Metabolism and Lipogenic Effects of the Cyclic Monomers of Methyl Linolenate in the Rat

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ABSTRACT

Cyclic fatty acids are absorbed by the rat, partially oxidized to CO_2, and a portion of the compound, presumably the ring structure, is excreted in the urine. Studies with uniformly labeled cyclic fatty acids showed that ca. 13-15% of $^{14}CO_2$ is expired by the animal in 48 hr with peak expiration occurring between 4-6 hr after ingestion. Approximately 40% of the total radioactivity is found in the urine after 48 hr with about 60% of that being excreted within 12 hr after ingestion. Decreased rates of lipogenesis were observed in livers of animals fed 8% and 10% protein and higher levels of cyclic fatty acids. An increased rate of lipogenesis was observed in adipose tissue of animals fed 10% protein and higher levels of cyclic fatty acids.

INTRODUCTION

Previous nutritional studies have shown that adverse physiological conditions were produced when varying amounts of cyclized fatty acids (or methyl esters) were present in the diets of experimental animals (1-7). In all of these studies, either an oil rich in cyclic fatty acids or a concentrated cyclic fatty acid fraction of the oil was fed to the animals. Studies conducted in this laboratory have shown that very low levels of methyl ω(2-alkyl cyclohexadienyl) carboxylic acids (cyclic fatty acids) incorporated into nutritionally adequate diets generally produced lowered weight gains and feed consumption in rats fed low levels of protein compared to control animals (8). Animals fed as low as 0.15% of this cyclic material in diets with low levels of protein (8-10%) also developed fatty livers (8). The present experiments describe the effects of dietary cyclic fatty acids on the metabolism of uniformly labeled cyclic fatty acids and on the rate of in vivo and in vitro lipogenesis in the rat.

MATERIALS AND METHODS

Experimental Diets and Animals

Male weanling SPF albino rats of Sprague-Dawley descent were used for all experiments and housed in galvanized iron wire cages with mesh bottoms. Animals had free access to water and different semipurified diets [Diet composition: (g/100g diet) casein, 8, 10, or 15; Wesson salts, 3.5; vitamin premix, 1.0; choline chloride, 0.15; corn oil containing either 0, 0.05, 0.15, or 1.0% cyclic fatty acid methyl ester to a total oil level of 15%, dextrose, to 100. Vitamin premix, (mg vitamin/kg diet): retinyl acetate, 20,000 IU; ergocalciferol 2,000 IU; α-tocopherol, 100 IU; ascorbic acid, 22; inositol, 112; menadione bisulfite, 58; para-amino benzoic acid, 22; riboflavin, 22; pyridoxine HCl, 22; calcium penthothenate, 67; thiamine HCl, 100; biotin, 0.45; folic acid, 4; cyanocobalamine, 0.03.] The animals were fed these diets until their weights reached 180-200 g.

Synthesis of Uniformly Labeled Cyclic Fatty Acids

Uniformly labeled cyclic monomers were synthesized from uniformly labeled linolenic acid (600 μCi, 720 μCi/mM) (DHOM Laboratories, North Hollywood, CA) according to a previously described general method (9).

After purification, the uniformly labeled cyclic fatty acids obtained (as the methyl esters) had a specific activity of about 500 μCi/g. The cyclic compound was diluted in corn oil to a specific activity of 59 μCi/g when fed to experimental animals. Uniformly labeled methyl linoleate (DHOM Laboratories, North Hollywood, CA) was diluted with pure methyl linoleate to a specific activity of 516 μCi/g and further diluted in fresh corn oil to 49 μCi/g. Chemical and radiochemical purity of cyclic fatty acids and methyl linoleate was checked by thin layer and gas chromatography.

Procedure for In Vivo Catabolism

Animals used in this experiment were fed complete diets as described previously containing 0, 0.0075, 0.0225, or 0.1500% cyclic esters. Each experiment used two control animals fed no cyclic esters and one animal each fed a different level of the cyclic esters (5 animals/experiment/5 experiments). Each animal was weighed, put under slight ether anesthesia, and uniformly labeled cyclic fatty acid esters diluted in corn oil (0.2 ml) were administered orally to one control and to each of the rats which had been fed different levels of cyclic esters in the diet. The other control animal was fed 0.2 ml of uniformly labeled methyl linoleate diluted in corn oil. The animals were then placed in all-glass metabolic cages for 48 hr. Exhaled carbon dioxide was freed of moisture by passing it through a series of four tubes containing $CaSO_4$. The moisture-free air was then allowed to bubble through a fritted glass tube into 150 ml test tubes containing 100 ml of distilled ethanolamine-ethylene glycol monomethyl ether solution (1:2 v/v) (10). The trapping solution was changed every 8 hr to assure complete collection of radioactive carbon dioxide. The trapping solution (3 ml) was removed from each test tube every 2 hr for 24 hr with a volumetric pipette and placed in a scintillation vial which contained 15 ml of scintillation solution (9). The samples were then counted in a liquid scintillation spectrometer. Urine from each animal was collected every 12 hr from the bottom of the cage, and the collecting apparatus was rinsed twice with small portions of distilled water. The urine was then diluted with distilled water to 25 ml, and one ml was removed, placed in a scintillation vial containing 9 ml of Aquasol (New England Nuclear, Boston, MA), and counted. At the end of 48 hr, the animals were killed, and the stomach, small intestine, caecum, epididymal fat pad, perirenal fat, and livers quickly were excised from each rat and stored at 0 C for further analysis. The feces trapped on a wire screen in the metabolic cages were also collected and stored in the same manner.

Acetate Metabolism

Rats fed 8, 10, and 15% protein (casein) and different levels of cyclic fatty acids as part of a complete diet as described earlier were utilized for these experiments.

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TABLE I

Percentage of Administered Radioactivity Recovered after 48 Hours in CO_2 and in Urine from Rats Fed 15% Protein and Various Levels of Cyclic Fatty Acids[a]

Group	% Cyclic monomer in diet	Substance administered	% Radioactivity recovered in urine[b]	% Radioactivity recovered in CO_2[c]
A	0.0000	Methyl linoleate	1.1 ± 0.1	35.8 ± 4.3
B	0.0000	Cyclic fatty acid	40.5 ± 2.9	13.4 ± 2.0
C	0.0075	Cyclic fatty acid	37.3 ± 2.7	14.4 ± 1.0
D	0.0225	Cyclic fatty acid	40.9 ± 0.8	15.1 ± 1.4
E	0.1500	Cyclic fatty acid	42.3 ± 2.2	13.1 ± 1.7

[a]As the methyl ester.
[b]Mean total percentage of four rats ± SEM.
[c]Mean total percentage of three rats ± SEM.

TABLE II

Percentage of Total Expired $^{14}CO_2$ Collected in 2 Hour Intervals from Rats Fed 15% Protein and Different Levels of Monomers[a]

Time (hours)	% Cyclic monomer fed in diet				
	0.000[b]	0.000	0.0075	0.0225	0.1500
2	6.3 ± 2.7	12.6 ± 1.8	13.4 ± 2.0	9.2 ± 4.0	15.0 ± 2.0
4	24.6 ± 5.8	23.0 ± 8.3	23.6 ± 4.8	13.8 ± 1.2	23.4 ± 6.7
6	18.8 ± 4.4	19.6 ± 3.3	18.7 ± 4.9	13.9 ± 6.0	16.0 ± 3.6
8	8.5 ± 1.0	11.5 ± 2.4	8.2 ± 1.8	10.4 ± 1.0	10.1 ± 2.2
10	4.5 ± 0.7	5.8 ± 1.2	6.9 ± 2.8	8.9 ± 3.0	4.2 ± 0.9
12	4.8 ± 0.5	3.9 ± 0.4	5.4 ± 0.4	5.8 ± 1.3	3.7 ± 0.6
14	3.9 ± 0.8	2.8 ± 0.1	3.2 ± 0.6	5.4 ± 0.4	3.7 ± 1.0
16	3.3 ± 0.6	2.8 ± 0.3	2.6 ± 0.1	3.6 ± 0.5	3.8 ± 0.8
18	1.6 ± 0.5	1.9 ± 0.3	2.0 ± 0.2	1.9 ± 0.1	2.4 ± 0.5
20	2.0 ± 0.6	1.7 ± 0.4	1.2 ± 0.2	1.3 ± 0.4	2.6 ± 0.9
22	2.0 ± 0.1	1.6 ± 0.6	1.5 ± 0.3	1.6 ± 0.2	2.5 ± 0.6
24	2.0 ± 0.1	2.0 ± 0.1	1.5 ± 0.4	1.4 ± 0.2	1.2 ± 0.4
% Expired during 24 hr	83.2	89.2	88.2	77.2	88.6

[a]Mean of three animals ± SEM.
[b]Fed uniformly labeled methyl linoleate.

Sodium-1-^{14}C-acetate (250 μCi, 59 mCi/mM) was dissolved in saline solution (0.9%) in a 25 ml volumetric flask. Each set of three animals was individually weighed and injected intraperitoneally with 1.0 ml of sodium acetate-1-^{14}C (10 μCi/ml). Each rat was immediately placed in an all-glass metabolic cage, and CO_2 was collected as described above. The animals were kept in the metabolic cages for 3 hr, then killed, and their livers were quickly excised, blotted, weighed, and stored at 0 C.

In Vitro Metabolism

Rats used in this study were fed 8, 10, and 15% protein diets and different levels of cyclic fatty acids as part of a complete diet as described earlier. Assays for fatty acid synthesis in vitro were conducted on liver and epididymal adipose tissue. The rats were killed just prior to an experiment by cervical dislocation followed by decapitation, and the liver and adipose tissues were quickly removed. Adipose tissues were kept at 37 C in saline (0.9%), and liver tissue was kept in saline on ice. The adipose tissues were cut into thin sections, and the liver was sliced with a Stadie-Riggs hand microtome. Tissue slices (150-200 mg) were transferred to 25 ml Erlenmeyer flasks containing 3 ml of Krebs Ringer bicarbonate buffer (pH 7.4). The buffer contained 10 mM acetate, 5 mM glucose, 0.3 units of insulin, and 1 μCi of radioactive substrate. Each flask was flushed with 95% O_2 and 5% CO_2 and stoppered. Incubations were carried out in a shaking water bath at 37 C for 3 hr. At the end of the incubation, the contents of the flask were saponified by adding 5 ml of 10% KOH in methanol and heating under reflux for 3 hr. After cooling, the mixture was acidified with 50% HCl and the fatty acids were then extracted three times with petroleum ether. The petroleum ether extracts were combined and washed with an equal volume of 1N acetic acid solution to eliminate the residual radioactive substrate. The petroleum ether was evaporated, and the remaining lipids were dissolved in scintillation solution and counted with a liquid scintillation counter.

Thin Layer Chromatography

Liver lipids were separated on glass plates (20 cm x 20 cm) coated with 0.5 mm Silica Gel G. The solvent system employed was hexane-diethyl ether-glacial acetic acid (90:10:1). Separated lipid classes were visualized by spraying lightly with 2'7'dichlorofluoroscein (0.2% in 95% ethanol) and dried under untralviolet light to determine the position of the bands. Bands were scraped directly into scintillation vials containing 10 ml of Brays solution (11) and shaken and counted in a liquid scintillation counter.

RESULTS AND DISCUSSION

Metabolism of Uniformly Labeled Cyclic Fatty Acids

The metabolism of the radioactive cyclic fatty acids was studied to determine the rate of CO_2 expiration, the rate of appearance of radioactive urinary metabolites, and the distribution of radioactivity in the lipids of selected tissues.

The percentages of administered radioactivity recovered in both urine and $^{14}CO_2$ are shown in Table I. Animals fed

TABLE III

Percentage of the Total Radioactivity in the Urine in 12 Hour Intervals for 48 Hours after Administration of Labeled Cyclic Monomer[a,b]

Hours	% Cyclic monomer fed in the diet			
	0.0000	0.00075	0.0225	0.1500
12	25.8 ± 3.0	22.9 ± 1.7	23.5 ± 1.7	24.9 ± 1.4
24	6.2 ± 0.9	6.0 ± 0.8	7.8 ± 0.6	7.3 ± 0.7
36	3.7 ± 0.4	4.0 ± 0.3	5.2 ± 0.8	4.6 ± 0.6
48	2.7 ± 0.4	2.7 ± 0.4	2.4 ± 0.4	3.3 ± 0.6

[a]Mean % values ± SEM.
[b]Averages of five animals.

TABLE IV

Percentage of Administered Radioactivity Recovered from Lipids of Selected Organs 48 Hours after Administration of Labeled Cyclic Monomer[a]

Group	Radioactive material	% Cyclic monomers in diet	Stomach (%)	Small intestine (%)	Caecum (%)	Liver (%)	Epididymal fat (%)	Perirenal fat (%)
A	Methyl linoleate	0.0000	0.22 ± 0.2[b]	1.10 ± .16	1.24 ± .21[b]	1.66 ± .18	1.16 ± .29	2.46 ± .09[b]
B	Cyclic ester	0.0000	0.28 ± .11[b]	0.45 ± .12	0.88 ± .15[b]	0.84 ± .17	0.27 ± 1.2	0.31 ± .10[b]
C	Cyclic ester	0.0075	0.26 ± .07[b]	0.41 ± .08	0.81 ± .06[b]	0.79 ± .07	0.64 ± .21	0.99 ± .23[b]
D	Cyclic ester	0.0225	0.27 ± .14	0.39 ± .07	1.00 ± .17[b]	0.99 ± .12	0.31 ± .06	0.39 ± .08
E	Cyclic ester	0.1500	0.24 ± .06	0.42 ± .05	1.09 ± .15	1.27 ± .14	0.29 ± .08	0.65 ± .20

[a]Mean total percentage ± SEM of five animals.
[b]Mean of four animals.

radioactive cyclic fatty acids excreted ca. 40% of the total counts in the urine and elminiated 13-15% as $^{14}CO_2$. No significant trend was seen which correlated the previous dietary conditioning of the animal with different levels of cyclic fatty acids and the amount of the total radioactivity recovered in the urine or expired ad $^{14}CO_2$.

Distributions of the expired $^{14}CO_2$ for each 2-hr period after administration of the radioactive samples are shown in Table II. It was observed that peak expiration of $^{14}CO_2$ took place between 4-6 hr for both the control animals and two of the three groups of animals consuming different levels of cyclic esters in the diet. Approximately 70% of the total $^{14}CO_2$ expired occurred within the first 12 hr and ca. 85% was expired after 24 hr for all groups of animals. Although the percent distribution of expired $^{14}CO_2$ between 4-12 hr was approximately the same for all groups of animals (Table II), the absolute amounts of radioactivity were different between the animals fed linoleate and those fed cyclic monomers (see Table I, last column). However, since the peak expiration values occurred at the same time, it was probable that absorption, transport, and the rate of fatty acid catabolism in various tissues were similar. The lower amount of actual CO_2 recovered from the animals fed cyclic fatty acids would be expected since β-oxidation processes would terminate when the ring was reached.

The appreciable absorption followed by degradation of cyclic fatty acids observed by the appearance of expired $^{14}CO_2$ can be seen further by noting the radioactivity recovered in the urine of animals fed radioactive cyclic fatty acids (Table III). For all sets of animals, over 60% of the total radioactivity in the urine was excreted from 0 to 12 hr. However, there was no significant difference among the values obtained in the urine. The excretion rates were similar for the conditioned and nonconditioned animals probably because of the small amounts of cyclic fatty acids used in the diets. Administration of a larger single dose or successive doses might result in a higher excretion rate in animals fed different levels of cyclic acids primarily because of a greater enzyme capacity to attack the cyclic acids (7).

The radioactivity recovered from the total lipids of selected organs 48 hr after administration of labeled cyclic monomers is shown in Table IV. It was not possible to determine whether the radioactivity found in the gastrointestinal tract was due to unabsorbed incubated material or to lipid that was resynthesized from radioactive acetate obtained from the degradation of cyclic compounds. Radioactivity was higher in the epididymal and perirenal fats for the methyl linoleate fed animals possibly because of the direct transport and storage of the unchanged acid. Lower amounts of radioactivity were found in these adipose tissues for rats fed cyclic fatty acids. This may be due to direct storage of unchanged cyclic acids or resynthesized "normal" lipids. Specific accumulation of radioactivity in the liver or in other selected tissues did not seem to occur, whereas nonspecific or random incorporation of unchanged cyclic fatty acids into various body lipids was possible as observed by Van Tilborg et al. (13).

Results of Van Tilborg et al. (13) obtained with aromatic fatty acids revealed that only minor differences were observed between adapted and nonadapted animals. Radioactivity recovered in the urine showed 89% for the adapted and 93% for the nonadapted animals after 30 hr. Metabolites excreted by the rats fed tritiated aromatic acids were partially identified and include ω,ω' dicarboxylic acids, phenyl acetate, or propionate with *o*-methyl, *o*-ethyl, and *o*-propyl side chains and hydroxy and dihydroxy cyclic acids. Van Tilborg et al. (13) postulated from their study that ω-oxidation occurs at the nonpolar *ortho* alkyl side chain, and normal β-oxidation takes place at the carboxyl containing side chain.

Fatty Acid Synthesis

In vitro fatty acid synthesis was carried out to compare the net rate of lipogenesis in rats fed different levels of cyclic compounds. Results from assays of fatty acid synthesis carried out in liver and in epididymal adipose tissues are presented in Table V. The amount of substrate converted in liver tissue was predictably low since ad libitum feeding was employed and a high fat diet was fed to the animals. There appeared to be a slight decrease in the rate

TABLE V

Utilization of Acetate-1-^{14}C for Fatty Acid Synthesis in Liver and Epididymal Adipose Tissues from Rats Fed Cyclic Fatty Acid Methyl Esters[a]

Cyclic esters in diet (wt %)	Substrate converted (nmoles converted/100 mg tissue/3 hr)					
	8% Protein in diet		10% Protein in diet		15% Protein in diet	
	Liver tissue	Adipose tissue	Liver tissue	Adipose tissue	Liver tissue	Adipose tissue
0.0000	26 ± 3	171 ± 22	62 ± 7	387 ± 51	31 ± 6	112 ± 6
0.0075	24 ± 4	161 ± 29	55 ± 8	490 ± 44[c]	23 ± 4	115 ± 10
0.0225	18 ± 2[b]	164 ± 13	22 ± 4[b]	613 ± 59[c]	29 ± 4	114 ± 7
0.1500	18 ± 4[b]	174 ± 25	38 ± 7[b]	575 ± 28[c]	29 ± 4	128 ± 6

[a]Values are means ± SEM for three animals.
[b]Significance $P < 0.01$ compared to 0.000 group.
cSignificance $P < 0.025$.

TABLE VI

Percentage of Total Radioactivity Recovered as $^{14}CO_2$ in Animals Injected with Acetate-1-^{14}C and Fed Fifferent Levels of Protein and Cyclic Monomer in the Diet[a,b]

% Protein in diet	Cyclic monomer in diet			
	0.0000	0.0075	0.0225	0.1500
8	71.6 ± 0.2	69.0 ± 1.7	70.1 ± 1.2	71.3 ± 1.2
10	70.8 ± 3.2	71.1 ± 3.4	74.3 ± 1.0	70.9 ± 1.1
15	52.0 ± 0.1	54.4 ± 0.6	54.2 ± 0.1	52.4 ± 0.1

[a]Three hour collection period.
[b]Mean values ± SEM (three animals/group).

of fatty acid synthesis in livers of rats fed higher concentrations of cyclic fatty acids in the diets with lower levels of protein. There was a significant difference ($P<0.01$) for animals fed 8% and 10% protein and with 0.0225% and 0.15% cyclic fatty acids in the diets. There was no difference in the rate of conversion of acetate-1-^{14}C into fatty acids among groups of rats fed 15% protein with different levels of cyclic monomer in the diet.

However, fatty acid synthesis in adipose tissue of rats fed 10% protein exhibited the opposite effect. There was an increase in synthesis with higher levels of cyclic fatty acids in the diet. Values obtained for rats fed 10% protein were 2-3 times higher than those obtained for the rats fed other levels of protein. No explanation for the higher levels can be given. Values obtained for fatty acid synthesis in adipose tissue are within the range of values obtained by Leveille (14) for nibbling rats fed diets containing 10% and 20% dietary fat, but a 20% protein level. Higher values obtained for fatty acid synthesis in adipose tissue rather than liver are consistent with the findings that adipose tissue is the major site of fatty acid synthesis in the rat and accounts for from 50-90% of the total fatty acids synthesized (15,16).

Numerous reports have demonstrated an inhibition of lipogenesis in rat liver slices by dietary fat (17-19). Others have reported similar observations for rat adipose tissue (20,21). Thus, while it is difficult to draw definite conclusions from small changes observed in organs in which lipogenesis was suppressed, there appeared to be a decrease in hepatic synthesis when 8% or 10% protein diets were fed and a slight increase in synthesis in the adipose tissue when a 10% protein diet was fed with increasing levels of cyclic fatty acids in the diet.

Such depression in hepatic lipogenesis may be attributed to the action of cyclic acids directly or indirectly on fatty acid synthesis and the related supporting systems, i.e., reducing equivalent production, etc. The presence of cyclic fatty acids or long chain fatty acids derived from the diet or the CoA derivatives in the cytoplasm may also contribute to the depression in enzyme activity (22,23). The fact that significant differences were observed in livers of rats fed 8% and 10% protein and not for animals fed 15% protein may be related to earlier observations of fatty lvier production in rats fed lower protein and 0.15% cyclic monomer diets compared with control animals fed the same protein level and no cyclic monomer who did not develop fatty livers (8).

The difference in rates of fatty acid synthesis in livers may also be due to differences in cell numbers since in vitro incubation results are expressed as substrates converted per unit weight of tissue. However, fatty infiltration in liver cells may account for difference in lipogenic rates since there would be fewer liver cells per unit weight of tissue.

Acetate Metabolism

The matabolism of acetate-1-^{14}C was studied to determine the effects of cyclic fatty acids in the diet on the rate of $^{14}CO_2$ formation, as well as the rate and extent of incorporation of ^{14}C in various lipid classes. The total expired radioactivity recovery data 3 hr after intraperitoneal injection of acetate-1-^{14}C are shown in Table VI. Animals fed 8% and 10% protein converted about 70% of the injected acetate to $^{14}CO_2$ with different levels of cyclic fatty acids in the diets. However, animals fed 15% protein only converted about 50% of the acetate-1-^{14}C to CO_2 for all levels of cyclic fatty acids in the diet. These values of total expired $^{14}CO_2$ obtained were in reasonable agreement with Govid Rao et al. (24) who conducted similar acetate metabolism studies with fresh and heated fats in the diet, as well as with different levels of dietary protein. Detailed analysis of the percentage distribution of expired $^{14}CO_2$ for each 10 min period for all groups indicated no consistent relationship between acetate metabolism and level of cyclic fatty acids in the diet.

The percentage distribution of radioactivity from administered acetate-1-^{14}C in lipid classes in livers of rats fed different levels of protein and increasing levels of cyclic fatty acids in the diet is shown in Table VII. The phospholipid fraction had the highest percentage of the radio-

TABLE VII

Percentage Distribution of Radioactivity from Acetate-1-^{14}C Metabolism in Various Lipid Classes in Livers of Rats Fed Different Levels of Protein and Cyclic Fatty Acid Methyl Esters[a]

Group	Protein diet (wt %)	Cyclic fatty esters (wt (%)	Lipid classes[b] PL (%)	(MG (%)	DG-C (%)	FFA (%)	TG (%)	CE (%)	HC (%)
1	8	0.0000	62.2	1.8	9.2	2.0	17.0	2.7	4.9
2	8	0.0075	67.0	1.8	5.4	4.2	18.1	1.6	2.6
3	8	0.0225	48.6	3.4	4.7	3.9	23.7	1.2	2.6
4	8	0.1500	53.6	3.4	8.9	2.7	22.6	1.1	7.7
5	10	0.0000	58.5	1.7	4.9	2.4	25.2	3.0	2.8
6	10	0.0075	62.9	2.3	5.7	1.9	20.3	1.5	2.0
7	10	0.0225	56.2	3.2	6.7	4.0	23.8	2.1	2.2
8	10	0.1500	48.0	2.6	10.5	3.6	29.3	1.4	3.2
9	15	0.0000	70.2	2.5	5.0	6.4	10.4	1.6	2.2
10	15	0.0075	64.7	3.6	3.6	9.0	11.0	2.5	2.2
11	15	0.0225	67.7	2.6	3.5	3.6	15.1	2.9	1.7
12	15	0.1500	65.2	2.8	4.2	5.2	16.3	1.2	2.6

[a]Mean of three animals/group.

[b]PL = phospholipids, MG = monoglyceride, DG-C = diglyceride-cholesterol, FFA = free fatty acids, TG = triglycerides, CE = cholesterol esters, HC = hydrocarbon.

activity of all the lipid classes; values ranged from 50-70% of the total counts. No trend could be correlated with different levels of dietary protein or amounts of cyclic fatty acids in the diets.

The triglyceride fraction contained the second highest percentage of recovered radioactivity. In most cases, an increasing amount of recovered radioactivity in the triglyceride fraction with increasing amounts of cyclic fatty acids in the diet was observed. Phospholipid and triglyceride fractions together comprised from 75-85% of the total radioactivity recovered in the liver lipids and were in agreement with the data of Evans and Norcia (25) who found high percentages of radioactivity in the phospholipid and triglyceride fractions of liver lipids from animals killed 1 hr after intraperitoneal injection of acetate-1-^{14}C.

Although there have been numerous studies on the adverse effects of partially purified cyclic fatty acids on experimental animals, this is the first report in which pure cyclic fatty acids were used. We have shown that very low levels of cyclic fatty acids caused decreased lipogenesis in livers of rats fed 8% and 10% protein and elevated lipogenesis in adipose tissue of animals fed 10% protein. These results also showed that although over 50% of the radioactivity from ingested cyclic acids were either expired as $^{14}CO_2$ or excreted in the urine, cyclic fatty acids nevertheless adversely affected animals fed low levels of protein.

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