Effect of propionate on lipogenesis in adipose tissue

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ABSTRACT The metabolism of propionate in adipose tissue and its effect on lipogenesis was investigated. Fasting induced changes in propionate metabolism of adipose tissue, drastically reducing higher fatty acid synthesis and increasing glyceride-glyerol formation from low concentrations of propionate (0.25 mM). Propionate also promoted lipogenesis from acetate-1-^{14}C in tissues of fasted rats, while it inhibited lipogenesis and CO_2 formation from acetate in the fed animal. Treatment with actinomycin D or ethionine abolished both the increased glyceride-glycerol formation from propionate and the promoting effect on lipogenesis from acetate. Synthesis of long-chain fatty acids from propionate-1-^{14}C was increased by actinomycin treatment. The change in propionate metabolism induced by fasting is, however, not entirely due to its conversion to glyceride-glycerol, since the latter was almost completely blocked by malonate while part of the promoting effect on fatty acid synthesis persisted.

KEY WORDS propionate metabolism · adipose tissue · promotion of lipogenesis · adaptation of adipose tissue · malonate · succinate · actinomycin D · ethionine

THE METABOLISM OF PROPIONATE in adipose tissue has been the subject of several investigations. Feller and Feist (1) demonstrated that the conversion of this acid into long-chain fatty acids is much more efficient in adipose tissue than in liver. They presented evidence to show that the intact 3-carbon unit is incorporated by a metabolic pathway differing from that of propionate metabolism in the liver (2). The importance of this incorporation of a 3-carbon unit into fatty acids in vivo was questioned by Masoro and Porter (3), who found that propionic acid injected into a rat is converted into long-chain acids primarily after decarboxylation to form acetyl CoA. Favarger and Gerlach (4) found that propionate-1-^{14}C was incorporated in vivo at one-third the rate of propionate-2-^{14}C, and appeared mainly in the odd-chain acids.

The metabolism of propionate and other short-chain acids is of major importance in ruminants. In these animals, dietary carbohydrate is fermented in the rumen to volatile fatty acids (5). Propionate, in addition to its possible contribution in fatty acid synthesis, serves as a major glucose precursor in ruminants (6). In other mammals propionate is derived mainly from the catabolism of isoleucine (7) and may serve as a gluconeogenic substrate during fasting.

Additional aspects of the metabolism of propionic acid, and in particular its effect on lipogenesis in rat adipose tissue, will be presented in the present paper.

MATERIALS AND METHODS

Groups of male albino rats weighing either about 100 or 150 g were used. Fasting animals were deprived of food for 24 hr but had free access to water.

At the beginning of the fast and 5 hr later, DL-ethionine (Aldrich Chemical Co.) was administered intraperitoneally, each injection containing 75 mg. Only those animals that revealed extensive fatty livers at the end of the 24-hr fast were examined further.

Actinomycin D (Merck), when given, was administered intraperitoneally (25 μg to each 100 g rat) 4 hr before the beginning of the fast.

Rats were killed by decapitation and the epididymal fat pads were quickly separated and put into 0.9% NaCl solution at room temperature. The distal finger part of the pad was cut into 2–4 equal pieces. Each piece was incubated in 2 ml of Krebs-Ringer phosphate buffer pH 7.4 with the indicated additions.

Incubation was carried out at 37°C with continuous shaking for 2 hr. When $^{14}CO_2$ was collected, the incu-

Abbreviations: PPO, 2,5-diphenyloxazole; POPOP, *p*-bis[2-(5-phenyloxyazolyl)]benzene.

bation was carried out in Warburg flasks sealed with rubber stoppers. The reaction was stopped by the injection of 0.3 ml of 1.5 N H_2SO_4 into the incubation medium, and 0.2 ml of Hyamine 10X [*p*-(diisobutylcresoxy-ethoxyethyl)dimethylbenzylamine] (Packard) was put into the center well. Shaking was resumed for an additional 10 min. The Hyamine was then quantitatively transferred to counting vials which contained 10 ml of scintillation fluid (PPO, 0.3%, and POPOP, 0.01% in toluene) and counted in a liquid scintillation counter (Packard Tri-Carb).

Radioactive compounds were purchased from the Radiochemical Centre, Amersham, England and were chromatographically pure.

Analysis of Radioactivity in the Fat

The tissues were washed several times in 0.9% NaCl, dried, weighed, and extracted with acidic isopropane–heptane extraction mixture according to Dole (8). The heptane phase was separated and washed three times with isopropanol–0.07 N H_2SO_4 4:3. A portion of the heptane phase was counted to give the radioactivity of total fat. Zero-time experiments showed that none of the original radioactive short-chain fatty acids contaminated this fat fraction. To separate the glycerol and fatty acid moiety, we evaporated another 2 ml portion to dryness and hydrolyzed it in 0.625 N alcoholic KOH at 70°C for 30 min, washed it once more with heptane to remove the residual unhydrolyzed esters, heated the residue to 80°C to evaporate the alcohol, and added 4 ml of 0.5 N H_2SO_4. The long-chain fatty acid fraction was extracted with two 3-ml portions of heptane. These were pooled and an aliquot was taken into toluene scintillation fluid for radioactivity counting. The water phase, which contained the glycerol fraction, was once more washed with heptane and a sample of the water phase was added to 5 ml of dioxane scintillation fluid (PPO, 0.33%, and POPOP, 0.0042% in naphthalene–dioxane 9:1). Counts were corrected for the lower efficiency of this scintillation mixture.

Short-chain, water-soluble acids, which might have been included in the glycerol determination, were found to be present in the glycerol esters formed from propionate or pyruvate in negligible amounts. The bulk of the radioglycerol could also be precipitated as the tribenzoate.

Statistical Evaluation

Figures in tables present means ± SEM. The experimental samples were treated as independent. Presentation in terms of the effect of the various additions on tissues of the same rat would give still higher significance and less variation. However, this would not give the mean activities. In the figures, all the points were obtained from tissues of the same rat or of pooled and mixed samples.

RESULTS

Effect of Sodium Propionate on CO_2 Formation and Lipid Synthesis from Acetate and Pyruvate

Epididymal fat pads (60–100 mg) obtained from fed animals were incubated in 2 ml of Krebs-Ringer phosphate buffer, 7.5 mM with respect to acetate-1-^{14}C. Approximately 3% of the radioactivity was assimilated and distributed about equally between $^{14}CO_2$ and tissue triglycerides. As low a concentration as 0.25 mM sodium propionate in the incubation medium inhibited both $^{14}CO_2$ production and lipid synthesis (Fig. 1) from acetate. Inhibition of the $^{14}CO_2$ production was the more pronounced, especially with higher propionate concentrations. On the other hand, the conversion of pyruvate-2-^{14}C both into $^{14}CO_2$ and into triglycerides was accelerated by the addition of sodium propionate to the incubation medium (Fig. 2).

Epididymal fat pads from fasted animals incorporated much less acetate-^{14}C into lipids than did tissues from fed rats. Addition of propionate, glucose, or pyruvate to the incubation medium of tissues from fasted rats increased lipid synthesis from acetate-1-^{14}C (Table 1). Propionate was added at a concentration of 0.25 mM, which usually caused inhibition of lipid synthesis from acetate in tissues from fed animals (Fig. 1).

Treatment with DL-ethionine abolished the effect of propionate on acetate incorporation into lipid by tissues from fasted rats. The effect of pyruvate, on the other hand, was depressed at most to a limited extent (Table 1).

Similar results were obtained in another group of rats, weighing 100 g, to which actinomycin D was administered (Table 2). The stimulatory effect of propionate on the incorporation of acetate into lipids by tissues of fasted rats was abolished by actinomycin D. The stimulatory effect of pyruvate was also diminished. but was still present, after actinomycin treatment.

Actinomycin D administered to fed animals also depressed the incorporation of acetate into lipids (Table 2). Addition of sodium propionate did not increase lipid synthesis in adipose tissue thus obtained. DL-Ethionine or actinomycin D added in vitro to the incubation medium had no effect on lipogenesis from acetate in the presence or absence of propionate.

It is generally accepted that propionate metabolism leads to succinate formation in mammals (9). Succinate might therefore be expected to imitate sodium propionate in enhancing lipid synthesis from acetate in the fasted rats. The effect of succinate was, however, ir-

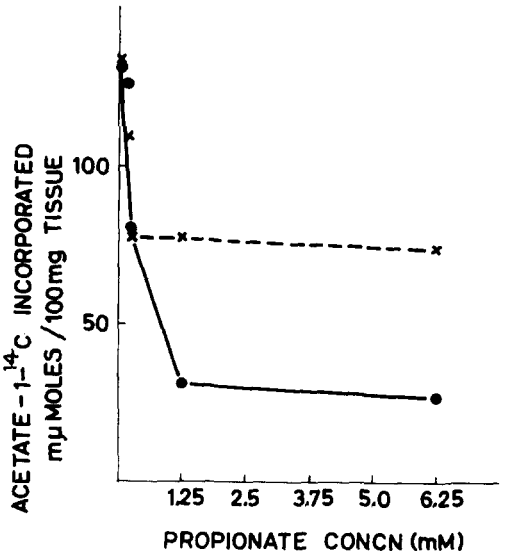

FIG. 1. Effect of propionate concentration on metabolism of acetate-1-^{14}C by epididymal fat pads from fed rats. The tissues were incubated in 2 ml of Krebs-Ringer phosphate buffer pH 7.4 with 7.5 mM acetate-1-^{14}C (1 μc/30 μmoles) in the presence of 0.125–6.25 mM propionate for 2 hr at 37°C. ●, acetate converted to $^{14}CO_2$; ×, acetate incorporated into lipid. Points were obtained from pooled tissues of two or three rats and are representative of three experiments.

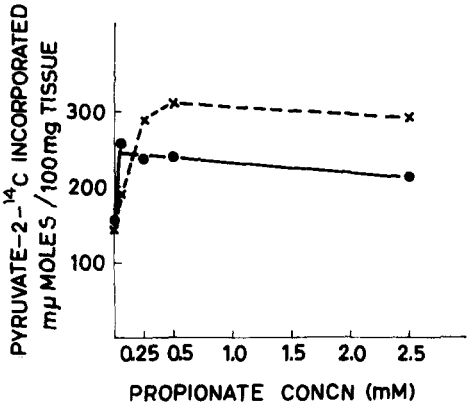

FIG 2. Effect of propionate on metabolism of pyruvate-2-^{14}C in fed rats. The tissues were incubated in 2 ml of Krebs-Ringer phosphate buffer pH 7.4 with 2.5 mM pyruvate-2-^{14}C (1 μc/20 μmoles) in the presence of 0.125–2.5 mM propionate for 2 hr at 37°C. ●, pyruvate converted to $^{14}CO_2$; ×, pyruvate incorporated into lipid. Points were obtained from pooled tissues of two or three rats and are representative of nine experiments.

regular and not significant when all the results were pooled. An increase in acetate incorporation in fasted rats was found in 5 out of 14 cases, when the effect on the same tissue was examined (Table 2). In these cases stimulation persisted even after actinomycin D treatment.

In order to test the assumption that propionate serves as source for glyceride-glycerol, we investigated the

TABLE 1 EFFECT OF PYRUVIC ACID, PROPIONIC ACID, AND GLUCOSE ON THE INCORPORATION OF ACETATE-1-^{14}C INTO LIPID IN ADIPOSE TISSUES FROM DIFFERENTLY TREATED RATS

Addition	No. of Expts.	Fed	Fasted	Fasted, DL-Ethionine Treated
		mμmoles of acetate incorporated/100 mg tissue		
None	16	213 ± 37	13 ± 2.4	16 ± 2.2
Pyruvic acid (0.25 mM)	8	229 ± 48	41 ± 7*	32 ± 3.7*
Propionic acid (0.25 mM)	16	170 ± 38†	30 ± 3.9*	16 ± 2.1
Glucose (6.25 mM)	4	323 ± 50‡	62 ± 18*	

Epididymal fat pads of 150-g rats (60–100 mg wet weight) were incubated at 37°C for 2 hr in 2 ml of Krebs-Ringer phosphate buffer (pH 7.4) in the presence of 7.5 mM acetate-1-^{14}C (S.A. 1 μc/30 μmoles) and the indicated compounds.

* $P < 0.01$ when compared with values obtained after no additions to the media.

† $P = 0.4$–0.5 when compared with values obtained after no additions to the media.

‡ $P = 0.1$ when compared with values obtained after no additions to the media.

metabolism of propionate-1-^{14}C in tissues of normally fed, fasted rats, and rats subjected to actinomycin treatment. The effect of malonate, which would poison the succinate pathway, was also studied. As expected, $^{14}CO_2$ production, from either acetate-^{14}C or propionate-^{14}C, was inhibited by the addition of malonate to the incubated tissues (Tables 3 and 4). Glyceride-glycerol synthesis from propionate-^{14}C in tissues from fasted animals was almost completely blocked by malonate (Table 4). However, in these tissues malonate only partly depressed the stimulatory effect of sodium propionate on the incorporation of acetate-^{14}C into lipids (Table 3, last column). Most of the radioactivity of propionate-1-^{14}C metabolized by the adipose tissue was recovered as $^{14}CO_2$, and much less was found in lipid. The dependence of $^{14}CO_2$ formation, and glyceride-glycerol synthesis, on the concentration of propionate-^{14}C in the medium is illustrated in Fig. 3. It is evident that $^{14}CO_2$ formation is less in tissues from fasted animals, at all concentrations of propionate tried. Glyceride-glycerol synthesis, on the other hand, was elevated in the fasted state, at least when low concentrations of propionate were used (also Table 5).

In fasted animals, actinomycin D treatment (Table 5) depressed the elevated glyceride-glycerol production from propionate-1-^{14}C. No effect was observed on the $^{14}CO_2$ formation, while fatty acid synthesis (which was small in the fasted rats) was significantly elevated. In fed animals, actinomycin D treatment had no effect on glyceride-glycerol formation but reduced both $^{14}CO_2$ and fatty acid synthesis.

TABLE 2 INFLUENCE OF ACTINOMYCIN D ON THE PROPIONATE AND PYRUVATE EFFECTS; AND EFFECT OF SUCCINATE ON LIPID SYNTHESIS

Addition	No. of Expts.	Fed	Fed, Actinomycin D-Treated	Fasted	Fasted, Actinomycin D-Treated
		mμmoles acetate incorporated/100 mg tissue			
None	18	262 ± 56	56 ± 6.6	12 ± 1.4	27 ± 3.8
Pyruvic acid (0.25 mM)	13			64 ± 10.8*	44 ± 4.5*
Propionic acid (0.25 mM)	18	188 ± 60	44 ± 8.2	33 ± 1.7*	21 ± 3.8
Succinic acid‡ (6.25 mM)	14			17 ± 2.8†	36 ± 4.7†

Experimental conditions as in Table 1. The animals weighed 100 g.
* $P < 0.01$ when compared with values after no addition.
† $P = 0.1$–0.2 when compared with values after no addition.
‡ Only in five experiments was the incorporation of acetate-^{14}C stimulated, when the effect of the addition was observed in tissues of the same rat.

TABLE 3 INFLUENCE OF MALONATE ON THE PROPIONATE EFFECT

Addition	No. of Expts.	Malonate (12.5mM)	Fed		Fasted	
			CO_2	Lipid	CO_2	Lipid
			mμmoles of acetate incorporated/100 mg tissue			
None	4	−	171 ± 47.3	196 ± 58	159 ± 26	7 ± 1.4
None	4	+	71 ± 33.5	206 ± 48.5	110 ± 31	4 ± 1.3
Propionic acid (0.25 mM)	4	−	148 ± 32.8	180 ± 58	110 ± 28.4	33 ± 2 4*
"	4	+	112 ± 30.0	90 ± 42.4	87 ± 35	17 ± 4.7†

The tissues were incubated in Warburg Flasks and $^{14}CO_2$ was collected. Other experimental details as in Table 1.
* $P < 0.01$ when compared with values obtained without propionate. The significance was much greater when the effect on individual samples of tissue was considered.
† $P = 0.05$ with same conditions as above.

TABLE 4 EFFECT OF MALONATE ON METABOLISM OF PROPIONATE-1-^{14}C IN ADIPOSE TISSUE FROM FED AND FASTED RATS

Treatment of Rat	No. of Expts.	Malonate (12.5 mM)	$^{14}CO_2$ Production	Fatty Acid	Glyceride-Glycerol
			mμmoles propionate converted/100 mg tissue		
Fed	7	−	128 ± 26	27 ± 9	10 ± 3.5
		+	66 ± 3.5	25 ± 6.4	7 ± 3.5
Fasted	7	−	74 ± 13.8	2.2 ± 0.27	12 ± 0.7
		+	35 ± 4.5	2.0 ± 0.12	2 ± 0.6

Epididymal fat pads of 150-g rats were incubated with 0.625 mM propionate-1-^{14}C.

TABLE 5 EFFECT OF ANTINOMYCIN D ON THE METABOLISM OF PROPIONATE-1-^{14}C

Treatment of Rat	No. of Expts.	$^{14}CO_2$ Production	Fatty Acids	Glyceride-Glycerol
			mμmoles propionate incorporated/100 mg tissue	
Fed	23	106 ± 7	11 ± 1.4	6.4 ± 0.9
Fed, actinomycin D-treated	6	57 (48−65)	2.3 ± 0.9	6.5 ± 1.6
Fasted	25	72 ± 6	0.6 ± 0.1	9.0 ± 0.4*
Fasted, actinomycin D-treated	19	70 ± 8	2.5 ± 0.9	4.0 ± 0.7

Tissues were incubated with 0.25 mM propionate-1-^{14}C under the conditions described for Table 1. $^{14}CO_2$ was measured in two experiments only for each group.
* $P < 0.01$ when compared with tissues of fed animals.

DISCUSSION

The inhibition caused by propionate of both lipid synthesis and $^{14}CO_2$ formation from acetate-1-^{14}C in adipose tissue of the fed rat, as well as of $^{14}CO_2$ formation in tissues of the fasted rat, indicated that in both types of tissue an initial common step of acetate metabolism, probably acetyl CoA synthesis, is impaired. This is further substantiated by the fact that propionate did not inhibit lipogenesis and CO_2 formation from pyruvate (Fig. 2). The demonstration that lipid synthesis is activated by propionate in the fasted rat indicates that propionate causes a marked effect on one of the steps leading from acetate to lipid, which compensates for the inhibition of acetyl CoA formation.

The change that occurs in the metabolism of the tissue from fasted rats, which makes its acetate metabolism susceptible to propionate activation, seems to be adaptive, since it is abolished by actinomycin D or by ethionine treatment.

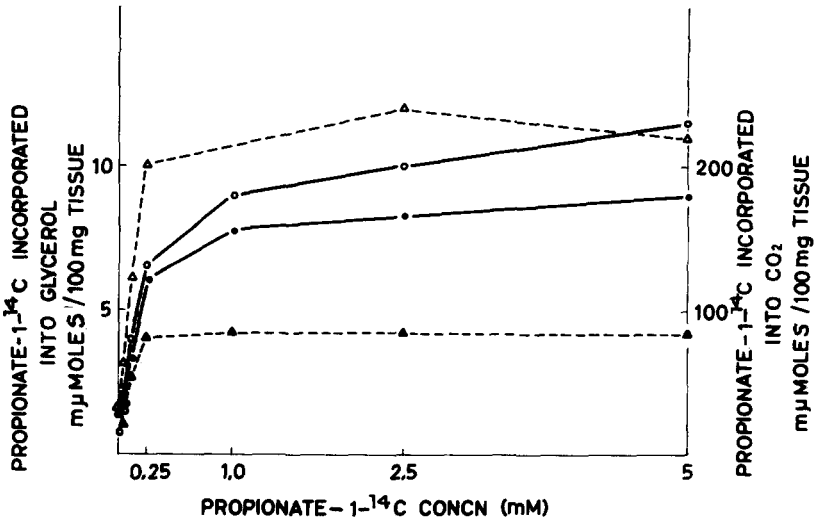

FIG. 3 Incorporation of propionate-1-^{14}C into CO_2 and glyceride-glycerol in tissues from fed and fasted rats. Incubation in 2 ml of Krebs-Ringer phosphate buffer pH 7.4 with 0.05–5 mM propionate-1-^{14}C for 2 hr at 37°C. ●, propionate converted to CO_2, fed animals; ○, propionate incorporated into glyceride-glycerol, fed animals; ▲, propionate converted to CO_2, fasted animals; △, propionate incorporated into glyceride-glycerol, fasted animals. Points were obtained from pooled tissues of two or three rats and are representative of five experiments.

One possible mechanism of such an activation may be the formation of α-glycerophosphate from propionate. This would aid in removal of the free fatty acids that accumulate in the tissues of starved rats and inhibit acetyl CoA carboxylase (10).

Propionate has been shown to be converted into glyceride-glycerol; the conversion tends to be more active in tissues of fasted rats than in those from fed animals. This increased glyceride-glycerol synthesis on fasting is also blocked by actinomycin D treatment. However, the finding that malonate addition caused almost complete blockage of glyceride-glycerol formation from propionate-1-^{14}C while a considerable part of the activation of lipogenesis from acetate-1-^{14}C still persisted, indicates that increased glyceride-glycerol synthesis is not the entire basis of propionate action.

Other metabolites known to be formed from propionate, i.e. citrate or malate, could enhance lipid synthesis by activation of acetyl CoA carboxylase (11) or by regeneration of NADPH (12), respectively. However, this also is not likely to be the basis of propionate action, since malonate would interfere with the formation of these metabolites too. Furthermore, succinate in most cases did not replace propionate as activator and in those cases in which it did act as promoter, its effect was not abolished by actinomycin treatment. Actinomycin D treatment of the fasted rat did not inactivate CO_2 formation from propionate by adipose tissue. This would exclude the succinate pathway of propionate metabolism from a role in the activation described.

Of the alternative pathways of propionate metabolism that might cause this activation, we consider the suggestion of Feller and Feist (2) of direct interaction between propionyl CoA and acetyl CoA and the effect of propionate metabolism on pyruvate the most worthy of attention. Propionate has been shown to accelerate pyruvate metabolism in adipose tissue (Fig. 2) both in the direction of CO_2 and of lipid synthesis. Propionate and other short-chain acids have been shown to accelerate gluconeogenesis from lactate by kidney slices (13) and from pyruvate by the lactating cow (14). Pyruvate can serve as source of glycerophosphate in adipose tissue (15). Glycerophosphate formation from pyruvate has therefore to be considered as a possible means of the propionate effect on lipogenesis from acetate. Experiments along this line are reported in the following communication (16).

No explanation can, so far, be provided for the acceleration of fatty acid synthesis in tissues of actinomycin D-treated rats.

This study was supported by a grant from the National Institutes of Health, PHS, U.S.A. under section 104 (k) of P.L. 480.

Manuscript received 3 April 1967; accepted 8 August 1967.

REFERENCES

1. Feller, D. D., and E. Feist. 1957. *J. Biol. Chem.* **228:** 275.
2. Feller, D. D., and E. Feist. 1959. *J. Lipid Res.* **1:** 90.
3. Masoro, E. J., and E. Porter. 1961. *J. Lipid Res.* **2:** 177.
4. Favarger, P., and J. Gerlach. 1960. *Bull. Soc. Chim. Biol.* **42:** 327.
5. Annison, E. F., and D. Lewis. 1959. Metabolism in the Rumen. Methuen, London.

6. Lindsay, D. B. 1959. *Vet. Rev. Annotations*. **5:** 103.
7. Robinson, W. G., B. K. Bachawat, and M. J. Coon. 1956. *J. Biol. Chem*. **218:** 391.
8. Dole, V. P. 1956. *J. Clin. Invest*. **35:** 150.
9. Lardy, H. A., and R. Peanasky. 1953. *Physiol. Rev*. **33:** 560.
10. Masoro, E. J. 1965. *Ann. N.Y. Acad. Sci*. **131:** 199.
11. Vagelos, P. R., A. W. Alberts, and D. B. Martin. 1962. *Biochem. Biophys. Res. Commun*. **8:** 4.
12. Wise, E. M., Jr., and E. Ball. 1964. *Proc. Natl. Acad. Sci. U.S*. **52:** 1255.
13. Krebs, H. A., R. N. Speake, and R. Hems. 1965. *Biochem. J*. **94:** 712.
14. Black, A. L., J. Luick, F. Moller, and R. S. Anand. 1966. *J. Biol. Chem*. **241:** 5233.
15. Rose, G., and B. Shapiro. 1960. *Bull. Res. Council Israel*. **9A:** 15.
16. Reshef, L., J. Viv, and B. Shapiro. 1967. *J. Lipid Res*. **8:** 688.