Tracer Experiments to Assess Metabolic Conversions of Polyunsaturated Fatty Acids[1]

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Abstract

Fifteen groups of rats were maintained on diets free of fat or supplemented with different levels of linoleate, arachidonate or linolenate. After 100 days on the diets the rats were sacrificed and liver slices were incubated with acetate-1-^{14}C. The individual fatty acids were separated by gas chromatography and their radioactivity was determined by liquid scintillation counting. The C_{20} esters were separated by GLC, collected, and the structures of the components were determined by ozonolysis. The acetate incorporation into the various polyunsaturated fatty acids was influenced by the previous dietary conditioning. The distributions of radioactivity indicated that metabolic reactions taking place in the rat liver are modified by dietary supply of essential fatty acids.

Introduction

ACETATE IS KNOWN to be the basic building block of fatty acids (1). Incubation of animal and human organs with acetate-1-^{14}C in vitro has helped to elucidate the biosynthesis of polyunsaturated fatty acids (PUFA). Klenk incubated rat liver slices with acetate-1-^{14}C and isolated the highly unsaturated fatty acids of the liver lipids. After ozonization and oxidative cleavage he found radioactivity only in the carboxyl end of the molecule, whereas the malonic acid derived from the carbon atoms between the double bonds of the PUFA had only traces of activity. Thus, it was shown that the common PUFA of normal rat tissue could not be totally synthesized in the rat (2–4). These investigations were extended to a number of other vertebrate animals (5) and humans, (6,7) with the same conclusions.

Recently we have studied the influence of various levels of dietary essential fatty acids (EFA) upon fatty acid composition of the rat liver (8) and other organs (9–11). The results of these experiments have confirmed previously known metabolic pathways, and a theory of competitive inhibition has been proposed concerning the interaction of different dietary polyunsaturated acids (12,13). In the course of these experiments, liver slices of rats conditioned on a fat-free diet or on supplements with various dose levels of EFA were incubated with acetate-1-^{14}C to gain further insight into the conversions of dietary EFA to tissue PUFA. The incorporation of acetate-1-^{14}C into tissue PUFA may be a measure of the rates of their syntheses from dietary precursors, the rates of their de novo synthesis, or the rates of their degradation and resynthesis.

Experimental Methods

Animals and Diets. Weanling male rats, 27 days old, of the Sprague-Dawley strain, were kept on a basal fat-free diet and were given daily supplements of linoleate, arachidonate and linolenate as described in detail previously (8). The number of animals and intake of PUFA in percent of calories are stated in Table I. After 100 days on the diet the animals were sacrificed by ether anesthesia, and portions of the livers were removed quickly and were sliced to a thickness of 0.5 to 1 mm. with a razor blade.

Incubation with Acetate-1-^{14}C. Liver slices (15 to 40 g) were pooled according to groups, and were incubated in 10 times their volume of Ringer solution prepared according to Krebs and Henseleit (14). Sodium acetate-1-^{14}C (Applied Science Laboratories, State College, Pa.), specific activity 5.28 mc/mM, was added in amounts given in Table I. The liver suspension was then shaken gently in an incubator at 37C for 6 hr. The aqueous phase was then filtered off, and the liver slices were washed with Ringer solution and water. Until analyzed, the livers were stored in saline solution at −20C.

Isolation of Methyl Esters of Unsaturated Fatty Acids. The incubated liver slices were homogenized and extracted with chloroform-methanol, 2:1, according to Folch et al. (15). The lipids were transesterified by refluxing for one hour with 30 volumes of a 5% solution of dry HCl in methanol. For structure determination, samples of the methyl esters were saponified by refluxing with 30 vols of ½ N NaOH in methanol. A 10% solution of these fatty acids in acetone was cooled to −40C in dry ice/methanol to precipitate the saturated fatty acids. The liquid fraction was separated by suction through a fritted glass tube and the unsaturated fatty acids were esterified with diazomethane in ether solution. These concs were used for preparative GLC and ozonolysis (*vide infra*).

Analytical Gas Chromatography (GLC) and Liquid Scintillation Counting of GLC-fractions. The methyl esters were analyzed by GLC using a Barber Coleman Model 10 apparatus with argon ionization detector. A 210 cm glass column of 5 mm i.d., packed with 20% ethylene-glycol-succinate polyester (EGS) (Applied Science Laboratories, State College, Pa.) coated on Gas Chrom P, 80–100 mesh (Applied Science Laboratories), was used. The flow rate was 60 ml argon/min at an inlet pressure of 16 psi. Temps were: inlet heater 270C; detector cell 250C; column 180C for esters with retention time shorter than that of 18:3, and 200C for long-chain esters. The individual esters were identified by equivalent chain length and by internal standards, and quantification was carried out by triangulation. Fatty acid composition is reported as area percent.

In order to measure the radioactivity of the GLC fractions, a collecting device described by Dutton (16) was adopted. A three-way syringe valve was connected to a 50 cc syringe as the storage vessel for scintillation solution. A 1 cc syringe was used for flushing the outlet. The third valve-outlet was fitted with 1/16 in. stainless steel tubing to a 1/16 in. Swagelok T which was connected with the GLC outlet and the counting vial. The outlet of the GLC apparatus and the Swagelok T were inserted into a ¼ in. aluminum tube which was heated to 250C and acted as a heat sink. The use of small bore tubing provided a very rapid and thorough hot flushing of the outlet system thus minimizing the trailing of radioactivity.

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TABLE I

Incorporation of Radioactivtiy into the Liver Fatty Acids of Rats by in vitro Incubation of Liver Slices with Acetate-1-^{14}C

Diet supplement	% of Calories	Number of rats per group	Amount of liver incubated (g)	Acetate-1-^{14}C incubated (mc)	Amount of F.A. isolated (g)	Fatty acids % of liver weight	Fatty acids, specific activity (cpm[a]/mg)	% of activity incorporated
Fat-free	—	10	37.0	1.0	1.75	4.8	10401	1.46
Linoleate	0.01	6	34.3	0.4	1.08	3.1	2055	0.44
	0.05	6	37.2	0.4	1.52	4.1	860	0.26
	0.2	6	35.9	0.4	1.02	2.8	7344	1.51
	0.8	6	40.1	0.4	1.75	4.4	1796	0.63
	1.5	2	15.9	0.2	0.45	2.8	3797	0.69
	2.0	2	15.8	0.2	0.42	2.7	4820	0.81
Arachidonate	0.01	6	31.3	0.3	1.06	3.4	842	0.24
	0.05	6	24.6	0.3	0.96	3.9	1328	0.34
	0.2	6	36.4	0.4	1.19	3.3	610	0.13
	0.8	6	35.9	0.4	0.97	2.7	4408	0.79
Linolenate	0.1	6	29.7	1.0	1.27	4.3	10851	1.10
	0.4	6	31.7	1.0	0.94	3.0	9578	0.72
	2.0	4	28.9	1.0	0.86	3.0	17994	1.24
	5.0	4	29.6	1.0	0.80	3.0	11875	0.76

[a] Counting yield 57%.

The GLC effluent was collected in 15 ml scintillation solution containing 5 g PPO and 0.5 g POPOP (PPO and POPOP, obtained from Packard Co., LaGrange, Ill.) as primary and secondary scintillators in 1 liter of toluene. The sampling device was flushed with 0.5 ml of scintillation solution by means of the 1 ml syringe immediately before the vials were changed. The temporary back pressure at the time of flushing resulted in a little mark on the GLC chart. Thus, sample change and the GLC chart were synchronized. These analyses employed samples of 0.6 to 1.0 μl neat methyl esters. The collected samples were counted in a Packard Tricarb liquid scintillation counter, Model 314X. The counting yield was checked with a standard benzoic acid sample and found to be 57%. Each sample was counted 3 times for 10 min, the background was subtracted, and counts per minute of each GLC fraction for 1 min of collecting time were calculated. The activity level observed for each fraction was plotted on the GLC charts. Thus, it can be seen directly how much activity is associated with each fatty acid. Besides the activity per peak, the activity in each fatty acid as percent of the total could be calculated. Since the GLC column had to be overloaded in order to collect enough effluent for liquid scintillation counting, the fatty acid composition of the liver fatty acids was determined in separate analytical GLC measurements.

Preparative Gas Chromatography. For preparation of GLC fractions on larger scale (1–10 mg) a Research Specialties apparatus with thermal conductivity detector was used. Fractions were collected using the method described by Schlenk and Sand (17). A 10ft × 3/8 in. aluminum column packed with 20% β-cyclodextrin acetate on Chromosorb P (20–60 mesh) was used. The gas flow was 100 ml of helium/min, column temp was 182C, the inlet heater was kept at 300C and the cell at 250C.

Structural Analysis of Unsaturated Fatty Acids. The positions of the double bonds of fatty acids isolated by preparative GLC were determined by ozonolysis and reduction of the ozonides followed by identification of the resulting aldehydic and ester-aldehydic fragments by GLC analysis according to Privett and Nickell (18).

Shorthand Notation. Substances to be discussed without reference to degree of unsaturation will be denoted as C_6, C_{18}, etc. Acids and esters to be discussed without reference to position of double bonds will be represented as 18:1, 20:4, etc. Inasmuch as metabolic relationships are a major point of discussion in this presentation, the "ω-notation" will be used in which the chain length and the number of double bonds precede the ω, which is followed by a number indicating the position of the first double bond counting from the terminal methyl group. All double bonds are assumed to be *cis* and methylene-interrupted. Thus, 5,8,11,14-eicosatetraenoic acid, arachidonic acid, is written as 20:4ω6.

Results and Discussion

In Table I the amts and specific activities of individual fatty acids isolated from each group of rat livers are given together with information about the EFA supplements animals received, the number of animals pooled in each group for incubation and the amount of liver incubated. The uptake of radioactivity is rather inconsistent. This may be due to differences in dietary status of the animals or to the primitive slicing procedure which made the incubations difficult to reproduce. Because the specific activities of the unsaturated fatty acids were rather low when linoleate and arachidonate were fed, the amts of acetate-^{14}C were doubled for the groups of rats kept on fat-free diet or fed linolenate, thus yielding higher specific activities of the liver fatty acids.

In order to check the GLC sampling and radioactivity counting procedures for reproducibility, a mixture was prepared consisting of 29.4% methyl stearate-1-^{14}C (3300 cpm/mg) and 70.6% randomly labeled methyl oleate-^{14}C (2591 cpm/mg). The mixture, with a measured specific activity of 2799 cpm/mg, was analyzed by GLC in the usual manner. The GLC effluent was collected for periods of 1 min, and at the critical portions of the chromatogram for 30 sec in order to describe the profile between two peaks. Chang-

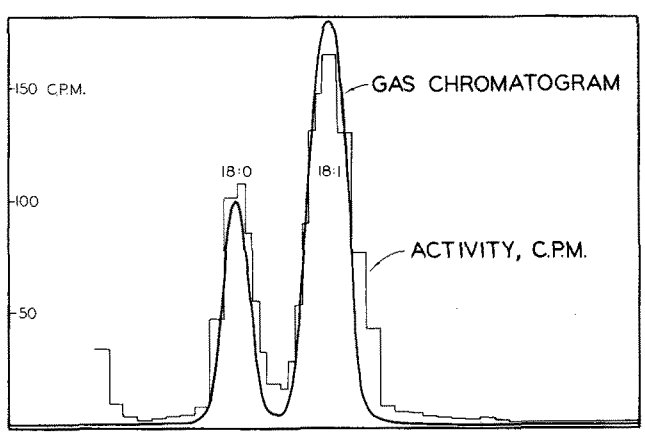

FIG. 1. Gas chromatogram and radioactivity curve for separation of model mixture of methyl stearate-1-^{14}C and randomly labeled methyl oleate-^{14}C.

ing counting vials takes less than 1.5 sec which is 2.5% of a 1 min collecting time. Thus, only very small amts of effluent escape to the air. Figure 1 shows the chromatogram obtained with the activity levels drawn in. It can be seen that the activity curve matches the GLC curve almost ideally. There is only very little trailing of activity. The activity level between the peaks is only 20 cpm which indicates a rather good separation. From the curve in Figure 1 the amts of stearic and oleic acids were calculated to be 27.4 and 72.6%, respectively. The activities recovered for stearate and oleate collected were 254 and 736 cpm, respectively. This represents 32.5 and 67.5% of the total radioactivities of the sum of the two GLC peaks. This compares favorably with the corresponding values 34.6 and 65.4% for the original mixture. Although stearate-oleate separation is a simple example, the test run described here shows that the procedure employed is adequate to assess radioactivity of GLC fractions. With longer chain, more unsaturated esters, the tailing effect may be expected to be greater.

In Table II the fatty acid compositions of lipids from incubated liver slices are given in percent of the total fatty acids. The radioactivity of each fatty acid collected is given in percentage of the sum of the radioactivities of all fatty acids in that chromatogram. Figure 2 shows the chromatograms and radioactivity curves on those samples from rats fed the fat-free diet and the highest levels of the three supplements. In order to get a higher activity count on the long chain unsaturated fatty acids, a second chromatogram was obtained with 6 to 10 times the amount of fatty acids injected in the normal run.

No consistent changes in radioactivity were seen with increasing amounts of different dietary fatty acids, possibly because the differences in level of EFA intake were too small. However, large differences in radioactive acetate uptake in tissue fatty acids were observed as a function of kind of dietary fatty acid. As was expected from previous incubation experiments and from the theory of fatty acid synthesis, most of the acetate was incorporated into the saturated fatty acids of 14, 16 and 18 carbon chain length. The monoenoic acids of 16 and 18 carbon atoms are directly synthesized from the respective saturated fatty acids by dehydrogenation. Thus, they, too, show a considerable portion of the activity incorporated. The peaks identified as 18:2 and 18:3 in the chromatograms show an activity which is definitely above the background level. This is surprising since it has been shown (2,3) that linoleic and linolenic acids cannot be synthesized *de novo* by animals. Thus, the peaks designated as 18:2 and 18:3 may contain other fatty acids of similar retention volumes which have been synthesized by the rat liver from the incubated acetate. For instance, 18:2ω7 and 18:2ω9 acids could have arisen from endogenous palmitoleate and oleate, respectively.

Fat-Free Diet. The chromatograms of the highly unsaturated long-chain fatty acids show the previously described metabolic changes corresponding to the dietary fatty acids (5–7). In the chromatogram from rats fed the fat-free diet, the 20:3 peak shows a small shoulder which is less than 10% of the total 20:3 but which carries approx 60% of its radioactivity. This suggests that the isomer occurring in smaller quantity has the higher rate of turnover. To determine the structures of the 20:3 isomers the labeled highly unsaturated acids from the rat liver lipids were obtained by deep cooling fractionation and submitted to preparative GLC. The 20:3 fraction was analyzed by

TABLE II

Fatty Acid Composition [a] of Liver Lipids and Incorporated Radioactivity [b]

Fatty acid Fed	% of Calories	14:0		16:0		16:1ω7		18:0		18:1ω9		18:2ω6		18:3ω3		20:3ω9		20:3ω6+ 20:3ω7		20:4ω6		22:3ω9		22:5ω6		22:5ω3		22:6ω3	
		Amt.	Act.	Amt.	Act.	Amt.	Act.	Amt.	Act.	Amt.	Act.	Amt.	Act.	Amt.	Act.	Amt.	Act.	Amt.	Act.	Amt.	Act.	Amt.	Act.	Amt.	Act.	Amt.	Act.	Amt.	Act.
Fat-free		0.9	25.0	27.6	35.2	14.5	7.5	9.5	12.7	38.5	13.6	38.5	13.6	0.7	0.9	5.4	0.5	0.4	0.7	1.3	0.6	0.2	2.0	0.2	0.2		0.2	0.2	0.2
Linoleate	0.01	0.8	23.3	22.8	35.4	11.6	5.9	12.8	15.8	38.7	14.9	1.0	0.5			8.3	0.8	0.4	1.0	2.2	0.9	0.4	0.5	0.4	0.5	0.1	0.2	0.3	0.6
	0.05	0.9	24.8	22.8	39.0	11.2	4.1	12.5	12.5	40.0	10.2	1.1	0.0			7.3	0.5	0.6	0.8	2.2	2.3	0.4	1.2	0.4	1.2	0.2	3.3	0.3	0.8
	0.2	0.7	18.0	23.2	37.9	10.6	8.6	13.7	14.7	36.7	14.1	1.5	0.6			7.2	0.5	0.4	0.8	4.0	0.9	0.8	0.5	0.5	0.5	1.4	5.9	0.4	0.3
	0.8	0.7	20.6	22.6	33.7	10.0	5.5	14.7	15.8	35.5	14.6	2.3	1.4			4.2	1.1	0.7	1.7	6.5	2.1	1.5	0.9	0.9	0.3	2.0	4.8	0.4	0.3
	1.5	0.9	20.6	22.6	38.2	11.8	5.4	17.3	16.3	39.4	15.2	2.1	0.5			1.6	0.5	0.4	0.5	5.5	0.9		0.3	0.4	0.4	1.4	5.9	0.4	0.4
	2.0	0.8	24.7	25.4	34.4	8.9	5.4	16.1	16.9	33.2	14.9	3.1	0.2			1.2	0.4	0.4	1.0	9.6	1.4	1.8	0.6	0.6	0.2	2.0	4.8	0.5	0.2
Arachidonate	0.01	0.7	13.2	21.9	31.2	12.2	9.4	12.5	12.6	38.9	17.6	0.5	2.4			9.2	0.9	0.5	1.5	2.8	3.2	0.4	1.9	0.4	1.9	0.1	0.2	0.4	3.0
	0.05	0.8	23.6	22.3	31.7	10.7	5.3	14.3	21.4	37.5	11.5	0.6	0.9			8.8	0.5	0.4	1.6	3.0	0.5	0.6	0.6	0.6	0.6	0.2	0.5	0.3	0.2
	0.2	0.5	34.0	17.5	18.6	10.0	2.3	15.3	14.8	36.1	8.4	0.6	2.6			7.4	0.9	0.5	3.9	6.9	3.9	1.7	6.1	0.5	0.9	0.3	0.9	0.5	0.9
	0.8	0.7	17.6	20.5	39.9	9.2	6.1	14.1	17.4	33.0	14.7	0.3	0.8			2.0	0.3	0.2	0.7	12.7	0.9	3.1	0.5	0.3	0.3	0.1	0.3	0.3	0.3
Linolenate	0.1	0.6	23.6	11.8	6.4	11.8	14.6	40.5	17.4	0.6	0.6	0.3	1.6	6.2	0.6	0.4	0.9			1.2	0.4	0.4	0.3	0.1	0.1	0.1	1.2	1.7	0.6
	0.4	0.8	17.9	29.6	36.9	11.4	6.4	10.1	14.6	40.2	14.3	0.6	0.6	0.4	1.8	2.5	0.3	0.1	0.6	0.7	0.4	0.9	0.3	0.2	0.2	0.2	3.3	2.5	1.2
	2.0	0.7	18.5	26.7	37.9	11.6	5.9	10.9	13.8	34.9	14.3	0.2	0.2	1.2	2.4	1.2	0.3	0.1	0.2	0.9	0.4	4.3	0.9	1.4	1.4	0.2	5.9	5.8	0.7
	5.0	0.7	12.7	30.5	38.8	11.3	8.6	10.0	13.6	30.9	13.7	0.3	0.4	4.8	2.3	0.3	0.1	0.1	0.2	0.7	0.5	5.1	1.0	2.0	4.8	0.1	0.5	4.5	0.5
		12.9		43.6		9.4		11.9		12.0		0.2		2.3		0.1		0.1		0.7		0.7							

[a] Area percentage on GLC.
[b] Percent of activity in all peaks collected.

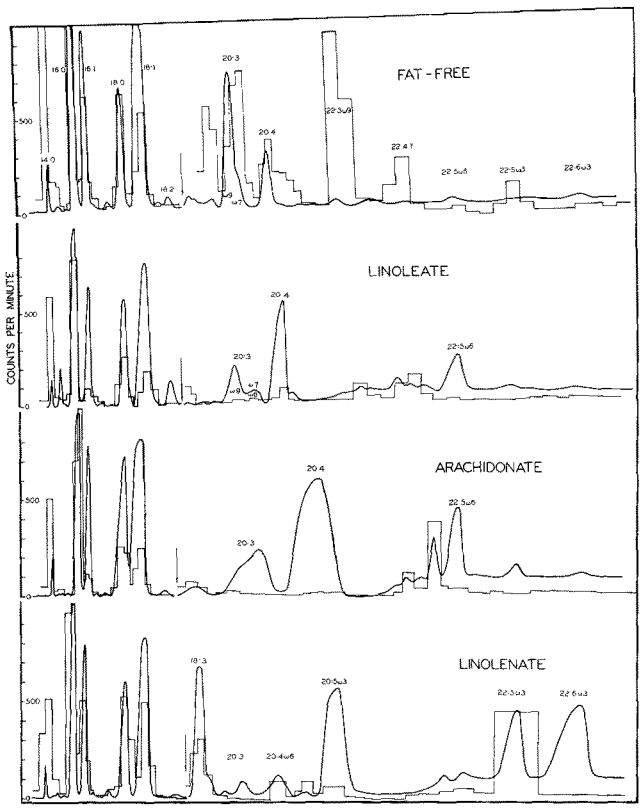

FIG. 2. Gas chromatograms and activity curves of the methyl esters obtained from the liver lipids of rats on a fat-free diet, as supplemented with linoleate, arachidonate or linolenate. Two chromatograms were used for making each composite drawing, and the break between the two is indicated by an arrow. The second chromatogram is for a 6–10 times larger sample, and the counts per minute have been multiplied by 10 to permit graphic presentation on the same scale as the first chromatogram. Differences in position of single esters are due to differences in temp programming.

GLC and was resolved into two major peaks 86% and 10% of the total fatty acids, respectively. Impurities present were found to be 0.5% 16:1, 2.0% 18:1 and 2% of an unidentified C_{20} acid. Ozonolysis of this 20:3 fraction yielded 90% C_9 aldehyde and 10% C_7 aldehyde; and the aldehyde esters were 90% C_5 and 10% C_7. Thus, the two isomers are 20:3ω9 and 20:3ω7 which are derived metabolically from 18:1 and 16:1, respectively. The higher activity in the 20:3ω7 shows that this isomer has the higher rate of turnover.

The 20:4 peak of the chromatogram of the methyl esters from rats fed fat-free diet likewise has a small shoulder which is associated with high radioactivity. By ozonolysis the 20:4 fraction was found to consist mainly of arachidonic acid, 20:4ω6. However, about 20% of the fraction representing the trailing shoulder is 20:4ω7, derived metabolically from palmitoleic acid. The higher radioactivity of the 20:4ω7 shows an appreciable rate of synthesis of 20:4ω7 from its precursor, 16:1ω7, which has a high radioactivity.

The second small peak following 20:4 is associated with a high level of radioactivity. The amt of this acid was too small to permit analysis by ozonolysis. Its equivalent chain length on EGS, 23.17, was found to be identical to an authentic 22:3ω9 isolated from beef testis lipids (19,20). The high level of its radioactivity confirms this identification, for 22:3ω9 would arise from 20:3ω9 by acetate chain elongation. The next small peak associated with significant radioactivity corresponds most closely to 22:4. This could be 22:4ω9 derived from 22:3ω9 by dehydrogenation but conclusive identification could be made neither by ozonolysis for lack of sample, nor from equivalent chain length data for lack of the authentic standards. The level of radioactivity of 22:5ω3 is significantly greater than background, indicating some synthesis of this metabolite of linolenate even on a fat-free diet.

Linoleate Supplement. The chromatogram from this group of rats revealed a smaller total amount of 20:3 separated into two more distinct peaks. The radioactivity is low, but is elevated somewhat in the second peak. Enrichment of the polyunsaturated acids by low temp crystallization followed by preparative GLC yielded a composite 20:3 fraction which was subjected to ozonolysis. It was found to consist of 60% 20:3ω9 and approx 20% each of 20:3ω7 and 20:3ω6. The radioactivity is associated mainly with the second peak which, according to equivalent chain length studies (20), is probably 20:3ω7. The 20:4 peak is associated with little radioactivity and this is found in the second half of the peak. Ozonolysis revealed that the 20:4 was 20:4ω6 with less than 1% contamination by 20:4ω7. The 22:5ω6 had no perceptible radioactivity. Thus, in a rat adequately supplemented with linoleate, the higher metabolites of linoleate appear to incorporate little or no acetate, indicating a low rate of turnover. Between 20:4 and 22:5 two appreciable peaks of radioactivity are observed, and these probably correspond to 22:3ω9 and 22:4ω9 (?) identified in the chromatogram of the fat-free group. Strict identification by equivalent chain length was not possible because temp programming was employed in GLC analysis of this sample.

Arachidonate Supplement. When arachidonate was fed, the content of arachidonate in the liver lipid methyl esters was so great that in preparative GLC the 20:3 fraction could not be separated from 20:4. Therefore, the identification of the isomers of 20:3 could not be made by ozonolysis. The 20:4 peak was principally arachidonate with only traces of 20:4ω7, neither of which were appreciably radioactive. The final metabolite of arachidonate, 22:5ω6, also had a rather low level of radioactivity whereas two peaks immediately before it had distinctly higher levels of radioactivity. Temp programming was used in the GLC analysis of the samples and therefore these components could not be identified by ECL values.

Linolenate Supplement. In rats conditioned on a linolenate supplement, the 18:3ω3 in the liver lipids became markedly radioactive when the livers were incubated with acetate-1-^{14}C, and the radioactivity of the 18:3ω3 increased with the increase in level of supplementation. This might be interpreted as indicating degradation by β-oxidation and resynthesis with acetate-1-^{14}C. The amts of 20:3 and 20:4 in the liver lipids of linolenate supplemented rats are proportionately less than in the other groups, as might be expected by the competitive inhibition exerted by 18:3 (12,13) and, therefore, these components could be identified more exactly. The three principal metabolic products of 18:3ω3, that is, 20:5ω3, 22:5ω3 and 22:6ω3, have differing radioactivities. The 20:5, although synthesized in large amt, has a much smaller activity than 22:5ω3 which requires 2 acetate molecules in its synthesis from 18:3. The 22:6ω3 has a low level of radioactivity indicating a low rate of turnover of this terminal metabolite.

The experiments reported here are of a preliminary nature but they have yielded positive conclusions regarding PUFA metabolism. They have demonstrated that in vitro incubation experiments with liver slices permit sufficient acetate uptake to assess the relative

rates of incorporations of acetate into various fatty acids of liver separated by analytical GLC. Dietary differences have been shown to cause marked differences in these relative rates of uptake. The positive results gained in this investigation suggest further experiments of this nature.

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Potential Synthetic Lubricants: Esters of C_{18}-Saturated Cyclic Acids[1]

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Abstract

A series of 16 esters of C_{18}-saturated cyclic acids (HCal) were prepared, and partial evaluation showed that several have qualities that recommend them as potential low-temp lubricants. Starting materials used were primary, straight, and branched chain alcohols C_4–C_7; perfluoro alcohols; phenol; cyclohexanol; and C_{18}-saturated cyclic alcohols prepared from cyclic acids. Viscosities were measured at –40, 100, and 210F. Their viscosity indexes ranged from 26 to 143. Pour points or melting points of the esters ranged from –27 to –96F. The oxidative stability of these esters measured at 347F according to a modification of the test method for military specification MIL–L–7808 was in nearly all cases equal or superior to the control bis-2-ethylhexyl sebacate. More severe oxidation tests showed the esters of HCal to be slower than the control in the development of acidic decomposition products.

Introduction

AN INVESTIGATION of the derivatives of C_{18}-saturated cyclic acids was undertaken as a part of the program to find new and extended uses for linseed oil. The C_{18}-cyclic acids are derived from the linolenic component of linseed oil (12,16,17). The preparation and evaluation of cyclic acids and their derivatives have been described in earlier publications from this laboratory (4,6,7,11,14,15,18) including a review paper (9). Initially the hydrogenated C_{18} cyclic acids (HCal) and their derivatives appeared to show poor oxidative stability. It was later found that under certain conditions of "hydrogenation" HCal could contain significant quantities of an aromatic acid (5,10). Aromatic structures containing long alkyl side chains are known to be quite unstable to oxidation. Our recent work has demonstrated that HCal can be prepared free of aromatic acid, enabling us to investigate HCal esters as synthetic lubricants.

Ester lubricants, particularly dibasic acid esters, date back to World War II, when a lubricant was needed that combined low pour point, good stability, and high viscosity index in addition to having good lubricity. Mono- and diesters of organic acids were investigated and as a class of compounds proved to be promising. In the late 1940's, development of the gas turbine placed such severe requirements on lubricants that diesters became increasingly important. The development of the turbo-jet and the necessity of the military to operate under arctic conditions placed emphasis on low-temp properties and less on film strength. The requirements were set down in U.S. military specification MIL–L–7808 (1953).

Many synthetic lubricants have been tested on the basis of this specification. The most widely known of these are diesters of adipic, azelaic, and sebacic acids and glycol esters of pelargonic acid. Because of the cost of raw material and processing, sebacic acid is relatively high priced. The alcohols used in these diester lubricants are principally C_8–C_{10} oxo alcohols and 2-ethylhexanol. Branched chain alcohols are necessary to produce the desired low-temp viscosities. It is well known that the oxidative stability of alcohols containing tertiary hydrogens (branched chain alcohols) is less than that of their straight chain isomers. Thus the present dibasic acid ester lubricants are a compromise between low-temp fluidity or pour point and oxidative stability. In contrast to the dibasic acids, saturated C_{18}-cyclic acids have unique inherent low-temp properties; also their short straight-chain primary alcohol esters have good low-temp properties as well as superior high-temp oxidative stability as determined by formation of acidic products.

With the advent of the space age and new supersonic (mach 3) aircraft the search for better lubricating fluids has been intensified and now includes highly refined petroleum fractions, silicones, polynuclear aromatics, and fluoroesters, to name a few (1). Although the ester linkage lacks the stability required for many of these new applications, the need for good ester lubricants, particularly in subsonic aircraft, is expected to continue.

Preliminary evaluations of several esters prepared from HCal are reported in this paper. Some of these esters are of sufficient interest to warrant their further evaluation as potential lubricants.

Starting Materials

C_{18}-Saturated cyclic acids were prepared by alka-

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