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Biosynthesis of Fatty Acids in Perfused Hearts of Rats Fed a Fat-Supplemented or a Fat-Free Diet[1] (39620)

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The heart is dependent on lipid metabolism for structural components as well as for some of its caloric requirements. Fatty acids for these needs are furnished to the heart from absorbed dietary lipids, from metabolic products of these absorbed lipids, and from lipids synthesized or interconverted from other components by the heart itself. The types of fatty acids biosynthesized and the lipids into which these fatty acids are incorporated are, therefore, of interest. As part of a continuing investigation of lipid metabolism in the heart, we have made such studies in the rat heart perfused with labeled acetate or butyrate. Because dietary factors influence many aspects of lipid metabolism, we have used hearts from rats fed a fat-free diet as well as from rats fed the same diet supplemented with corn oil.

Methods and materials. A purified diet containing 66% sucrose, 24% casein, 3% minerals, 5% corn oil, and complete vitamin mix was used as the fat-supplemented diet. For the fat-free diet, the corn oil was omitted and an equal weight of sucrose was substituted. Male, weanling Sprague-Dawley rats were maintained on either of these diets for 3 to 6 months before the perfusion studies were done. Perfusion with a recirculating system was done as reported previously (1).

Methods for the extraction of total lipids and separation into lipid classes and for the isolation of fatty acids after hydrolysis of total lipids have been described (2). Lipid classes were separated by thin-layer chromatography using silica gel HF_{254} (E. Merck, West Germany) and the solvent system: petroleum ether-diethyl ether-glacial acetic acid (80:20:1, by volume). Bands of silica gel containing the individual components were immediately scraped from the plates and the lipids were eluted according to the technique of Biezenski (3). The recovery of radioactivity from the plates was greater than 85% in all cases and usually greater than 90% of the amount applied to the plates. Gas-liquid chromatography and gas-liquid radiochromatography were done as described previously (4).

Perfusion of [1-^{14}C]sodium acetate and of [1-^{14}C]sodium butyrate was done using a concentration of 5 m*M* and 20 μCi of radioactivity in 25 ml of perfusion medium (oxygenated Krebs-Henseleit bicarbonate buffer, pH 7.4, containing glucose at a concentration of 5 m*M*). Perfusion was carried on for 2 hr in an apparatus similar to that described by Morgan *et al.* (5).

Results. Body and heart weights of rats fed the fat-free diet were lower than those of rats fed the fat-supplemented diet (body weight averages: 248 vs 344 g; heart weight averages: 1.74 vs 2.03 g). Incorporation of [1-^{14}C]acetate into fatty acids of perfused hearts was not significantly different from the value of 0.2 to 0.3% of the added substrate reported previously (1), and there was no difference between hearts of rats fed the different diets. Incorporation of [1-^{14}C]butyrate averaged 0.44 $\pm$ 0.12 in the rats fed the supplemented diet versus 0.81 $\pm$ 0.13% of added substrate in hearts of rats fed the fat-free diet. This difference was of borderline statistical significance with a *P* value of 0.07.

The distribution of radioactivity in the various lipid classes of hearts of rats fed the fat-supplemented diet is shown in Table I. A greater percentage of the ^{14}C incorporated into total lipids was incorporated into phospholipids when [^{14}C]acetate was the substrate than when [^{14}C]butyrate was the substrate. ^{14}C from the latter was incorporated into all other lipid fractions to a greater extent than [^{14}C]acetate. However, the differences in values for cholesteryl esters and for unesterified fatty acids were not statistically significant.

With [^{14}C]acetate as substrate, no differ-

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TABLE I. DISTRIBUTION OF ^{14}C IN LIPIDS OF HEARTS OF RATS FED A FAT-SUPPLEMENTED DIET AND PERFUSED WITH [1-^{14}C]ACETATE OR [1-^{14}C]BUTYRATE

	Total ^{14}C (%)				
Substrate	Phospholipids	Cholesterol plus diglycerides	Unesterified fatty acids	Triglycerides	Cholesteryl esters
[1-^{14}C]Acetate (3)[a]	74.4 ± 3.7[b]	1.6 ± 0.17	5.1 ± 1.5	15.1 ± 3.8	0.4 ± 0.12
[1-^{14}C]Butyrate (6)	52.3 ± 1.5	4.3 ± 0.35	13.0 ± 2.7	23.4 ± 1.7	1.0 ± 0.27
P value	<0.01	0.03	n.s.[c]	0.05	n.s.

[a] (*n*) = Number of animals.
[b] Mean ± SE.
[c] n.s. = Not significant.

TABLE II. DISTRIBUTION OF ^{14}C IN FATTY ACIDS OF HEARTS OF RATS FED A FAT-SUPPLEMENTED DIET AND PERFUSED WITH [1-^{14}C]ACETATE OR [1-^{14}C]BUTYRATE

	^{14}C in total fatty acids (%)							
Substrate	<14:0	14:0	16:0 16:1	18:0 18:1	18:3 20:1	20:2	20:4	>20:4
[1-^{14}C]Acetate (3)[a]	12.1 ± 0.35[b]	9.7 ± 1.6	32.3 ± 5.1	27.2 ± 0.96	3.1 ± 0.23	2.4 ± 0.23	1.9 ± 0.40	2.4 ± 0.23
[1-^{14}C]Butyrate (6)	31.8 ± 4.0	11.6 ± 0.67	20.8 ± 1.8	25.0 ± 1.6	2.3 ± 0.71	3.6 ± 1.2	0.7 ± 0.24	1.6 ± 0.55
P value	0.01	n.s.[c]	0.04	n.s.	n.s.	n.s.	0.04	n.s.

[a] (*n*) = Number of animals.
[b] Mean ± SE.
[c] n.s. = Not significant.

ences were observed between rats fed the supplemented and the fat-free diets with respect to relative incorporation of ^{14}C into the different lipid classes. With [^{14}C]-butyrate as substrate there was more ^{14}C incorporated into phospholipids of hearts of rats fed the fat-free than in those fed the fat-supplemented diet (62.5 ± 2.8 vs 52.3 ± 1.5% of total ^{14}C incorporated; P = 0.01). Although no other differences were statistically significant, less ^{14}C was present in the triglycerides of the deficient compared to those of the supplemented group.

The relative distribution of the incorporated ^{14}C into the individual fatty acids of perfused hearts of rats fed the supplemented diet is given in Table II. Use of [^{14}C]-butyrate as substrate resulted in more ^{14}C incorporation into shorter chain fatty acids (<14:0)[2] and less ^{14}C incorporation into palmitic acid (16:0) and into arachidonic acid (20:4) than use of [^{14}C]acetate. In the combined 16:0, 16:1 fraction nearly all the [^{14}C]acetate was in 16:0, while in the combined 18:0, 18:1 fraction the activity was divided about equally between the saturated acid and the monoene.

[2] Number of carbon atoms in chain: number of double bonds.

With either [^{14}C]acetate or [^{14}C]butyrate as substrate there were no statistically significant differences between rats fed the fat-supplemented and those fed the fat-free diet in the distribution of ^{14}C into individual fatty acids.

Biochemical signs of deficiency were evident in the hearts of the rats fed the fat-deficient diets. About 20% of the phospholipid and about 7% of the triglyceride fatty acids were 20:3w9, the fatty acid which accumulates in essential fatty acid deficiency. No measurable amount of this fatty acid was present in the heart lipids of the rats fed the fat-supplemented diet.

Discussion. The main difference in the incorporation of these two ^{14}C-labeled substrates into perfused heart lipids was the relatively greater incorporation of [^{14}C]-acetate into phospholipids and the greater incorporation of [^{14}C]butyrate into triglycerides. The use of [^{14}C]butyrate as substrate resulted in less ^{14}C in palmitic and in arachidonic acids while [^{14}C]butyrate yielded more ^{14}C-shorter chain acids than did [^{14}C]acetate. It is known that in the perfused heart of the rat, [^{14}C]acetate can be incorporated into fatty acids both by the *de novo* and elongation pathways (1). The manner by which ^{14}C from butyrate enters

longer chain fatty acids is not known, but presumably the butyrate may be oxidized to acetyl CoA units which then can form fatty acids, or butyrate itself may be elongated to longer chain fatty acids.

Lipogenesis in some tissues is affected by such factors as diet and conditions such as diabetes. Whereat and Orishimo observed that the heart mitochondrial system for synthesis of fatty acids was not affected by fasting and by diabetes (6). It is not known whether cytosol synthetic activity in heart tissue is subject to the same regulations present in other tissues. Likewise, the effect of essential fatty acid deficiency on fatty acid synthesis by heart mitochondria or microsomes apparently has not been investigated (7). Our studies with perfused heart indicate that the feeding of a fat-free diet, though it resulted in a decrease in arachidonic acid and an increase in 5,8,11-eicosatrienoic acid in the heart, apparently did not affect the metabolism of the [^{14}C]acetate perfused for 2 hr. In the case of the butyrate, however, there was increased incorporation in hearts of rats fed the fat-free diet, a result which is similar to that seen with cytosol fatty acid-synthesizing systems of organs such as the liver. In rats fed the fat-supplemented diets, more of the ^{14}C incorporated into lipids was in phospholipids than was observed in rats fed the fat-free diet. However, there was no effect on the individual fatty acids synthesized. It was observed that the hearts of rats fed the fat-free diet had a weaker contraction and a slightly lower rate of contraction than did hearts from rats fed the fat-supplemented diet. Although this is not a precise quantitative observation, it is interesting in view of the finding reported by Caster and Ahn (8) of a notch in the QRS complex of the electrocardiograms of rats fed diets deficient in essential fatty acids. The notching was prevented by the addition of linoleate, linolenate, or arachidonate to the diet.

Summary. Incorporation of ^{14}C from [1-^{14}C]butyrate into lipids of the perfused rat heart was slightly greater than that from [1-^{14}C]acetate. Of the ^{14}C incorporated into total lipids, a greater proportion was in phospholipids when [^{14}C]acetate was the substrate than when [^{14}C]butyrate was used as substrate. Perfusion with [^{14}C]butyrate resulted in more ^{14}C incorporation into shorter chain fatty acids and less incorporation into palmitic and arachidonic acids than with [^{14}C]acetate. Feeding a fat-free diet did not affect the metabolism of acetate in the perfused heart. However, it did increase the relative incorporation of ^{14}C from butyrate into phospholipids as well as the total ^{14}C incorporation into total lipids of perfused hearts of rats compared to those fed a fat-supplemented diet.

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