for the view that propionate supplies the terminal three carbons for these odd-chain fatty acids.

EXPERIMENTAL

Substrates

Sodium [1-^{14}C]propionate was synthesized from ethyl bromide and $^{14}CO_2$ by the Grignard procedure[6]. The sodium salts of [2-^{14}C]- and [3-^{14}C]propionic acid, the zinc salts of [1-^{14}C]-, [2-^{14}C]- and [3-^{14}C]lactic acid and [1-^{14}C]-, [2-^{14}C]- and [3-^{14}C]alanine were kindly supplied by Dr. R. LEMMON of the Donner Laboratory, University of California, Berkeley. The zinc salts of the ^{14}C-labeled lactates were converted to the sodium salts by means of Dowex-50 (Na^+ form). [1-^{14}C]- and [2-^{14}C]succinic acids were synthesized by the method of JORGENSON *et al.*[7].

Treatment of rats

Multiparous lactating rats of the Long-Evans strain that had suckled at least six pups were used in this study. Pup growth was taken as evidence of adequate lactation. Lactating rats were killed 15–20 days postpartum, by a blow on the head. The mammary glands were quickly excised and placed in ice-cold KREBS–HENSELEIT bicarbonate buffer[8] (pH 7.3–7.4).

Incubation procedure

Slices of mammary tissue were prepared with the aid of a MCILWAIN–BUDDLE tissue chopper[9], and washed with the ice-cold buffer until milk no longer appeared in the wash. The slices were gently blotted with filter paper, and 500-mg portions were weighed and incubated as described[10].

Analytical procedures

Carbon dioxide was collected and assayed for ^{14}C-activity as described[11]. Fatty acids were isolated and assayed for ^{14}C-activity as follows: The contents of each incubation flask were transferred to a glass funnel provided with a glass wool plug. The slices and tissue fragments enmeshed by the glass wool were exhaustively washed with distilled water until no ^{14}C-activity could be detected in the wash water. The funnel contents, including the glass wool, were then transferred to a test tube, and

3 ml of 30% KOH were added. The tube was stoppered and the tissue was saponified overnight at 90°. The saponification mixture was cooled to −10° and to it were added 3 ml of ether. 1 ml of ice-cold, concentrated sulfuric acid was then added slowly, with shaking, to dispel heat. Care was taken to avoid localized heating, which causes loss of volatile fatty acids. The tubes were stoppered and shaken vigorously. The two liquid phases were separated by centrifugation, and the ether layer was quantitatively transferred to a 10-ml volumetric flask. Two additional extractions with 3-ml portions of ether were carried out, and the three extracts were combined. Ether was added to the combined extracts to yield a total volume of 10 ml, and the entire volume was dried with anhydrous sodium sulfate. 0.5-ml aliquots of the dried ether extract were taken for ^{14}C-assay by adding them directly to 10 ml of toluene containing 24 mg PPO. All counting was done with the Packard tri-carb liquid scintillation spectrometer (counting error less than 3%). [^{14}C]Toluene was used as an internal standard, and no quenching was observed with the amounts of ether used.

Gas chromatographic analysis of the isolated [^{14}C]fatty acids

The ether extract containing the [^{14}C]fatty acids isolated from the mammary gland slices was transferred to a small test tube placed in a bath containing dry ice and isopropyl alcohol, and the solvent was cautiously evaporated under a gentle stream of dry nitrogen*. An ethereal solution of a mixture of pure fatty acids containing from two to eleven carbon atoms was added to the contents of the test tube, and aliquots were taken for (a) ^{14}C-assay, as described above, (b) gas-chromatographic analysis.

The free fatty acids were subjected to gas-chromatographic analysis** on a 6-foot stainless steel column packed with Celite-545, which had been coated with a polyester of diethylene glycol adipate (10%). The gas used for development was argon, and the flow was maintained at 67 ml/min. The effluent gas was passed through an ionization detector (10 mC of ^{90}Sr), and the fatty acids were detected by means of a standard recording potentiometer. The temperature of the column was programmed from 106 to 200° throughout the development period to allow for rapid separation of the individual fatty acids. The recovery of the injected [^{14}C]fatty acids ranged from 92 to 105% and was accomplished by leading the exhaust gas through a glass tube that dipped 2 cm below the surface of a solution containing 5 ml of toluene and 24 mg PPO, in a scintillation counting vial. After each fraction was collected, the tube and vial were removed and immediately replaced with a new tube and vial assembly. The tube was rinsed with 5 ml of toluene and the toluene collected in the same scintillation vial used for collecting the effluent gas. The contents of each vial were assayed for ^{14}C-activity as described above.

Each analysis was run at 106–110° for about 1 h, which allowed the short-chain fatty acids to emerge from the column, and then the temperature was raised to 200° to expel the long-chain fatty acids. After 1 h at this elevated temperature, no more ^{14}C-activity came off.

---

* The loss of acetic acid was about 30% by this procedure, but the longer chain fatty acids were almost quantitatively retained.

** Research Specialties Co., Richmond, Calif. (U.S.A.).

RESULTS

Relative incorporation of the three carbons of propionate into fatty acids in the presence and absence of glucose in the medium

Tables I and II show the results of experiments in which the variously labeled propionates were incubated with mammary glands of lactating rats. Although the

TABLE I

INCORPORATION OF ^{14}C OF [1-^{14}C]-, [2-^{14}C]- AND [3-^{14}C]PROPIONATE INTO FATTY ACIDS BY MAMMARY-GLAND SLICES PREPARED FROM LACTATING RATS, IN THE ABSENCE OF ADDED GLUCOSE

500 mg of mammary-gland slices prepared from lactating rats (15–20 days postpartum) were incubated for 3 h at 37° in 5.0 ml of Krebs–Henseleit bicarbonate buffer (pH 7.4) containing 2.5 μmoles of labeled sodium propionate. Gas phase was O_2–CO_2 (95:5).

Rat	*Per cent ^{14}C incorporated into fatty acids from propionate*			*Relative incorporation into fatty acids [2-^{14}C]Propionate = 100*	
	[1-^{14}C]	*[2-^{14}C]*	*[3-^{14}C]*	*[1-^{14}C]*	*[3-^{14}C]*
2	6.4	11.4	7.7	56	68
3	1.3	2.2	2.3	59	101
4	2.6	3.3	2.9	79	88
5	2.9	8.0	4.0	36	50
6	4.2	10.4	10.1	40	97
7	4.5	17.6	11.8	25	67
8	1.5	5.0	3.5	30	70
9	0.7	3.1	3.0	23	97
10	0.5	2.2	1.1	23	46
11	0.9	4.0	1.7	23	43
12	0.7	2.7	1.4	26	52
13	0.4	3.7	2.6	11	70
14	1.0	6.8	3.6	15	53

TABLE II

INCORPORATION OF [1-^{14}C]-, [2-^{14}C]- AND [3-^{14}C]PROPIONATE INTO FATTY ACIDS BY MAMMARY-GLAND SLICES PREPARED FROM LACTATING RATS, IN THE PRESENCE OF ADDED GLUCOSE

50 μmoles of unlabeled glucose were added to the incubation medium at the start of each experiment. For other incubation conditions see Table I.

Rat	*^{14}C-labeled propionate added (μmoles)*	*Per cent ^{14}C incorporated into fatty acids from propionate*			*Relative incorporation into fatty acids [2-^{14}C] propionate = 100*	
		[1-^{14}C]	*[2-^{14}C]*	*[3-^{14}C]*	*[1-^{14}C]*	*[3-^{14}C]*
16	10	8.1	9.2	10.8	88	117
17	10	3.1	4.0	3.3	78	83
18	10	6.1	8.2	6.2	74	76
19	10	5.6	8.0	6.4	70	80
20	10	5.6	9.2	8.4	61	91
21	10	8.7	10.6	12.4	82	117
22	10	19.0	25.6	25.2	74	98
23	10	29.4	39.4	35.9	75	91
24	10	12.1	12.4	15.0	98	121
25	2.5	58.3	73.7	77.9	79	106
26	2.5	44.9	66.1	61.8	68	93
27	2.5	42.2	60.7	65.9	70	109

[^{14}C]fatty acid recovered from each labeled substrate, both in the presence and absence of glucose, differed widely from animal to animal, less variation was observed in the relative activities recorded in the last two columns.

In the absence of glucose, the ^{14}C of [2-^{14}C]propionate was incorporated into fatty acids to a greater extent than was the ^{14}C of [1-^{14}C]- or [3-^{14}C]propionate, except in one animal.

The addition of glucose to the medium resulted in a pronounced increase in the amount of each propionate carbon incorporated into fatty acids. The stimulation of lipogenesis by glucose from other precursors (acetate, butyrate, lactate and pyruvate) has been repeatedly observed in mammary tissue[10–14], particularly in the lactating gland[15,16].

Interestingly enough, the addition of glucose to the incubation medium also changed the pattern of fatty acid labeling from [1-^{14}C]-, [2-^{14}C]- and [3-^{14}C]propionate. In the presence of glucose the recoveries of [^{14}C]fatty acids from [2-^{14}C]- and [3-^{14}C]-propionate were nearly the same, and these recoveries were significantly higher than those observed with [1-^{14}C]propionate. Thus glucose promoted almost equal labeling of fatty acids from the second and third carbons of propionic acid.

FLAVIN AND OCHOA[17–19] have demonstrated that propionate forms succinyl-CoA in liver extracts. Our results with lactating rat mammary-gland slices incubated with glucose are consistent with formation of fatty acids from propionate via succinyl-CoA together with the simultaneous formation of fatty acids by direct condensation of propionyl-CoA with malonyl-CoA. If the fatty acids had been synthesized solely via the intermediary formation of succinyl-CoA and the Krebs cycle, no ^{14}C should have been found in the fatty acids recovered in the experiments with [1-^{14}C]propionate, whereas equal ^{14}C activities should have been observed with [2-^{14}C]- and [3-^{14}C]-propionate. On the other hand, fatty acids derived from propionate solely by direct condensation would contain equal activities from each of the three carbons. Thus, provided these are the only two paths for fatty acid synthesis from propionate, our results indicate that, in the presence of glucose, from 70 to 80 % of the labeled fatty acid was synthesized by direct condensation, and the remainder by two-carbon additions via succinyl-CoA

The above analysis does not explain the results obtained with propionate incubated in the absence of glucose in the medium. In this case an additional pathway must be postulated to account for the increased activity found in fatty acids derived from [2-^{14}C]propionate as compared with [3-^{14}C]propionate*. An oxidative mechanism has been suggested by JAMES *et al.*[3].

The various carbons of propionate compared with those of lactate and alanine in their conversion to CO_2 and fatty acids

Table III shows the results of experiments comparing the conversion of the three carbons of lactate, alanine and propionate to fatty acids and CO_2 by mammary gland slices prepared from the *same* lactating rat. Some striking differences as well as similarities in the metabolism of these three carbon compounds were revealed by this study. No fatty acids were synthesized from the carboxyl carbons of either lactate (see ref. 10) or alanine. As already noted above, the ^{14}C of [1-^{14}C]propionate was, how-

* A more complete analysis of the participation of the various pathways of propionate conversion to fatty acids is presented in another section.

ever, recovered in the isolated fatty acids. The addition of glucose to the medium augmented the amounts of ^{14}C of [2-^{14}C]lactate, [3-^{14}C]lactate (see ref. 10), [2-^{14}C]-alanine and [3-^{14}C]alanine converted to fatty acids, but did not change the relative pattern of this conversion.

The addition of glucose increased the oxidation of the carboxyl carbons of lactate, alanine and propionate but reduced the conversion of the α- and β-carbons of all three 3-carbon compounds to CO_2. This action of glucose could increase the availability of 2-carbon units from these compounds, thereby making them more readily convertible to fatty acids.

TABLE III

COMPARISON OF THE CONVERSION OF THE VARIOUS CARBONS OF LACTATE, ALANINE AND PROPIONATE TO CO_2 AND FATTY ACIDS BY MAMMARY-GLAND SLICES PREPARED FROM LACTATING RATS

500 mg of mammary-gland slices excised from a lactating rat (17 days postpartum) were incubated for 3 h at 37° in 5.0 ml of Krebs–Henseleit bicarbonate buffer (pH 7.4) containing 50 μmoles of the labeled substrates in the absence or presence of 50 μmoles of unlabeled glucose as indicated below. Gas phase was O_2–CO_2 (95:5).

Rat	*Labeled substrate added to medium*	*Per cent of added ^{14}C recovered as:*			
		CO_2		*Fatty acids*	
		Absence of unlabeled glucose	*Presence of unlabeled glucose*	*Absence of unlabeled glucose*	*Presence of unlabeled glucose*
1	[1-^{14}C]Lactate	36.9	55.0	0	0
	[2-^{14}C]Lactate	17.0	6.8	5.5	21.6
	[3-^{14}C]Lactate	12.9	5.3	5.6	22.0
	[1-^{14}C]Alanine	12.0	26.6	0	0
	[2-^{14}C]Alanine	4.8	3.6	0.20	8.6
	[3-^{14}C]Alanine	3.5	2.2	0.20	8.3
	[1-^{14}C]Propionate	7.0	9.9	0.10	6.9
	[2-^{14}C]Propionate	4.8	3.9	0.27	7.4
	[3-^{14}C]Propionate	4.1	3.9	0.20	7.4

Dilution experiments with succinate, lactate, pyruvate and methyl malonate

If succinyl-CoA serves as an important carbon precursor of fatty acids from propionate, it must form acetyl-CoA or malonyl-CoA before it is converted to fatty acids. A likely pathway for this conversion would involve entry of succinyl-CoA into the Krebs cycle, with subsequent formation of pyruvate from either malate or oxaloacetate. In order to test for such a pathway in lactating mammary gland, labeled propionate was incubated in the presence of unlabeled succinate, lactate, pyruvate and methyl malonate. The results are shown in Table IV. No decrease in the incorporation of propionate carbon into fatty acids was noted with any of these unlabeled substrates, at the concentrations studied. That lactate and succinate can be utilized by the slice and converted to fatty acids is shown in Tables III and V. Thus, it is unlikely that large amounts of propionate traverse the Krebs cycle in the conversion of its carbons to fatty acids. Our failure to observe a decrease in fatty acid synthesis when methyl malonate was added to the incubation medium (Table IV) does not necessarily rule out the conversion of propionate to succinyl-CoA, for methyl malonate may not have been

TABLE IV

EFFECT OF ADDITIONS OF UNLABELED SUCCINATE, PYRUVATE, LACTATE AND METHYL MALONATE TO THE MEDIUM ON THE RELATIVE INCORPORATION OF THE ^{14}C OF [1-^{14}C]-, [2-^{14}C]- AND [3-^{14}C]-PROPIONATE INTO FATTY ACIDS BY LACTATING RAT MAMMARY-GLAND SLICES

See Table I for experimental details and incubation conditions.

Rat	*Expt.*	*Propionate carbon labeled with ^{14}C*	*Glucose (μmoles)*	*Compounds added to medium*	*Per cent of added ^{14}C recovered as:**	
					Fatty acids	*CO_2*
10, 11, 12	1	1	None	None	0.68	39.7
		1	None	10 μmoles methyl malonate	0.77	51.8
		1	None	10 μmoles succinate	0.81	52.8
		2	None	None	2.96	38.7
		2	None	10 μmoles methyl malonate	2.80	41.8
		2	None	10 μmoles succinate	3.37	39.7
		3	None	None	1.41	24.9
		3	None	10 μmoles methyl malonate	1.57	26.9
		3	None	10 μmoles succinate	1.73	27.0
25, 26	2	1	50	None	51.6	5.9
		1	50	10 μmoles succinate	53.3	4.9
		1	50	10 μmoles pyruvate	49.4	6.2
		2	50	None	69.9	1.15
		2	50	10 μmoles succinate	71.7	1.20
		2	50	10 μmoles pyruvate	70.9	0.77
		3	50	None	69.9	1.00
		3	50	10 μmoles succinate	71.1	0.85
		3	50	10 μmoles pyruvate	70.6	1.05
28, 29	3	1	50	None	38.0	
		1	50	10 μmoles methyl malonate	43.0	
		2	50	None	69.0	
		2	50	10 μmoles methyl malonate	65.0	
30, 31, 32	4	1	50	None	58.4	
		1	50	25 μmoles lactate	57.2	
		1	50	25 μmoles succinate	54.3	
		1	50	25 μmoles methyl malonate	53.6	
		3	50	None	68.4	
		3	50	25 μmoles lactate	68.8	
		3	50	25 μmoles succinate	69.3	
		3	50	25 μmoles methyl malonate	67.0	

* Average values obtained from individual determinations.

activated to methyl malonyl-CoA at a rate adequate to dilute the ^{14}C-activity derived from propionate. It is also conceivable that methyl malonate does not penetrate the cells of the rat mammary-gland slice.

Experiments with added acetate in the presence and absence of glucose

At low concentrations (2.5 μmoles/5.0 ml of medium), when the molar concentration ratio of propionate to acetate was 1 to 1, there was little change in the con-

version of any of the carbons of propionate to fatty acid in the presence of glucose (Expt. 1, Table VI). The oxidation of the propionate carbons to CO_2 was also unaffected by the addition of acetate in the presence of glucose under these conditions (Expt. 1). However, at higher propionate concentrations (10 μmoles/5.0 ml of medium, Expt. 3) addition of acetate depressed the incorporation of propionate carbon into both CO_2 and fatty acids. This effect of acetate was particularly apparent when unlabeled glucose was also present in the incubation medium.

TABLE V

COMPARISON OF THE CONVERSION OF THE VARIOUS CARBONS OF PROPIONATE AND SUCCINATE TO CO_2 AND FATTY ACIDS BY MAMMARY-GLAND SLICES PREPARED FROM LACTATING RATS

The labeled substrates were added in 10-μmole amounts, and the unlabeled glucose in 50-μmole amounts. See Table I for other experimental details and incubation conditions.

Rat	*^{14}C-labeled substrate added to medium*	*Per cent of added ^{14}C recovered as:*			
		CO_2		*Fatty acids*	
		Absence of unlabeled glucose	*Presence of unlabeled glucose*	*Absence of unlabeled glucose*	*Presence of unlabeled glucose*
33	[1-^{14}C]Propionate	27.5	33.7	0.08	19.9
	[2-^{14}C]Propionate	16.3	8.7	0.74	38.9
	[3-^{14}C]Propionate	18.8	8.5	0.33	36.0
	[1-^{14}C]Succinate	5.3	5.4	0	0
	[2-^{14}C]Succinate	3.9	1.8	0.21	2.3
34	[1-^{14}C]Propionate	28.5	35.3	0.09	20.8
	[2-^{14}C]Propionate	17.3	9.0	0.70	39.3
	[3-^{14}C]Propionate	18.9	8.8	0.30	37.3
	[1-^{14}C]Succinate	5.4	5.4	0	0
	[2-^{14}C]Succinate	4.0	2.1	0.22	2.5

When the amount of unlabeled acetate was increased to four times (Table VI, Expt. 1) or five times (Expt. 2) that of propionate, a significant reduction in the fatty acid recoveries from propionate was observed. Even at these high acetate levels, the addition of glucose still augmented fatty acid synthesis from propionate.

Experiments with malonate

The results of experiments in which unlabeled malonate, a well-known inhibitor of the succinic dehydrogenase system[20], was incubated with the variously ^{14}C-labeled propionates are shown in Table VII. The addition of malonate resulted in a decrease in both fatty acid synthesis and CO_2 production with all three carbons of propionate. This may have been due to diminished production of ATP and of the reduced form of pyridine nucleotides (both needed for fatty acid synthesis) as a consequence of an inhibition of Krebs cycle activity. The lowered recoveries of [^{14}C]fatty acids and $^{14}CO_2$ could not have resulted from dilution by the added malonate since [1-^{14}C]-malonate is not metabolized by slices of lactating rat mammary glands.

Chain lengths of the fatty acids synthesized from the three carbons of propionate in the absence and in the presence of glucose in the medium

The individual fatty acids synthesized by the lactating rat mammary-gland slices from [1-^{14}C]-, [2-^{14}C]- and [3-^{14}C]propionate in the presence and the absence of glucose

TABLE VI

EFFECT OF ADDITION OF UNLABELED ACETATE ON THE INCORPORATION OF THE VARIOUS CARBONS OF PROPIONATE TO FATTY ACIDS AND CO_2 IN THE ABSENCE AND THE PRESENCE OF GLUCOSE

See Tables I and II for incubation conditions and experimental details.

Expt.	Rat	Substrate: Labeled: Propionate carbon labeled	Substrate: Labeled: Amount (μmoles)	Substrate: Unlabeled: Glucose (μmoles)	Substrate: Unlabeled: Acetate (μmoles)	Per cent of added ^{14}C recovered as: Fatty acids	Per cent of added ^{14}C recovered as: CO_2
1	8,9	1	2.5	0	0	1.1	
		1	2.5	50	0	51.6	5.9
		1	2.5	50	2.5	44.8	5.8
		1	2.5	50	10	20.1	4.1
		2	2.5	0	0	4.1	
		2	2.5	50	0	69.9	1.2
		2	2.5	50	2.5	64.3	1.2
		2	2.5	50	10	45.2	1.2
		3	2.5	0	0	3.3	
		3	2.5	50	0	69.9	1.0
		3	2.5	50	2.5	68.8	1.2
		3	2.5	50	10	31.8	1.0
2	35, 36	1	10	0	0	0.9	
		1	10	50	0	5.6	
		1	10	0	50	0.4	
		1	10	50	50	0.7	
		2	10	0	0	0.9	
		2	10	50	0	6.6	
		2	10	0	50	0.6	
		2	10	50	50	1.5	
		3	10	0	0	0.7	
		3	10	50	0	7.0	
		3	10	0	50	0.5	
		3	10	50	50	1.6	
3	38, 39	1	10	0	0	2.1	7.1
		1	10	10	0	15.6	10.2
		1	10	0	10	1.4	2.5
		1	10	10	10	2.0	3.0
		2	10	0	0	2.7	3.5
		2	10	10	0	18.6	3.0
		2	10	0	10	2.1	1.2
		2	10	10	10	3.2	1.1

in the medium are shown in Fig. 1. The predominant fatty acids that arose from all three propionate carbons were of odd-chain length (Table VIII). [2-^{14}C]Propionate and [3-^{14}C]propionate yielded the highest amounts of fatty acids of even-chain length, but [1-^{14}C]propionate also served to a small extent as a precursor for ^{14}C-labeled fatty acids of this type. [1-^{14}C]-, [2-^{14}C]- and [3-^{14}C]propionate, in the absence of glucose,

yielded ^{14}C-activity mostly in the hepatanoic acid fraction; in the presence of glucose the ^{14}C-activity was present predominantly in the nonanoic acid fraction.

TABLE VII

EFFECT OF ADDED UNLABELED MALONATE ON CONVERSION OF PROPIONATE CARBONS TO FATTY ACIDS IN THE PRESENCE AND THE ABSENCE OF GLUCOSE

Incubation conditions are given in Table I. Each flask contained 2.5 μmoles of propionate and the other additions given below.

Rat	*Labeled substrate*	*Unlabeled substrate*	*Per cent ^{14}C recovered as:*	
			Fatty acids	*CO_2*
3, 4, 5, 37*	[1-^{14}C]Propionate	None	2.43	20.8
	[1-^{14}C]Propionate	50 μmoles malonate	0.98	16.2
	[2-^{14}C]Propionate	None	3.93	15.2
	[2-^{14}C]Propionate	50 μmoles malonate	2.28	13.2
	[3-^{14}C]Propionate	None	2.70	10.2
	[3-^{14}C]Propionate	50 μmoles malonate	1.18	7.3
40	[1-^{14}C]Propionate	None	7.1	19.2
	[1-^{14}C]Propionate	10 μmoles glucose	50.7	19.5
	[1-^{14}C]Propionate	50 μmoles malonate	1.9	12.4
	[2-^{14}C]Propionate	None	13.0	11.0
	[2-^{14}C]Propionate	10 μmoles glucose	67.8	6.1
	[2-^{14}C]Propionate	50 μmoles malonate	5.2	6.1
	[3-^{14}C]Propionate	None	9.6	7.7
	[3-^{14}C]Propionate	10 μmoles glucose	67.4	7.6
	[3-^{14}C]Propionate	50 μmoles malonate	2.5	2.5

* Each value is the average of 4 results obtained in 4 separate experiments.

TABLE VIII

INCORPORATION OF PROPIONATE CARBONS INTO EVEN- AND ODD-CHAIN FATTY ACIDS BY LACTATING RAT MAMMARY-GLAND SLICES

Labeled propionate (2.5 μmoles) and unlabeled glucose (10 μmoles) were added as indicated below. These experiments were carried out with slices prepared from the mammary glands of rat No. 40.

Substrate		*Propionate carbon incorporated into fatty acids*		*Isolated [^{14}C]fatty acids recovered as:*			
				Even-chain		*Odd-chain*	
Labeled	*Unlabeled*	*Per cent*	*mμmoles*	*Per cent of total*	*mμmoles*	*Per cent of total*	*mμmoles*
[1-^{14}C]Propionate	None	7.1	178	15	27	85	151
[2-^{14}C]Propionate	None	13.0	325	51	166	49	159
[3-^{14}C]Propionate	None	9.6	240	31	75	69	165
[1-^{14}C]Propionate	Glucose	50.7	1270	14	178	86	1090
[2-^{14}C]Propionate	Glucose	67.8	1700	27	459	73	1240
[3-^{14}C]Propionate	Glucose	67.4	1690	23	389	77	1300

DISCUSSION

The results suggest that lactating rat mammary-gland can convert propionate carbon to longer chain fatty acids by at least three separate pathways: (a) the direct conversion of the 3 propionate carbons, (b) oxidation of propionate in such a way as to yield 2-carbon units from the alpha and beta carbons and (c) the formation of 2-carbon units from the alpha and carboxyl carbons by the oxidative removal of the beta (methyl carbon). Pathway a would lead to the formation of odd-chain fatty acids while pathways b and c would yield even-chain fatty acids.

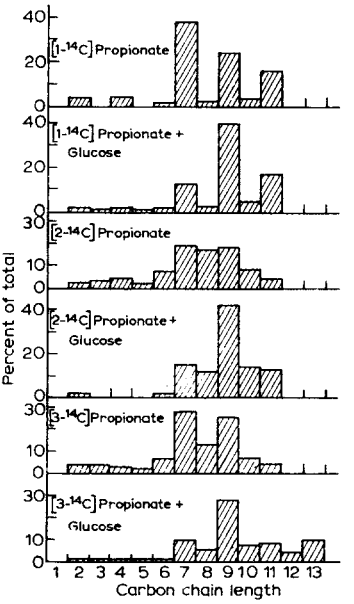

Fig. 1. Conversion of propionate carbons to fatty acids by lactating rat mammary-gland slices. The mixture of fatty acids synthesized from [1-^{14}C]-, [2-^{14}C]- and [3-^{14}C]propionate by the mammary-gland slices (lactating rat No. 40) were chromatographically separated into their individual components. The results presented above are given as a percentage of the total [^{14}C]fatty acids recovered. See Table VII for experimental details and the incubation conditions.

The appearance of large quantities of ^{14}C-activity in even-chain fatty acids from incubated [2-^{14}C]propionate probably represents the production of two-carbon units from the alpha and beta carbons of propionate (pathway b) in addition to the production of two-carbon units from the carboxyl and alpha carbons (pathway c). The latter pathway cannot contribute to the activity of fatty acids derived from [3-^{14}C]-propionate. This is reflected in a recovery of even-chain fatty acids from [3-^{14}C]-propionate lower than that from [2-^{14}C]propionate (Table VIII).

Evidence for pathway a comes from the observation that [1-^{14}C]propionate is converted to longer chain fatty acids by slices prepared from the mammary glands of lactating rats. These fatty acids have been identified as predominantly of the odd-chain variety.

Pathway b may, in part, be identical with the metabolic scheme for the oxidation of propionate proposed by FLAVIN AND OCHOA[17–19]. However, provided the results of

our dilution experiments are valid indicators, it is conceivable that free succinate is not an obligatory intermediate in fatty acid synthesis from propionate. If any succinyl-CoA is formed as a result of the FLAVIN–OCHOA mechanism, it should proceed to fatty acids by some pathway other than entry into the Krebs cycle. KATZ AND KORNBLATT[21] have come to a similar conclusion from their observations that the chromatographic patterns of the water-soluble compounds resulting from propionate metabolism are not at all similar to those from succinate metabolism by slices prepared from the mammary glands of lactating rats. Of interest in this connection is the work of FELLER AND FEIST[22] who have demonstrated that the conversion of [2,3-$^{14}C_2$]succinate to fatty acids by mouse adipose tissue and liver is negligible, although [^{14}C]propionate readily yielded [^{14}C]fatty acids. These same tissues, however, did utilize methyl malonate carboxyl carbon for fatty acid synthesis, and unlabeled methyl malonate markedly decreased the incorporation of [1-^{14}C]propionate into fatty acids[22]. This latter finding is in direct contrast to the present results obtained with lactating rat mammary gland, and may be explained on the basis of permeability and activation differences between the two types of tissue studied.

Our findings with mammary tissue are compatible with those of MASORO AND PORTER[23], who determined the conversion of the carbons of propionate into fatty acids by surviving rat adipose tissue. Equal labeling of fatty acids from incubated [2-^{14}C]- and [3-^{14}C]propionate was observed. Furthermore, the first carbon of propionate was converted to fatty acids much less readily than was either of the other carbons of propionate.

If, as stated above, propionate is converted to fatty acids by a process which involves its incorporation as an intact three-carbon unit as well as a two-carbon unit, one would expect the addition of unlabeled acetate to depress this conversion by at least two different mechanisms. The first, since it involves competition for the available cofactors needed for activation (CoA, ATP etc.), should result in a decreased recovery of [^{14}C]fatty acids from all three labeled species of propionate. The other, a dilution process, would be expected to decrease only the conversion of [2-^{14}C]propionate and [3-^{14}C]propionate to fatty acids. Since, at high acetate concentrations (molar ratio of acetate to propionate greater than 5), the conversion to fatty acids of all three propionate carbons was depressed, acetate apparently competes with propionate for the cofactors needed for activation of these acids.

The results obtained in the experiments with added acetate suggest a pathway for propionate conversion to fatty acids that does not proceed through succinate. Since acetate is an effective diluent on the second and third carbons of propionate only at high concentrations, we may speculate that these carbons do not pass through acetate, as such, on their way to fatty acids, but rather through a compound derived from acetate. Such a compound might be malonyl-CoA. Thus, a possible mechanism could involve the conversion of propionyl-CoA to malonyl-CoA. A similar scheme has been proposed for propionate metabolism in plants[24,25] and bacteria[26].

Free malonic acid is probably not involved in the conversion of propionate to fatty acids, for the ^{14}C of [1-^{14}C]malonic acid is not incorporated into fatty acids by slices or homogenates[27] prepared from lactating rat mammary glands. In addition, since malonate can permeate the slice in amounts sufficient to inhibit fatty acid synthesis and decrease the CO_2 production from propionate, it is likely that a malonic acid activating enzyme is absent in this tissue.

Evidence for pathway c (formation of two-carbon units from the carboxyl and alpha carbons of propionate) is suggested by the marked isotopic dilution of the first and second carbons of propionate by small amounts of acetate. Thus, acetate apparently could be formed from the first two carbons of propionate by oxidation of the third. Such an oxidative mechanism has been proposed by JAMES *et al.*[3] to account for the isolation of labeled acetate from cow udders perfused with [1-^{14}C]propionate. In addition, YAMADA AND JAKOBY[28] have demonstrated a direct conversion of malonic semialdehyde to acetyl-CoA in extracts from a strain of *Pseudomonas fluorescens*. A similar reaction may occur in mammary tissue, and might provide the mechanism for two-carbon formation from the first and second carbons of propionate.

The direct condensation pathway (a) would yield only odd-chain fatty acids from propionate. However, the production of two-carbon units (acetyl- or malonyl-CoA) from propionate derived from either the second and third carbons (pathway b) or the first and second carbons (pathway c), could give rise to both even- and odd-chain fatty acids. The condensation of a propionate-derived two-carbon unit with other two-carbon units could yield even-chain fatty acids, and condensation with propionyl-CoA could yield odd-chain acids. The fate of the two-carbon unit will probably depend upon the relative pool sizes of two-carbon units and propionyl-CoA. If we assume that the two-carbon unit pool is large with respect to the propionyl-CoA pool, an approximate evalution of the relative significance of each of the three pathways of propionate conversion to fatty acids becomes possible. Thus the formation of odd-chain fatty acids reflects a direct condensation, pathway a, while the formation of even-chain fatty acids reflects primarily two-carbon unit production. In addition, the first carbon of propionate can produce only even-chain fatty acids by formation of two-carbon units from the first and second carbons of propionate (pathway c). Similarly the third carbon of propionate can produce only even-chain fatty acids by the formation of two-carbon units from the second and third carbons of propionate (pathway b). Finally the second carbon of propionate can give rise to two-carbon units formed by both pathways b and c. Thus, the even-chain fatty acids formed from [1-^{14}C]propionate must have arisen via pathway c, the even-chain fatty acids from [3-^{14}C]propionate must have arisen from pathway b, whereas the odd-chain fatty acid from any carbon of propionate reflects the direct pathway a.

Table IX shows the results of a calculation based on the above considerations. Of interest is the finding that the addition of glucose to the incubation medium does not significantly change the routes by which propionate is incorporated into fatty acids, but appears to stimulate all three mechanisms equally.

TABLE IX

RELATIVE SIGNIFICANCE OF THE THREE PROPOSED PATHWAYS OF PROPIONATE CONVERSION TO FATTY ACIDS

Calculated from data in Table VIII obtained with rat No. 40.

Proposed pathway	*Carbons derived from propionate*	*mμmoles traversing pathway*		*Per cent traversing pathway*	
		No glucose	*Plus glucose*	*No glucose*	*Plus glucose*
(a) direct	1, 2 and 3	159	1240	61	69
(b) two-carbon	2 and 3	75	389	29	21
(c) two-carbon	1 and 2	27	178	10	10

ACKNOWLEDGEMENT

This investigation was aided by a grant from the National Science Foundation.

REFERENCES

1 M. KLEIBER, A. L. BLACK, M. A. BROWN AND B. M. TOLBERT, *J. Biol. Chem.*, 203 (1953) 339.
2 M. LAURYSSENS, G. PEETERS, R. COUSSENS AND R. DELOOSE, in G. POPJÁK AND E. LEBRETON, *Biochemical Problems of Lipids*, Butterworths Scientific Publications, London, 1956.
3 A. T. JAMES, G. PEETERS AND M. LAURYSSENS, *Biochem. J.*, 64 (1956) 726.
4 M. G. HORNING, D. B. MARTIN, A. KARMEN AND P. R. VAGELOS, *J. Biol. Chem.*, 236 (1961) 669.
5 P. FAVARGER AND J. GERLACH, *Helv. Physiol. Pharmacol. Acta*, 18 (1960) 328.
6 M. CALVIN, C. HEIDELBERGER, J. C. REID, B. M. TOLBERT AND P. F. YANKWICH, in *Isotopic Carbon*, John Wiley and Sons, Inc., New York, 1949, p. 178.
7 E. C. JORGENSON, J. A. BASSHAM, M. CALVIN AND B. M. TOLBERT, *J. Am. Chem. Soc.*, 74 (1952) 2418.
8 H. A. KREBS AND K. HENSELEIT, *Z. Physiol. Chem.*, 210 (1932) 33.
9 H. MCILWAIN AND H. L. BUDDLE, *Biochem. J.*, 53 (1953) 412.
10 S. ABRAHAM, P. F. HIRSCH AND I. L. CHAIKOFF, *J. Biol. Chem.*, 211 (1954) 31.
11 P. F. HIRSCH, H. BARUCH AND I. L. CHAIKOFF, *J. Biol. Chem.*, 210 (1954) 785.
12 J. H. BALMAIN, S. J. FOLLEY AND R. H. GLASCOCK, *Biochem. J.*, 52 (1952) 301.
13 G. POPJÁK AND A. TIETZ, *Biochem. J.*, 56 (1954) 46.
14 P. F. HIRSCH, W. J. LOSSOW AND I. L. CHAIKOFF, *J. Biol. Chem.*, 221 (1956) 509.
15 S. ABRAHAM AND I. L. CHAIKOFF, *J. Biol. Chem.*, 234 (1959) 2246.
16 S. ABRAHAM, P. CADY AND I. L. CHAIKOFF, *Endocrinology*, 66 (1960) 280.
17 M. FLAVIN AND S. OCHOA, *J. Biol. Chem.*, 229 (1957) 965.
18 M. FLAVIN, H. CASTRO-MENDOZA AND S. OCHOA, *J. Biol. Chem.*, 229 (1957) 981.
19 W. S. BECK, M. FLAVIN AND S. OCHOA, *J. Biol. Chem.*, 229 (1957) 997.
20 J. N. QUASTEL AND W. R. WOOLRIDGE, *Biochem. J.*, 22 (1928) 689.
21 J. KATZ AND J. KORNBLATT, *Federation Proc.*, 21 (1962) 289.
22 D. D. FELLER AND E. FEIST, *J. Biol. Chem.*, 228 (1957) 275.
23 E. J. MASORO AND E. PORTER, *J. Lipid Res.*, 2 (1961) 177.
24 J. GIOVANELLI AND P. K. STUMPF, *J. Biol. Chem.*, 231 (1958) 411.
25 M. D. HATCH AND P. K. STUMPF, *Arch. Biochem. Biophys.*, 96 (1962) 193.
26 P. R. VAGELOS AND J. M. EARL, *J. Biol. Chem.*, 234 (1959) 2272.
27 R. DILS AND G. POPJÁK, *Biochem. J.*, 83 (1962) 41.
28 E. W. YAMADA AND W. B. JAKOBY, *J. Biol. Chem.*, 235 (1959) 589.