Incorporation of acetate-C^{14} into individual fatty acids of rat liver triglycerides and phospholipids

J. D. EVANS and L. N. NORCIA

Departments of Physiology and Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania

SUMMARY Control rats, and those made hyperthyroid or hypothyroid by dietary means, showed the same general pattern of C^{14} incorporation into liver lipid fractions following intraperitoneal injection of $C^{14}H_3COONa$, although the synthesis of phospholipids was apparently decreased in both hyper- and hypothyroid states. Liver triglyceride linoleate was greatly depleted in the hyperthyroid state.

The triglycerides and phospholipids together contained 97–99% of the counts from all the principal components recovered chromatographically from liver lipids. A variable fraction of these counts resided in the fatty acid portion. Of the counts in the liver fatty acids, saturated acids had the largest number, both in triglycerides and phospholipids. Monoenoic and tetraenoic acids had comparatively few counts; and dienoic acid only a negligible number.

STUDIES OF normal rats have demonstrated that incorporation of intraperitoneally injected acetate-C^{14} into fatty acids of liver lipids is rapid, attaining a maximum within the first 30 min after administration (1). Tove, Andrews, and Lucas (2) observed two peaks in specific activity–time curves of individual fatty acids from rat liver phospholipids and neutral fat, the first occurring approximately 2–9 hr after acetate-C^{14} injection, and the second approximately 25–35 hr after injection. The specific activity of palmitic acid exceeded that of other isolated acids.

Previous studies (3) have indicated that intraperitoneally injected acetate-C^{14} is rapidly incorporated into liver triglycerides and phospholipids but not into sterol ester fatty acids. Fatty acid composition was determined by alkali isomerization but no data were obtained concerning isotope incorporation into individual fatty acids. The initial purpose of the present work was to study, by utilizing gas–liquid chromatographic isolation, the incorporation of intraperitoneally injected acetate-C^{14} into fatty acids of normal, hyperthyroid, and hypothyroid rats. Although too few animals have been studied in the present observations to prove definite differences in regard to entrance of C^{14} into particular liver lipids, certain consistent patterns of incorporation have been observed.

METHODS

Two series of rats were studied. The first, composed of a single group of six rats maintained on laboratory chow checkers, provided illustrative data on the incorporation of C^{14} from acetate into different fractions of liver total lipids. The second series consisted of six groups with three rats in each group. Each of the latter received a basal diet of 50% sucrose, 20% casein, 20% corn oil,[1] 2.0% salt mix[2] (USP XIV), and 2.0% vitamin mix[2] in dextrose. Individual groups received supplements containing sufficient cellulose to bring their diet composition to 100% as follows. Control group D received 6% cellulose; group D + T, 5.5% by weight of cellulose and 0.5% by weight of desiccated thyroid powder; group D + P, 5.8% cellulose and 0.2% propyl thiouracil; group D + Ch, 5.0% cellulose and 1.0% cholesterol; group D + Ch + T, 4.5% cellulose, 1.0% cholesterol, and 0.5% thyroid powder; and group D + Ch + P, 4.8% cellulose, 1.0% cholesterol, and 0.2% thiouracil. Animals of each group were provided with 15 g of synthetic diet per day and were permitted to eat ad lib.

After 15 days on synthetic diets the animals were fasted for 20 hr. Two rats, selected from each group, were then injected intraperitoneally with 30 μc $C^{14}H_3COONa$ in 1.5 ml 0.9% NaCl. At the end of 1 hr, each injected animal was anesthetized with ether and its liver was removed, shaken several times in fresh saline,

[1] Composition: saturated acids 28.4%, monoenoic 12.3%, dienoic 54.0%, trienoic 0.8%.

[2] Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE 1 DISTRIBUTION OF C^{14} IN LIPID FRACTIONS OF RAT LIVER* FOLLOWING INTRAPERITONEAL INJECTION OF $C^{14}H_3COONa$

	Fraction Weight	Dpm in Fraction	Methyl Esters of Fatty Acids	Dpm in Methyl Ester	Dpm in Water Wash	Dpm in Water Wash
	mg		*mg*			%
Hydrocarbon	2.5	820				
Sterol Ester	4.8	670				
Glyceride	65.3	58,900	57.8	11,850	45,970	78.1
Free Sterol + FFA†	21.8	14,800				
Phospholipid	368.0	397,500	163.8	17,300	353,300	88.9
Total	462.4 (98.5% recovery)	472,690				

* Values in the table are from the lipids of six pooled rat livers. The "average" liver (7.43 g) contained 469.5 mg total lipids following purification by the Folch procedure.

† FFA = Free Fatty Acid.

and blotted. Livers from animals in the same group were combined, weighed, and homogenized in a Waring Blendor with ethanol–ether (v/v) 3:1. The material that was soluble in ethanol–ether was removed by evaporation and was taken up in petroleum ether, from which it was again recovered by evaporation. Total lipids thus obtained were weighed and stored in isooctane. On several occasions the lipids were water-washed or purified by the Folch procedure (4) to remove radioactivity that might be present as contaminant.

Aliquots of the total lipids were taken for separation by silicic acid chromatography (5) into the major lipid classes. Methyl esters from triglycerides and phospholipids were prepared and purified by the method of Luddy, Barford, and Riemenschneider (6). In this procedure, separation of methyl esters from reaction products of methylation was accomplished in part by means of a series of water washes. C^{14} in the combined water washes was determined by the method of Werbin, Chaikoff, and Imada (7). One aliquot of each purified methyl ester was analyzed for fatty acid composition by isomerization and UV spectrophotometry. A second aliquot (approximately 5 mg) of each methyl ester was counted in a Packard Scintillation Spectrometer in a toluene–PPO–POPOP[3] solvent–scintillator system. Little or no correction for quenching was necessary in these samples. Counting efficiency averaged 59%. From the exact weight of the sample, the specific activity of the mixed methyl ester was determined.

A third methyl ester aliquot (10–15 mg) was reduced under nitrogen to approximately 50 μl in a 15 ml conical tipped centrifuge tube. From this, 5–10 μl, containing 1–5 mg of methyl ester, was injected into the inlet chamber of the gas–liquid chromatography (GLC) column (Packard). The column, 150 cm × 5.0 mm i.d., contained a packing of 40% diethylene glycol adipate polyester and 4% H_3PO_4 on 60–80 mesh Gas Chrom P.[4] The high percentage of liquid phase was used in order that relatively large samples could be injected and chromatographed with minimum peak distortion. Tritium foil served as ionizing source in a detector maintained at 200°. Column temperature was 190°, and argon gas flow 20 ml/min. Under these operating conditions, C20:4 did not emerge completely from the column until 6–7 hr after injection time, and from published retention times, C22:5 and C22:6 would not have been expected to emerge until 13–16 hr after injection time. No peaks were observed for C22:5 and C22:6 acids, but from UV spectrophotometric analyses small amounts of these polyunsaturated fatty acids were known to be present. Methyl ester composition was obtained by triangulation.

A stream-splitter interposed between the column outlet and detector directed 19.5% of the column effluent to the ionization detector and 80.5% to a fraction collector where the methyl esters were collected in cartridges containing coated anthracene crystals as described by Karmen and co-workers (8, 9). Anthracene cartridges containing the trapped radioactive vapors were counted at an efficiency averaging 62%. Exploratory tests confirmed the observation of Karmen, Giuffrida, and Bowman (8) that the emergence of esters from the GLC column coincided with the appearance of their respective peaks on the mass record. In our experience with injected, labeled palmitate, 90% of that fraction of palmitate calculated to be directed to the anthracene cartridges by the stream-splitter was collected simultaneously with the appearance of the recorded peak. In the experiments reported here, 18–20 samples per chromatogram were collected in cartridges containing anthracene—some representing the duration of peak emergence, and others the intervening periods during which only base line was recorded. The same volume

[3] PPO, 2,5-diphenyloxazole. POPOP, 1,4-bis[2-(5-phenyloxazolyl)]-benzene. Packard Instrument Company, Inc., LaGrange, Ill.

[4] Applied Science Laboratories, Inc., State College. Pa.

TABLE 2 RAT LIVER LIPID COMPOSITION OBTAINED BY SILICIC ACID COLUMN CHROMATOGRAPHY (VALUES FROM POOLED LIVERS OF TWO ANIMALS)

	Diet*					
	D	D + T	D + P	D + Ch	D + Ch + T	D + Ch + P
Average Liver weight, *g*	6.02	6.45	6.25	7.50	7.10	6.20
	mg lipid per g wet liver					
Lipid Content						
Hydrocarbon	0.3	0.3	0.2	0.5	0.3	0.4
Sterol Ester	3.7	0.8	3.7	23.8	1.2	31.7
Triglyceride	19.4	6.6	18.2	31.0	7.2	45.7
Free Sterol + FFA	3.2	2.7	2.8	4.0	3.0	4.5
Phospholipid	47.1	42.4	51.4	52.6	46.9	52.4
Total Lipid	73.7	52.8	76.3	111.9	58.6	134.7

* D = synthetic control diet; T = 0.5% desiccated thyroid powder; P = 0.2% propyl thiouracil; Ch = 1.0% cholesterol.

of each methyl ester injected into the GLC column was also pipetted directly to anthracene crystals in a cartridge and counted. Each GLC analysis was continued for 24 hr. For 18 hr following the C20:4 peak, the column continued to bleed isotope at a rate averaging 1 cpm above background during each hour of column operation. Because tests had demonstrated that the number of disintegrations per minute (dpm) of sample could be obtained in either the PPO-POPOP system or the anthracene crystal system under appropriate conditions, it was possible to calculate the weight of methyl ester recovered from the GLC column. Thus, if the specific activity of a mixed methyl ester was 734 dpm/mg and if the net total counts collected from the column, corrected for stream-splitter, equalled 1032 dpm, the calculated amount of methyl ester recovered from the column was 1032/734 = 1.4 mg. From the percentage composition of methyl esters obtained by GLC, the weight of each individual ester was determined. This datum, together with the number of counts delivered to the fraction collector during the period of emergence of each peak, permitted the specific activity of each individual fatty acid to be calculated. For example, if GLC showed that C16:0 constituted 19.0% of the mixed methyl esters, and if 640 dpm were recovered in the cartridge collecting methyl palmitate effluent (corrected for stream-splitter), then 640/0.19 × 1.4 = 2400 dpm/mg of methyl palmitate.

RESULTS

The series of six rats that had been fed laboratory chow checkers yielded 3.068 g of liver total lipid by the ethanol–ether extraction procedure. An aliquot of 613.5 mg subjected to Folch purification yielded 563.2 mg. Of this, 225.3 mg placed on a silicic acid column provided data which have been expressed on the basis of lipids in the liver of the average of the six rats (Table 1). Counts determined on the water washes of the esters formed during methylation of the triglycerides and phospholipids showed that a large proportion of radioactivity in the total lipid sample was in the water fraction. In Table 1, 20.2% of the total triglyceride count was recovered in its methyl esters, and only 4.4% of the phospholipid count reappeared in the methyl esters of this lipid component. Equilibrium dialysis studies of the neutralized water wash phases showed that the radioactive material diffused readily across a cellulose membrane against distilled water.

Table 2 illustrates the distribution of lipid components in livers from each experimental group of the second rat series. The amounts of liver sterol esters and tri-

TABLE 3 RADIOACTIVITY IN DISINTEGRATIONS PER MINUTE PER TOTAL LIVER IN LIPID FRACTIONS (AVERAGE VALUES FROM POOLED LIVERS OF TWO ANIMALS)

	Diet*					
	D	D + T	D + P	D + Ch	D + Ch + T	D + Ch + P
	dpm					
Hydrocarbon	22	0	32	0	25	0
Sterol Ester	528	1,480	353	605	470	332
Triglyceride	51,770	76,900	48,000	39,500	66,800	35,400
Free Sterol + FFA†	1,600	5,150	1,425	2,290	1,610	1,085
Phospholipid	575,000	234,000	277,000	764,000	242,000	276,000

* See Table 2.
† Free Fatty Acids.

TABLE 4 AMOUNTS OF METHYL ESTERS FROM TOTAL FATTY ACIDS OF TRIGLYCERIDES AND PHOSPHOLIPIDS PER RAT LIVER (VALUES FROM POOLED LIVERS OF TWO ANIMALS)

	Diet*					
	D	D+T	D+P	D+Ch	D + Ch + T	D + Ch + P
			mg/liver			
Phospholipid Methyl Esters						
16:0	32.0	25.2	39.8	32.9	33.2	26.4
18:0	34.9	40.1	44.4	39.7	49.7	24.0
18:1	7.8	9.3	10.1	9.3	10.9	11.0
18:2	26.3	25.5	34.9	43.0	33.2	27.3
20:4	23.7	33.0	23.5	29.6	34.5	23.8
Total	124.7	133.1	152.7	154.5	170.2	125.5
Triglyceride Methyl Esters						
14:0	0.5	0.3	0.3	0.9	0.3	1.2
16:0	22.2	9.6	19.5	44.1	9.5	51.3
16:1	2.0	0.8	0.0	3.9	0.7	4.8
18:0	2.5	1.2	1.3	4.1	0.9	5.8
18:1	16.5	7.0	18.6	39.5	6.6	51.1
18:2	49.7	11.1	52.2	112.6	14.3	142.0
20:4	2.2	0.7	4.6	2.6	1.5	5.6
Total	95.6	30.7	96.5	207.7	33.7	261.8

* See Table 2.

glycerides were smaller after thyroid and greater than control levels after cholesterol feeding (10). Propyl thiouracil also elevated liver lipids in rats receiving dietary cholesterol. Because the quantity of liver sterol esters from the control rats (group D) was greater than expected, confirmation was sought by chemical analysis. By applying the Liebermann-Burchard reaction quantitatively to the chromatographically isolated sterol ester fraction and the Sperry-Webb method (11) to an aliquot of the original total lipid, corroboration was obtained. It thus appears that under the dietary conditions employed in these experiments the ratio of sterol esters to free sterols in the control group livers was relatively high.

TABLE 5 COUNTING AND RECOVERY DATA FROM GLC ANALYSIS OF METHYL ESTER FROM LIVER TRIGLYCERIDE

Cartridge No.	Collection Interval	Cpm above Background	Per Cent of Total Count	Identity	Per Cent Composition
	min				
1	30	0			
2	18	0			
3	11	38	6.5	14:0	1.0
4	7	0			
5	12	496	84.4	16:0	32.6
6	12	17	2.9	16:1	2.4
7	23	9		No peak	
8	5	11	1.9	18:0	3.9
9	3	0			
10	11	9	1.5	18:1	22.2
11	8	0			
12	33	6	1.0	18:2	35.4
13	30	0			
14	25	0			
15	35	0			
16	20	0			
17	88	1	0.3	20:4	2.3
18	1100	4		No peak	
		Sum 591			Sum 99.8

10 μl methyl ester injected; background = 20 cpm.
859 cpm above background obtained following direct injection of 10 μl methyl ester into anthracene cartridge.
859 × 80.5% = 693 cpm expected to go to fraction collector.
591/693 = 85% recovery of counts expected.

The distribution of dpm in the principal lipid components obtained by silicic acid chromatography is shown in Table 3. Since each rat was injected with 6.6×10^7 dpm, between 1/80 and 1/200 of the radioactive acetate was incorporated in liver lipid 1 hr after injection time. Of this, 97–99% resided in the triglyceride and phospholipid fractions. Radioactivity in the hydrocarbon fraction was negligible. Differences in average dpm per total liver are possibly ascribable to individual differences in animals rather than to effects of experimental conditions, especially since the over-all pattern of C^{14} incorporation in the different groups was somewhat similar.

Table 4 indicates the amounts of individual and total fatty acids (expressed as methyl esters) in phospholipids and triglycerides of average total livers. Entries represent minimum amounts based on recovery of the fatty acid esters following methylation and isolation. Average recovery of the phospholipid methyl esters was 44.8% of the original phospholipid weight, and that of the triglyceride methyl esters, 82% of the initial weight. If the data of Table 4 are placed on a percentage basis, it may be seen that 16:0 and 18:0 acids constituted about half of the total phospholipid fatty acids, but only 20–30% of triglyceride acids. Oleic acid (18:1) was the principal monoenoic acid of both triglycerides and phospholipids, but 16:1 was also present in triglycerides in small amounts. Although the essential constancy of phospholipid fatty acids is emphasized in Table 4 there may have been a small increase in phospholipid 20:4 in the hyperthyroid animal. In contrast, triglyceride linoleate decreased in the hyperthyroid rat livers from 49.7 to 11.1 mg per liver in one case and from 112.6 to 14.3 mg per liver in another. Such a decrease is interpreted as due to failure of assimilation to keep pace with degradative and interconversion processes.

For illustrative purposes, data from one GLC analysis and counting procedure are shown in Table 5. The distribution of counts is shown, as well as the correlation between appearance of peaks and collection of individual fatty acids. Also indicated are the total counts (859) observed following injection of 10 μl of methyl ester directly on the anthracene crystals of one cartridge. When correction was made for the counts deflected by the stream-splitter to the detector, the sum of counts recovered from the column totaled 85% of the number expected. The data show that the methyl myristate peak was well separated from the peak of methyl palmitate.

TABLE 6 PER CENT METHYL MYRISTATE IN METHYL ESTER MIXTURES* OF KNOWN COMPOSITION AS COMPARED WITH PER CENT AS DETERMINED BY GLC ANALYSIS

Mixture	I	II	III	IV
Prepared to Contain, %	3.86	1.58	0.80	0.40
By GLC Analysis, %	3.7	1.5	0.8	0.4

* Approximate compositions of mixture: methyl palmitate 19%, methyl oleate 35%, methyl stearate 43%; more exact compositions known but vary with the proportion of methyl myristate in the mixture.

Because a relatively high count was recovered from triglyceride methyl myristate it was important to know the accuracy of our GLC analysis of small percentages of this ester in mixed methyl ester sample. Accordingly, methyl esters of myristic, palmitic, stearic, and oleic acids of 99.8% purity[4] were prepared as mixtures in isooctane. GLC analyses were performed on the mixtures, employing the same conditions (including sample size) used in the animal experiments. The known percentages of methyl myristate in the prepared mixtures are compared in Table 6 with those obtained by GLC analysis. The percentage of methyl esters of fatty acids other than C14:0, obtained by analysis, were in good agreement with corresponding values in the prepared mixtures. GLC analyses were also made on standard test mixtures (K-101 and K-102[4]) under the operating conditions described in the previous section. Results agreed with stated compositions with average relative errors of 5% for components constituting >10% of the total mixture, and 12% for components constituting <10% of the total mixture.

To show how reliably counts could be recovered from the GLC column under operating conditions, a series of dilutions of C^{14}-labeled methyl palmitate of 99% purity were made in isooctane and added to a standard methyl ester mixture (K-102)[4] in isooctane. Following each dilution, the approximate number of methyl palmitate counts desired was added to 10 mg of the standard mixture in a conical tipped centrifuge tube, and the procedure followed of reducing solvent to small volume and taking two equal aliquots (4.0 μl), the first of which was injected directly on anthracene crystals in a cartridge, and the second into the inlet chamber of the GLC unit. The cpm directly pipetted on the anthracene crystals have been compared in Table 7 with cpm recovered during emergence of methyl palmitate from the column as evidenced by its peak on the recording chart. Counts recovered from methyl palmitate were corrected for the fraction of effluent deflected by the stream-splitter to the detector.

Specific activities from individual and mixed methyl esters derived from triglycerides and phospholipids are shown in Table 8. In general, specific activities of esters from saturated acids exceeded those from unsaturated

TABLE 7 RECOVERY OF RADIOACTIVITY IN METHYL PALMITATE-C^{14} FROM EFFLUENT OF GLC COLUMN

Cpm Injected ± Standard Deviation of Count	Cpm Recovered* ± Standard Deviation of Count	Recovery
		%
395 ± 4.5	377 ± 3.2	95
129 ± 3.8	115 ± 2.3	90
31 ± 1.4	26 ± 1.2	84
10 ± 0.8	11 ± 0.8	110
6 ± 0.7	6 ± 0.7	100

* Corrected for partition of flow by the stream-splitter.

acids. The highest specific activity was exhibited by methyl myristate, which exceeded that of methyl palmitate by 3- to 8-fold in these experiments. The data suggest that thyroid might increase and thiouracil decrease the specific activities of individual fatty acids.

The data of Table 9 essentially illustrate the capacity of liver for fatty acid synthesis from acetate. Proportional distribution of counts was basically unchanged by hyperthyroidism but the total number of dpm/liver was increased. In both glycerides and phospholipids, palmitic acid attained the highest total liver count, but in glycerides, myristic acid also showed considerable C^{14} incorporation in total liver despite the fact that this acid constituted less than 1% of liver acids recovered.

DISCUSSION

Analyses of fatty acids were made both by GLC and by alkali isomerization and UV spectrophotometry. The latter data are not presented here but in general these analyses gave slightly higher percentages for tetraenoic

TABLE 8 SPECIFIC ACTIVITIES (DPM/MG) OF INDIVIDUAL METHYL ESTERS (VALUES FROM POOLED LIVERS OF TWO ANIMALS)

	Diet*					
	D	D + T	D + P	D + Ch	D + Ch + T	D + Ch + P
Phospholipid						
16:0	275	2405	208	218	1010	186
18:0	58	455	42	41	105	23
18:1	0	146	16	0	216	0
18:2	2	52	5	0	15	0
20:4	16	69	26	20	24	27
Mixed	115	739	92	75	289	68
Triglyceride						
14:0	1470	7200	1545	670	5260	186
16:0	161	2750	230	69	1551	23
16:1	0	1308	0	0	357	0
18:0	151	520	306	47	372	0
18:1	0	72	0	7	28	5
18:2	2	31	5	0	15	0
20:4	67	81	134	36	161	81
Mixed	75	1080	87	22	565	12

* See Table 2.

TABLE 9 Disintegrations Per Minute Per Rat Liver in Individual Methyl Esters of Fatty Acids from Triglycerides and Phospholipids (Values Are from Two Pooled Rat Livers)

	Diet*					
	D	D + T	D + P	D + Ch	D + Ch + T	D + Ch + P
Phospholipid						
16:0	8,820	60,700	8,310	7,200	33,500	4,910
18:0	2,005	16,250	1,870	1,610	5,220	620
18:1	0	1,360	162	0	2,360	0
18:2	40	1,330	169	0	482	0
20:4	383	2,290	610	574	1,050	620
Total	14,300	97,600	13,900	11,600	49,200	8,510
Triglyceride						
14:0	675	2,310	495	581	1,430	230
16:0	3,550	27,500	4,500	3 040	14,700	1,180
16:1	0	915	0	0	242	0
18:0	371	630	398	192	338	0
18:1	0	481	0	273	181	246
18:2	79	336	251	0	352	0
20:4	147	57	619	94	242	452
Total	7,200	34,100	8,310	4,570	19,050	3,090

* See Table 2.

and lower percentage values for monoenoic and dienoic acids. In the present experiments, the GLC employed was not sensitive enough to detect penta- and hexaenoic acids in phospholipids whereas spectrophotometry gave average values for these acids of 2.6 and 4.3% respectively. It must be emphasized that in this work, preparative rather than analytical GLC was the prime objective.

Comparison of Tables 3 and 9 shows a larger number of disintegrations per minute in the individual lipid fractions than in the fatty acids obtained from them. Table 1 reveals that much of the radioactivity in total lipids resided in the water washes obtained following methylation. Nevertheless, the discrepancy (in Tables 3 and 9) between the ratios of phospholipid dpm to dpm of methyl esters of phospholipid fatty acids in groups D + T and D + Ch + P (234,000/97,600 and 276,000/-8,510, respectively) gave concern. Accordingly, phospholipids from these groups were again methylated, and dpm in methyl esters and in water wash determined. In this instance, the dpm (group D + T) in phospholipid, water wash, and methyl ester fatty acids of total liver were respectively: 228,000, 117,000, and 89,900. In group D + Ch + P, the dpm were respectively 287,900, 275,900, and 9070. We do not know that these ratios are typical of hyperthyroid and hypothyroid animals. In any case it appears that a varying fraction of C^{14} from acetate, obtained in liver phospholipid and triglyceride fractions under the experimental conditions, is released during methylation as a water-soluble substance.

The magnitude of specific activities of individual methyl esters (Table 8) bore no constant relationship to their concentration (Table 4). This fact probably indicates that liver fatty acid composition was only in part a reflection of the relative rates of synthesis of the individual acids as measured by the rates of incorporation of C^{14} from acetate.

The high specific activity of C14:0 from triglyceride probably has no unusual significance. The amounts of myristic acid originally present were small, and the amounts synthesized during the experiment were probably not sufficient to change fatty acid composition. Hence the resultant specific activity—higher than in palmitic acid—probably indicates only that the rate of synthesis is unrelated to pool size. On the other hand, the total radioactivity incorporated into triglyceride palmitic acid (Table 9) is 5 (D Group) to 12 (D + T Group) times as much as that incorporated into triglyceride myristic acid, just as might be expected from a chain-lengthening biosynthesis, ending usually at C_{16}.

The authors gratefully acknowledge the assistance of Mrs. Nadia Bulyk and Mrs. Barbara Newman.

This study was supported by PHS Research Grants H-4910 and H-5106 from the National Institutes of Health, U.S. Public Health Service.

Manuscript received August 5, 1963; accepted February 25, 1964.

References

1. Van Bruggen, J. T., T. T. Hutchens, C. K. Claycomb, and E. S. West. *J. Biol. Chem.* **200:** 31, 1953.
2. Tove, S. B., J. S. Andrews, Jr., and H. L. Lucas. *J. Biol. Chem.* **218:** 275, 1956.
3. Evans, J. D., and R. C. Baldridge. *Federation Proc.* **19:** 223, 1960.
4. Folch, J., I. Ascoli, M. Lees, J. A. Meath, and F. N. LeBaron. *J. Biol. Chem.* **191:** 833, 1951.
5. Luddy, F. E., R. A. Barford, R. W. Riemenschneider, and J. D. Evans. *J. Biol. Chem.* **232:** 843, 1958.
6. Luddy, F. E., R. A. Barford, and R. W. Riemenschneider.

J. Am. Oil Chemists' Soc. **37:** 447, 1960.
7. Werbin, H., I. L. Chaikoff, and M. R. Imada. *Proc. Soc. Exptl. Biol. Med.*, **102:** 8, 1959.
8. Karmen, A., L. Giuffrida, and R. L. Bowman. *J. Lipid Res.* **3:** 44, 1962.
9. Karmen, A., and H. R. Tritch. *Nature* **186:** 150, 1960.
10. Evans, J. D., N. O. Bulyk, P. J. Middleton, and H. G. Schwartz. *Endocrinology* **70:** 465, 1962.
11. Sperry, W. M., and M. Webb. *J. Biol. Chem.* **187:** 97, 1950.