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Propionic acid as a precursor in the biosynthesis of animal fatty acids*

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SUMMARY

The carboxyl carbon of propionate was found to be a poor precursor for the synthesis of fatty acids in the rat. The data indicate that propionate as a three-carbon unit is not incorporated into long-chain fatty acids by the intact rat to any appreciable extent. The results also suggest that the conversion of propionate to long-chain fatty acids in surviving adipose tissue occurs primarily by a mechanism involving the decarboxylation of the propionate. These data are consistent with the concept that the major pathway of lipogenesis in adipose tissue is not different from pathways described for the liver. A small fraction of the propionate is converted to long-chain fatty acids in adipose tissue *in vitro* by a mechanism that involves the use of propionate as an intact three-carbon unit, but in any case, its significance in the intact animal would appear to be negligible.

The liver was long considered as the major, if not the sole, site of lipogenesis in the mammalian organism (1). However, recently Favarger and Gerlach (2) and Favarger (3) have presented evidence that indicates that the liver does not play a major role in this process. These authors found that hepatic lipogenesis can account for only 4% of the total lipogenic activity of the mouse and that most of the remaining fatty acid synthetic activity is carried out in the adipose tissue.

In the last few years important advances in understanding the biochemical mechanism by which fatty acids are synthesized in the animal organism have been made (4, 5); however, most of these studies have been carried out on liver enzyme systems. It is of interest, therefore, that Feller and Feist (6, 7, 8) have presented considerable evidence indicating that adipose tissue can convert propionic acid as an intact three carbon unit into long-chain fatty acids, and have proposed a pathway of lipogenesis in adipose issue that is different from those described for liver (9). They also have shown that this pathway does not occur in the liver, because in this organ the carboxyl carbon of propionate is not incorporated into long-chain fatty acids. If all of the above assumptions are true and if the pathway proposed by Feller and Feist is a major one, it would be anticipated that most of the fatty acid synthesis in the intact animal would be found to occur via a pathway other than currently accepted ones. Since this pathway involves the use of propionic acid as a three-carbon unit, it would seem to be a corollary that administration of propionic acid labeled in the carboxyl position should lead to a large incorporation of the label into the body fatty acid.

METHODS

In Vivo Experiments. Male rats of the Wistar strain (inbred at Tufts for about 20 years) were kept at 25° for 10 days before the experiment and were allowed to eat the standard diet of the following composition: 25% casein, 10% lard, 50.95% glucose, 3% salt mix (10), 5% cellulose, 6% brewer's yeast, 0.04% cod liver oil concentrate, and 0.01% alpha tocopherol. The rats used in the experiments weighed 230 ± 10 g and two groups were fasted for 6 hours prior to the experiment. One group was given by stomach tube 1 g of triacetin plus 1.9 g dextrose containing a tracer dose of sodium acetate-1-C^{14}.[1] Another group was given 1 g of tripropionin plus 1.9 g dextrose containing sodium propionate-1-C^{14} in a tracer dose. The rats were then placed in a metabolism cage and their res-

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[1] The C^{14}-labeled compounds used in these experiments were purchased from the New England Nuclear Corporation, Boston, Mass.

piratory CO_2 was collected for 24 hours by the methods described in a previous paper (1). During the course of this metabolism study the rats had access to the diet described above.

The collected respiratory $C^{14}O_2$ was analyzed for its C^{14} content by a liquid counting method (11). At the end of the experiment the rats were killed by a blow on the head and the carcass was analyzed for lipids (12). The C^{14} content of the fatty acids was determined by pipetting 15 mg of the C^{14} fatty acids dissolved in $CHCl_3$ into a cupped nickel planchet and drying until the last trace of $CHCl_3$ had disappeared. The fatty acids were then counted at this mass and the counts were corrected to infinite thinness by a conversion factor. The C^{14} content of the labeled acetic acid and propionic acid was also measured (11).

A third group of rats was treated in a somewhat different manner. These rats were fed until the experiment was started. They were then injected intraperitoneally with 1 ml of saline containing either sodium acetate-1-C^{14} or sodium propionate-1-C^{14}. They were at once placed in wire cages and either fed or fasted. The respiratory $C^{14}O_2$ was not collected. The rats were sacrificed after 24 hours and the lipids isolated and analyzed from C^{14} content as described above.

In Vitro Experiments. Male rats of the above strain and male white Swiss mice (obtained from Mrs. Woodman, of Wayland, Mass.) were kept at 25° for 10 days on our standard diet. They were sacrificed by decapitation and the epididymal fat pad was excised and placed in a petri dish of Krebs-Henseleit bicarbonate buffer solution (13). A weighed piece of this adipose tissue (chosen randomly) was then placed into an incubation flask (14). The adipose tissue was kept at room temperature throughout this preincubation manipulation.

TABLE 1. METABOLISM OF GASTROINTESTINALLY-ADMINISTERED ACETATE-1-C^{14} AND PROPIONATE-1-C^{14} BY RATS

No. Rats Used*	Labeled Substrate†	Per Cent Labeled Substrate Converted to	
		CO_2	Fatty Acids
5	Acetate-1-C^{14}	78.8 ± 2.47‡	5.9 ± 0.75
5	Propionate-1-C^{14}	79.5 ± 7.07	0.3 ± 0.08

* Rats weighed 230 ± 10 g.

† 1 g of triacetin, 1.9 g of glucose and tracer dose of sodium acetate-1-C^{14} or 1 g of tripropionin, 1.9 g glucose and tracer dose of sodium propionate-1-C^{14} were administered by stomach tube; the rats were killed 24 hours later.

‡ S. E. (Note that in the case of the CO_2 analysis, the average value refers to only 4 rats.)

The main compartment of the incubation flask contained the adipose tissue, 4.5 ml of Krebs-Henseleit bicarbonate buffer containing glucose, 100 mg/100 ml, and 0.5 ml of saline containing 5 μmoles of the labeled substrate. The system was gassed for about 30 seconds with 95% O_2–5% CO_2 gas mixture, sealed and incubated for 3 hours at 37.5° with continuous shaking.

The $C^{14}O_2$ formed during the incubation was recovered by the method of Chernick *et al.* (15) and its C^{14} content was then determined (11).

At the end of the incubation the adipose tissue was removed from the incubation flask and saponified in 30% KOH made up in a solvent of 50% ethanol. The fatty acids were then extracted from this hydrolyzate of adipose tissue by a method described earlier (16). The C^{14} content of the fatty acid was determined by a previously described method (17).

The C^{14} content of the labeled acetic acid and propionic acid was then determined (11).

RESULTS

The results of experiments in which rats were given by stomach tube either (*a*) sodium acetate-1-C^{14}, triacetin, glucose, or (*b*) sodium propionate-1-C^{14}, tripropionin, glucose, are recorded in Table 1. More than 75% of the C^{14} content of each of the labeled compounds appeared in the respiratory CO_2 during the 24 hours of the experiment. Significant quantities of C^{14} from the acetate-1-C^{14} appeared in the long-chain fatty acids of the body while C^{14} from propionate 1-C^{14} was almost completely absent. These results were surprising in light of the *in vitro* mouse experiments of Feller and Feist (6 to 9), and it seemed possible that these findings were due to the trapping by the liver of the labeled propionate entering it from the portal vein, thus preventing the propionate from reaching the adipose tissue. To circumvent this possibility to some extent, carboxyl-labeled propionate was administered intraperitoneally (Table 2), and the rats were then either fed or fasted for 24 hours. Very little of the C^{14} of the administered propionate-1-C^{14} appeared in the body's fatty acids. In a recently published investigation (18) Feller reported that about 2% of the C^{14} of intraperitoneally administered carboxyl-labeled propionate was found in the body fatty acids of mice fasted for 24 hours following the isotope administration.

Since the results of our *in vivo* studies are not consistent with the *in vitro* results of Feller and Feist (6 to 9), it was decided that the *in vitro* system should be reinvestigated. As shown in Table 3, rat liver is able to convert large amounts of acetate-1-C^{14} into fatty

TABLE 2. METABOLISM OF INTRAPERITONEALLY-ADMINISTERED PROPIONATE-1-C^{14} BY RATS

Nutritional State of the Rat Following Propionate-1-C^{14} Administration*	Per Cent Propionate-1-C^{14} Converted to Fatty Acids
Fed	0.5
Fed	0.3
Fasted	0.2
Fasted	0.1

* Rats weighed 230 ± 10 g; propionate-1-C^{14} was administered intraperitoneally in a tracer dose and the rats were killed 24 hours later.

acids but is unable to convert any of the carboxyl of propionate into long-chain fatty acids. On the other hand, while adipose tissue uses considerable amounts of the carboxyl carbon of acetate for lipogenesis, it uses very little of the carboxyl carbon of propionate for the purpose.

This finding led to an investigation of the conversion of propionate-1-C^{14}, propionate-2-C^{14}, and propionate-3-C^{14} to long-chain fatty acids by surviving adipose tissue (Table 4). Propionate-2-C^{14} and propionate-3-C^{14} entered fatty acids to a considerable extent, and at practically the same rate. In contrast, the conversion of propionate-1-C^{14} was very small. Since these results are not in agreement with those of Feller and Feist (7) in their studies with mice, propionate-1-C^{14} and propionate-2-C^{14} metabolism experiments were carried out on mouse adipose tissue (Table 5). It can be seen that in the mouse, as in the rat, propionate-2-C^{14} appears to be a much better substrate than propionate-1-C^{14} for lipogenesis.

The incubation medium used by Feller and Feist differs from ours in two particulars, namely, it contains more glucose than our medium and it contains 0.01 M succinate while our medium contains none. Therefore experiments using the succinate-containing medium were carried out (Tables 6 and 7). Although the results are not quantitatively identical to those found in our medium, it can be seen that propionate-2-C^{14} is still a better precursor of long-chain fatty acids than is propionate-1-C^{14}. It should be noted that the succinate depressed propionate metabolism in the case of rat adipose tissue, but that it promoted mouse adipose tissue lipogenesis, while still not abolishing the difference in the behavior of the 1 and 2 carbons.

TABLE 3. ACETATE-1-C^{14} AND PROPIONATE-1-C^{14} METABOLISM BY SURVIVING RAT LIVER SLICES AND ADIPOSE TISSUE

No. Rats Used*	Labeled Substrate†	Tissue Used‡	Per Cent C^{14} Converted to	
			CO_2	Fatty Acids
5	Acetate-1-C^{14}	Liver	33.7 ± 2.61§	4.8 ± 1.51
	Propionate-1-C^{14}	Liver	66.2 ± 1.46	0.0 ± 0.0
	Acetate-1-C^{14}	Adipose tissue	5.8 ± 0.32	4.0 ± 1.12
	Propionate-1-C^{14}	Adipose tissue	14.8 ± 0.77	0.6 ± 0.09

* Each substrate was incubated with liver and adipose tissue from each of the five rats; therefore results are a comparison of incubation flasks, not a comparison of different rats.

† The incubation system contained the tissue in 5 ml of Krebs-HCO_3^- buffer containing 100 mg/100 ml glucose and 5 μmoles of the labeled substrate; system was incubated at 37.5° for 3 hours.

‡ 250 mg of tissue used.

§ S. E.

TABLE 4. PROPIONATE METABOLISM BY SURVIVING RAT ADIPOSE TISSUE

No. Rats Used*	Labeled Substrate†	Per Cent‡ C^{14} Converted to	
		CO_2	Fatty Acids
5	Propionate-1-C^{14}	19.9 ± 3.06§	0.9 ± 0.25
	Propionate-2-C^{14}	7.0 ± 0.94	7.5 ± 1.50
	Propionate-3-C^{14}	7.0 ± 0.88	7.6 ± 1.50

* Each substrate was incubated with adipose tissue from each of the five animals; therefore results are a comparison of incubation flasks, not a comparison of different animals.

† See footnote, Table 3.

‡ 250 mg of adipose tissue were used in each flask.

§ S. E.

DISCUSSION

The experiments presented above indicate that the

TABLE 5. PROPIONATE METABOLISM BY SURVIVING MOUSE ADIPOSE TISSUE

No. Mice Used*	Labeled Substrate†	Per Cent‡ C^{14} Converted to	
		CO_2	Fatty Acids
5	Propionate-1-C^{14}	17.6 ± 1.46§	1.7 ± 0.32
	Propionate-2-C^{14}	6.7 ± 0.58	7.3 ± 0.61

* See footnote, Table 4.

† See footnote, Table 3.

‡ 200 mg of adipose tissue used in each flask.

§ S. E.

TABLE 6. PROPIONATE METABOLISM BY SURVIVING RAT ADIPOSE TISSUE INCUBATED IN A SUCCINATE-CONTAINING MEDIUM

No. Rats Used*	Labeled Substrate†	Per Cent‡ C^{14} Converted to	
		CO_2	Fatty Acids
5	Propionate-1-C^{14}	7.2 ± 0.30§	0.6 ± 0.06
	Propionate-2-C^{14}	3.3 ± 0.30	2.3 ± 0.35

* See footnote, Table 4.

† The incubation system contained the tissue in 5 ml of the incubation medium of Feller and Feist (7) containing 5 μmoles of the labeled substrate. The system was incubated at 37.5° for 3 hours.

‡ 250 mg of tissue were used.

§ S. E. (Note that the value of 7.2 refers to only 4 rats.)

carboxyl carbon of propionate is not used to an appreciable extent in the biosynthesis of long-chain fatty acids in the intact rat. As a corollary of this finding, it can be concluded that in the intact organism a very small fraction of fatty acid synthesis occurs via a pathway utilizing propionate as an intact molecule. This is true whether the propionic acid is ingested or is administered to the animal by intraperitoneal injection.

In the case of surviving adipose tissue, it is evident that the carboxyl carbon of acetate is far more readily incorporated into long-chain fatty acids than is the carboxyl label of propionate. Furthermore, on the basis of varying the position in which propionate is labeled, it is evident that propionate-2 or -3 carbons are far better precursors of long-chain fatty acids than is the carboxyl carbon. It seems evident, therefore, that even in adipose tissue studied *in vitro*, most of fatty acid synthesis is not occurring via a pathway that involves the conversion of the propionate molecule as a unit into long-chain fatty acids, but rather by pathways not inconsistent with those described for the liver by Brady (4) and Wakil *et al.* (5).

TABLE 7. PROPIONATE METABOLISM BY SURVIVING MOUSE ADIPOSE TISSUE INCUBATED IN A SUCCINATE-CONTAINING MEDIUM

No. Mice Used*	Labeled Substrate†	Per Cent‡ C^{14} Converted to	
		CO_2	Fatty Acids
5	Propionate-1-C^{14}	15.7 ± 1.26§	3.1 ± 0.60
	Propionate-2-C^{14}	4.4 ± 0.10§	15.5 ± 1.85

* See footnote, Table 4.

† See footnote, Table 6.

‡ 100 or 150 mg of tissue used; all values are corrected to that of 100 mg of tissue.

§ S. E. (Note that the value of 4.4 refers to only 4 mice.)

It will be clear that some of the data presented in this paper lead to conclusions which are at variance with those advanced by Feller and Feist (7, 8, 18). In the case of the *in vivo* experiments, the possibility existed that the variance could be attributed to species difference since Feller (18) used mice and we used rats. However, in the case of the *in vitro* experiments, similar results were obtained by us with mice as with rats. It is true that minor differences between our technique and those of Feller and Feist exist, such as the composition of the diet, the region of the body from which the fat was taken, and the method of slicing and chilling the tissue. These differences, however, were not of a nature, it seems to us, to explain the discrepancies in our results.

Our data with propionate-1-C^{14} indicate that in isolated adipose tissue a small fraction of the propionate enters long-chain fatty acids by a mechanism involving the use of propionate as an intact three-carbon unit. This finding is compatible with the possibility that the pathway suggested by Feller and Feist (9) is operative to a limited extent in adipose tissue. However, it seems probable from our data that such a pathway has little or no activity in the intact organism.

Since the submission of this manuscript for publication, Favarger and Gerlach (19) have published a report of their studies on the synthesis of fatty acids from propionate by the intact mouse. They find that three times as much C^{14} is incorporated into fatty acids when propionate-2-C^{14} is administered intravenously to mice than when propionate-1-C^{14} is used. Propionate-1-C^{14} is primarily incorporated in the odd-chain fatty acids and on basis of degradation studies the authors have concluded that intact propionate molecules are incorporated in the ω position of odd-chain acids and that the 2 and 3 carbons of propionate are incorporated via acetyl-CoA throughout the carbon skeleton of odd- and even-chain fatty acids. Our results are completely compatible with the findings and conclusions of Favarger and Gerlach.

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