

98. Fairchild, H. E., U.S. 2,920,993 (1960); C. A. 54, 9196h (1960).
99. Sun, Yun-Pei, and E. R. Johnson, J. Econ. Entom. 53, 887 (1960).
100. Moorefield, H. H., Contribs. Boyce Thompson Inst. 19, 501 (1958). U.S. 2,904,465 (1959); C. A. 54, 808a (1960).
101. Eldefrawi, M. E., R. Miskus and W. M. Hoskins, Science 129, 898 (1959).
102. Eldefrawi, M. E., R. Miskus and V. SUTCHER, J. Econ. Entom. 53, 231 (1960).
103. Speirs, R. D., *Ibid.* 53, 974 (1960).
104. Clark, P. H., and M. M. Cole, U.S. Dept. Agr. ARS-33-62 (1960). C. A. 55, 21451f (1961).
105. Hartle, R. J., and H. I. Thayer, U.S. 2,951,850 (1960); C. A. 55, 12756i (1961).
106. Hartle, R. J., and H. I. Thayer, U.S. 2,990,320 (1961); C. A. 55, 22703b (1961).
107. Hartle, R. J., and H. I. Thayer, U.S. 3,035,969 (1962); C. A. 57, 8966d (1962).
108. Metcalf, R. L., T. R. Fukuto and M. Y. Winton, J. Econ. Entomol. 53, 828 (1960).
109. Hodgson, E., and J. E. Casida, Biochim. Biophys. Acta 42, 184 (1960). Biochem. Pharmacol. 8, 179 (1961).
110. Grunt, J. A., W. H. Knisely and R. J. Berry, Proc. Soc. Exptl. Biol. Med. 94, 649 (1957).
111. Calhoun, T. B., and C. A. Angerer, Endocrinology 46, 327 (1950).
112. Jasper, R. L., M. E. Denison, W. A. Hiestand and M. X. Zarrow, Proc. Soc. Exptl. Biol. Med. 95, 417 (1957).
113. Denison, M. E., R. L. Jasper, W. A. Hiestand and M. X. Zarrow, Am. J. Physiol. 186, 471 (1956).
114. DiCuattro, C., and T. Mercadante, Policlinico (Rome), Sez. Med. 62, 171 (1955); C. A. 49, 14172a (1955).
115. Mirand, E. A., M. C. Reinhard and H. L. Goltz, Exptl. Med. Surg. 11, 286 (1953).
116. Anon., Am. Vet. Assoc. J. 125, 464D (1954).
117. Bishoff, F., Fed. Proc. 16, 155 (1957).
118. Bishoff, F., E. W. Sheller, G. Lopez and R. Fraundorf, J. Natl. Cancer Inst. 19, 977 (1957).
119. Bishoff, F., J. J. Rupp, J. G. Turner, Jr., and G. Bryson, Proc. Am. Assoc. Cancer Res. 2, 281 (1958).
120. Bishoff, F., Progr. exp. Tumor Res. 3, 412 (1963).
121. Ambrose, A. M., A. J. Cox and F. DeEds, J. Agr. Food Chem. 6, 600 (1958).
122. Gangadharam, P. R. J., S. Natarajan, T. K. Wadhvani, K. V. Giri, N. L. Narayanamurthy and B. H. Iyer, J. Ind. Inst. Sci. 35A, 69 (1953); C. A. 47, 4413d (1953).
123. Ramaswamy, A. S., Naturwissenschaften 44, 380 (1957); Chemotherapy, Proc. Symposium Lucknow 1958, p. 46; C. A. 54, 4910g (1960).
124. Chou, T. C., and A. L. Marlatt, J. Nutr. 51, 305 (1953).
125. Ershoff, B. H., *Ibid.* 71, 45 (1960).
126. Benton, C. H., and C. D., Robeson, U.S. 2,835,679 (1958); C. A. 53, 2279c (1959).
127. Haroks, O. D., Brit. 778,753 (1957); C. A. 52, 2077i (1958).
128. Naidu, M. B., and S. H. Zaheer, Indian 69,325 (Appl. 1959); C. A. 56, 2519i (1962).
129. Moore, R. N., and W. G. Bickford, JAOCS 29, 1 (1952).
130. Sahasrabudhe, M. R., J. Sci. Ind. Res. (India) 12B, 63 (1953); C. A. 47, 7238b (1953).
131. Oikawa, T., Kagaku to Sosa 9, 15 (1956); C. A. 51, 8454h (1957).
132. Acharya, B. N., and B. G. Malpoorwala, J. Univ. Bombay, Sect. A, 21, 53 (1952); C. A. 47, 12548g (1953).
133. Roy, B. R., J. Indian Chem. Soc., Ind. News Ed. 15, 171 (1952); C. A. 49, 2098i (1955).
134. Roy, B. R., Ann. Biochem. and Exp. Med. (India) 12, 63 (1952); C. A. 48, 4231 (1954); J. Indian Chem. Soc., Ind. News Ed. 17, 90 (1954); C. A. 49, 5863c (1955).
135. Sampath, S. R., C. P. Anantkrishnan and K. C. Sen, Indian J. Dairy Sci. 10, 34 (1957); C. A. 51, 18369f (1957).
136. Mathur, L. B., K. S. Tilara and R. Sahai, J. Indian Chem. Soc., Ind. News Ed. 18, 123 (1955); C. A. 50, 5309e (1956).
137. Dane, B. S., and B. S. Anantanarayanan, J. Sci. Ind. Research (India) 17C, 18 (1958); C. A. 52, 12260b (1958).
138. Murray, M. D., R. T. O'Connor, C. C. Suarez and W. G. Bickford, JAOCS 30, 329 (1953).

[Received February 1, 1963—Accepted October 3, 1963]

The Effect of Dietary Fat on the Glyceride Structure of Rat Carcass Fat¹

E. G. PERKINS, The Burnsides Research Laboratory, University of Illinois, Urbana, Illinois

Abstract

Carcass fats were obtained from weanling rats fed a complete diet for 8 weeks, which consisted of 2% cottonseed oil and 10% of the following fats: (1) corn oil; (2) the fatty acids of corn oil; (3) triricinolein; (4) ricinoleic acid; (5) the hydrogenated fatty acids of castor oil; and (6) commercial hydrogenated shortening. The fats were subjected to both pancreatic lipase and nonspecific hydrolysis; the resulting acids converted into methyl esters by conventional methods, and subjected to gas chromatographic analysis. From these data, the positional distribution of the component fatty acids, glyceride types, and isomeric forms were calculated. The results indicated a preferential placement of unsaturated acids in the 2-position of the carcass triglycerides and that the carcass fat composition in terms of unsaturated (U) and saturated (S) fatty acid composition is not greatly influenced by the S and U compositions of the dietary fat. It was found that hydroxy acids or their triesters are metabolized much the same as are normal triglycerides and exert no particular influence upon the fat structure of the rat. Some type of relationship between the dietary U and the U₃ in the carcass fat appears to be present. The glycerides of the carcass fats examined here are essentially a random mixture of the major glyceride types, but the isomeric forms (SUS,

SSU, USU and UUS) are a definite non-random mixture.

Introduction

Numerous reports (1,2,3) concerning the effects of dietary fats upon the fatty acid composition of depot fat of animals have recently appeared. It is generally agreed that the fatty acid composition of the depot fat of an animal will reflect the fatty acid composition of the ingested fat. However, few reports concerning the effects of dietary fats upon the triglyceride structure of depot fat have appeared. The glyceride structure of depot fat has been studied by Hilditch (4), Kartha (5) and others. As a result of this work several theories have been proposed to account for the observed differences in depot fat glyceride structure found in various species of animals. These theories have been aptly reviewed by Deuel (6) and VanderWal (7). Reiser et al. (8) have studied, by means of isotope dilution techniques, the glyceride structure of chick and rat fats when the animals were fed fat-free diets. The influence of ingested fat, fed at the 20% level was studied in the same manner. These authors found that the glyceride structure of endogenous rat fat in terms of saturated components (S) and unsaturated components (U) conformed to a random distribution pattern and that ingested fat appeared to be deposited according to the even type of distribution.

Tove et al. (9) recently have shown that the distribution of linoleic acid in depot fat triglycerides of the mouse is apparently dependent on the level of linoleate fed. These authors (10) have also stated that "the effect of increased depot fat levels of linoleate and oleate, and the distribution of other fatty

¹ Carried out at the Food Res. Div., Armour & Co., and at The Burnsides Research Laboratory under research grant No. EF 225 from the National Institutes of Health, U. S. Public Health Service, and Department of Health, Education, and Welfare.

TABLE I

Total and Positional Composition of Fresh Corn Oil and Carcass Fats of Rats Fed Thereon

Diet supplement			Carcass fats			
Animal number Esterified at: Fatty acid component	C1,3	C2	1 C1,3	C2	2 C1,3	C2
	%	%	%	%	%	%
Linoleic.....	49.3	70.6	22.6	69.1	23.6	58.4
Oleic.....	28.7	29.9	28.8	36.0	25.2	31.3
Stearic.....	3.1	1.5	3.8	1.1	4.1	2.0
Palmitoleic.....	7.4	3.1	5.2	2.2
Palmitic.....	18.0	-0.6	31.9	3.4	29.2	4.0
Myristic.....	2.0	0.1	2.4	0.9
Lauric.....	3.5	0.1	0.3	0.3

acids indicates that the distribution of fatty acids in the depot fat is adjusted so that there is a tendency to maintain a homeostasis of the physical properties of the depot fat." It was reported (9) that both the 1- and 2-positions of the glycerol molecule became substituted with linoleic acid depending upon the dietary level of this acid.

Only recently have techniques been developed which permit a detailed analysis of the triglyceride structure of a natural fat. This was accomplished by the discovery that the primary hydroxyl groups of glycerol esters of fatty acids are specifically cleaved by pancreatic lipase (11,12). The application of pancreatic lipase hydrolysis to the determination of the specific distribution of fatty acids in triglycerides has been carried out by Mattson et al. (13,14) who state that "no general pattern of distribution prevails among animal fats except that a non-random type of distribution is evident."

VanderWal (15) has developed a method for the calculation of the glyceride types and isomeric forms of glycerides present in natural fats from pancreatic lipase hydrolysis data. It has been demonstrated by Youngs (16) and Boatman et al. (17) that the method of calculation developed by VanderWal (15) yields results in agreement with those obtained by determination of glyceride types and isomeric forms present in fats by oxidative methods.

The effects of ingested fatty acids and triglycerides on rat carcass fat triglyceride structure are reported herein. The effects of the ingested lipids discussed in this report on the growth, carcass and fecal fat composition have been reported earlier (18).

Experimental

All rat fat samples employed in the present study were samples derived from a previous study (18), where male weanling rats (five per group) were fed a complete diet containing 10% test fat, and 2% cottonseed oil as a source of essential fatty acids. The animals were sacrificed at the end of a 59-day test period and the carcass fat obtained by HCl digestion of the eviscerated carcass according to the procedure of Johnson et al. (19). The fat from two animals

TABLE II

Total and Positional Composition of Shortening and Carcass Fats of Rats Fed Thereon

Diet supplement			Carcass fats			
Animal number Esterified at: Fatty acid component	C1,3	C2	1 C1,3	C2	2 C1,3	C2
	%	%	%	%	%	%
Linoleic.....	3.5	5.6	5.2	21.7	6.4	9.7
Oleic.....	32.4	55.5	45.7	45.7	44.5	61.9
Stearic.....	28.1	19.7	8.7	0.3	6.3	2.7
Palmitoleic.....	2.7	6.6	2.7	8.5	2.5
Palmitic.....	33.6	11.7	30.3	14.1	30.2	8.3
Myristic.....	2.4	4.8	3.1	6.7	3.5	2.6
Lauric.....	0.4	-0.5	0.6	0.3

TABLE III

Total and Positional Composition of Triricinolein and Carcass Fats of Rats Fed Thereon

Diet supplement			Carcass fats			
Animal number Esterified at: Fatty acid component	C1,3	C2	1 C1,3	C2	2 C1,3	C2
	%	%	%	%	%	%
Linoleic.....	2.6	0.5	11.2	29.8	8.2	12.1
Oleic.....	2.8	0.4	42.1	53.5	37.7	51.5
Stearic.....	0.6	0.0	3.9	3.3	4.9	5.5
Palmitoleic.....	8.9	4.4	11.6	11.0
Palmitic.....	0.8	1.6	28.5	10.5	34.9	17.5
Myristic.....	3.5	1.4	2.1	3.3
Lauric.....	3.5	6.1	2.1	3.3
Ricinoleic.....	93.1	99.8

was utilized in each group. This is a sufficient number to give an indication of the effects of the diets employed. The rat carcass fats and all other fats and methyl esters subsequently obtained were stored at -20C under nitrogen gas when not in use. Fresh corn oil and shortening, utilized as dietary supplements, were obtained locally. Hydrogenated castor oil fatty acids (Emery Industries) were used as obtained and contained 84.3%, 12-hydroxy-stearic acid. Triricinolein was prepared from castor oil by solvent extraction, and the fatty acids of corn oil and triricinolein were prepared as previously described (18). The procedure for pancreatic lipase hydrolysis of groups in the 1- and 3- positions of triglycerides employed in this study and the subsequent preparation of the methyl esters of the liberated acids using diazomethane has been published by Ast and VanderWal (20). Methyl esters of the whole fats were prepared by interesterification with methanol and those of fatty acids by methylation with diazomethane. The fatty acid compositions of the methyl esters were determined by GLC. Analysis of methyl esters was carried out using a gas chromatograph employing a thermal conductivity detector system. A 5-ft 1/4-in. internal diam copper column was packed with 65% of 60-80 mesh chromasorb (Johns Mansville, Chromasorb W) which was impregnated with 35% by wt of a succinic anhydride-diethylene glycol polyester as the stationary phase. Helium gas at a 10-lb pressure was used as the carrier gas, column temp was maintained at 187-197C, and the detector system at 228-232C. Percentage composition was determined by the triangulation technique and the esters of component acids identified by comparison with known methyl esters.

The method of calculation of glyceride types and isomeric forms present in fats from pancreatic lipase hydrolysis data, as recently published by VanderWal (15) has been employed in the present study. Data required for these calculations are the percentage of saturated acyl groups among all the acyl groups in the 2- position. From these data the proportions of the glyceride types S₃, S₂U, SU₂, and U₃ and of the symmetrical and unsymmetrical isomers which make up the S₂U and SU₂ molecules were calculated for the dietary fats and rat carcass fats studied here.

Results and Discussion

In a previous study (18) it was found that rats fed diets containing fresh corn oil, the fatty acids of the corn oil, triricinolein, ricinoleic acid, hydrogenated castor oil fatty acids and a commercial hydrogenated shortening all grew well; only a small amount (1.5-7.5%) of the ingested hydroxylated material was deposited as such in the carcass fat.

Analysis of the methyl esters of the dietary supplement fats, carcass fats and of the acids separated by

TABLE IV
 Total and Positional Carcass Fatty Acid Composition of Rats Fed Fatty Acid Containing Diets

Diet supplement	Fresh corn oil fatty acids		Ricinoleic acid				Hydrogenated castor oil fatty acids			
	1 C1,3	C2	1 C1,3	C2	2 C1,3	C2	1 C1,3	C2	2 C1,3	C2
Animal number Esterified at: Fatty acid component	%	%	%	%	%	%	%	%	%	%
Linoleic	28.2	46.8	8.7	9.6	3.6	21.9	6.1	18.1	5.1	21.3
Oleic	30.1	29.8	34.6	61.6	38.3	53.9	37.6	57.4	36.4	52.0
Stearic	4.0	2.0	5.0	6.2	7.4	4.0	7.4	0.8	6.4	1.3
Palmitoleic	6.3	14.7	12.2	11.0	12.0	9.6	10.3	11.8	11.9	8.6
Palmitic	27.4	9.7	36.5	8.9	34.6	18.1	34.4	11.9	36.8	14.0
Myristic	3.3	0.3	2.7	2.4	3.4	1.0	3.5	0.2	2.9	0.8
Lauric	0.7	0.4	tr.	0.9	0.6	0.3	0.6	0.0	0.4	0.4

pancreatic lipase hydrolysis of the carcass fats provides a view of the positional distribution of the individual fatty acids in the carcass triglycerides.

Smaller percentages of linoleic acid were found in the 1- and 3- positions than in the 2- positions of the dietary corn oil while palmitic acid was found to be in the 1- and 3- position in greater proportion (Table I). The shortening supplement had essentially the same fatty acid pattern in all positions except that an increased proportion of palmitic acid was located in the 1- and 3- positions (Table II), while the purified triricinolein also had the same pattern of fatty acid distribution in all positions (Table III). This indicates that in the latter two fats, fatty acids were distributed throughout the triglyceride molecules in essentially a random fashion while those acids in corn oil are not distributed in the same manner. The carcass fat of rats fed diets containing fresh corn oil had less linoleic and less oleic, and more stearic, palmitoleic, palmitic and myristic acids in the 1- and 3- positions than in the 2- position (Table I). Similar preferential placement of fatty acids was found in the triglycerides of the carcass fat of rats fed a commercial hydrogenated vegetable-animal fat shortening or triricinolein (Tables II, III).

To compare the effects of dietary free fatty acids and their degree of saturation on carcass fat glyceride structure, different groups of animals were fed the fatty acids of fresh corn oil, ricinoleic acid, triricinolein, and the fatty acids of hydrogenated castor oil. Animals fed fresh corn oil fatty acids deposited less linoleic and palmitoleic acids in the 1- and 3-positions of the glycerol moiety of the triglycerides than was deposited in the 2- position. The levels of oleic acid and of lauric acid in the 1-, 2-, and 3- positions of the carcass fat were essentially distributed at random (Table IV). When animals were fed ricinoleic acid, the percentages of linoleic and oleic acids in the 1- and 3- positions of the carcass fat of one animal (animal 2) were significantly less and that of palmitic, significantly greater in the 1- and 3- positions. A similar pattern was found in the carcass fat of animals fed hydrogenated castor oil acids. In both groups fed ricinoleic acid and hydrogenated castor oil acids, palmitoleic acid in the 1-, 2-, and 3- positions remained about equal (Table IV).

Comparisons of the fatty acid composition of the dietary supplement fats with those of the carcass fats (Tables I-V) indicate that some type of general inter-relationship may exist between the dietary fat and carcass fat, in the types of fatty acids in the whole fat and the 1-, 3- positions. Although Craig et al. (21) found no direct relationship between the fatty acids in the 1-, 3-, and the 2- position in dietary and depot fats, some type of relationship is indicated by the results of Savary et al. (22). Lymph chylomicrons were found to have the same fatty acid composition

as ingested fat, and were formed mostly from dietary fats in the small intestine. These authors have also shown that intraluminal lipolysis of triglycerides is not complete and that the intestinal mucosa can absorb a mixture of free fatty acids and partial glycerides with the 2- position remaining intact. A more recent report by Mattson and Volpenhein (23) has definitively shown that digestion and absorption does not destroy the structure of ingested fats. These authors fed triglycerides of known structure to rats and determined the distribution of the fatty acids in the lymph. It was found that from 85-90% of the fatty acids occupy the same position on the lymph triglyceride molecules as they did in the dietary triglycerides before the process of digestion and absorption. Since it has been shown that digestion and absorption does not destroy completely the structure of ingested fats such fats could be deposited without further rearrangement. Rearrangement of this freshly deposited fat could then take place in the stored fat to yield a fat of a structure desired by the animal.

In the present study, rats fed corn oil or the corresponding acids exhibited a lower percentage of linoleic acid esterified at the 1-, 3- positions of the carcass fat than in the 2- position. Little change was found in the oleic acid levels in the 1-, 2-, and 3- positions of the triglycerides of rats fed the corn oil (Table I).

It appears from the preceding data that the rat, when fed a diet high in linoleic acid, as on the corn oil diet (10% of a fat containing 56.4% linoleate), deposits this acid preferentially in the 2- position of the carcass triglycerides. On this diet, the carcass fat linoleate was C_{1,3}, 22.6%; and C₂, 69.1% for one of the animals examined (Table I). Similar results were obtained when a diet low in linoleate was fed to the rat, as in the case of the shortening diet (10% of a shortening containing 4.2% linoleate) i.e., C_{1,3} was 5.2% and C₂, 21.7% for one of the animals examined (Table II). The same tendency was noted for the fats of animals fed ricinoleic acid and hydrogenated castor oil diets. Again, this indicates that preferential deposition of linoleate in the 2- position occurs. Although Tove and Smith (9,10) have found

 TABLE V
 Fatty Acid Composition of Diet Supplement Fatty Acids

Diet supplement	Fresh corn oil fatty acids	Ricinoleic acid	Hydrogenated castor oil fatty acids
Fatty acid component	%	%	%
Linoleic.....	56.4	1.9
Oleic.....	29.1	2.0
Stearic.....	1.9	0.4	10.9
Palmitoleic.....
Palmitic.....	11.8	5.1
Myristic.....
Hydroxy acid.....	95.3 ¹	84.3 ²

¹ 12-hydroxyoctadec-9-enoic acid.

² 12-hydroxystearic acid.

TABLE VI
Positional Distribution of Saturated and Unsaturated Fatty Acids in Dietary Supplement and Carcass Fats

Esterified at: Fatty acid type: ^{1,2}	C1,2,3		C1,3		C2 (Calcd.)	
	S	U	S	U	S	U
Fresh corn oil						
Diet supplement:	13.7	85.5	21.1	78.0	1.1	100.5
Carcass fat: 1	26.4	73.5	36.0	63.8	6.5	93.1
2	26.7	73.0	41.1	58.8	2.1	97.9
Shortening						
Diet supplement:	54.3	45.2	64.0	35.9	36.2	64.1
Carcass fat: 1	38.3	61.7	42.5	57.4	29.9	70.2
2	35.7	64.2	40.8	59.3	25.7	74.1
Triricinolein						
Diet supplement:	0.7	99.3	1.4	98.6	0.7	100.7
Carcass fat: 1	29.3	70.7	36.8	62.2	14.1	87.8
2	36.9	63.2	42.4	57.5	25.9	74.5
Fresh corn oil						
Fatty acids:						
Carcass fat: 1	26.4	73.5	35.4	64.5	8.5	91.5
Ricinoleic acid:						
Carcass fat: 1	35.7	64.3	44.3	55.4	18.5	82.1
2	35.6	64.3	45.9	54.0	14.9	84.9
Hydrogenated castor						
Oil acids:						
Carcass fat: 1	34.9	65.1	45.9	53.9	12.8	87.3
2	36.5	62.9	46.5	53.4	16.8	81.6

¹ S - Saturated fatty acids in the glycerides.

² U - Unsaturated fatty acids in the glycerides.

in mouse depot fat indications of a selective deposition of linoleate in the 2- position of the depot glycerides until a level of about 15% is reached, after which deposition in the 1-, and 3- positions predominates, the present results indicate that a specificity for the 2- position exists for linoleate in the rat, at both low and high linoleate levels. These results confirm those which appeared in a more recent paper by Tove (24) which demonstrated that the unsaturates of mouse depot fat are located to a large extent in the 2- position of the depot glycerides, while the saturates are largely located in the 1-, and 3- positions. It was noted at the same time that palmitoleic acid was randomly distributed among all the positions in the depot glycerides of the mouse. The results obtained in the present study tend to confirm this in the case of carcass fats of rats fed ricinoleic acid, triricinolein and hydrogenated castor oil acids. The distribution of palmitoleic acid in the carcass fats of animals fed shortening, fresh corn oil, and the corresponding acid was, however, definitely not random, since a greater than random concn of this acid was found in the 1-, and 3- positions (Tables I,II,III).

The effect of a change in the degree of unsaturation of dietary fat upon the glyceride structure of the corresponding carcass fat can be determined by an examination of the individual fatty acid distribution in the fat of animals fed a diet containing a commercial hydrogenated shortening. Considerably less stearic acid and much less palmitic acid was found in the 2- position of the carcass fats than was in the 1-, and 3- positions; and linoleic acid was present in this position in larger proportions than in the 1-, and 3- positions. Palmitic acid was found in a greater than random proportion in the 1-, and 3- positions of the carcass fat. Palmitoleic acid appeared to be of slightly increased proportion in the 1-, and 3- position (Table II).

Since the naturally occurring hydroxy acids, 12-hydroxystearic and ricinoleic acids have been shown to be readily metabolized by the rat (18) the effects of feeding these acids and triglycerides thereof on rat carcass fat structure were investigated. Analysis of the carcass fats of animals fed triricinolein indicates that octadecadienoic and octadecenoic acids are in greater than random proportion in the 2- position, while hexadecenoic acid was more nearly uniformly distributed as were myristic and lauric acids. Palmitic acid was found to be in the 1-, and 3- posi-

tions in considerably higher concentrations than in the 2- position (Table III).

A similar distribution of the individual acids was found in the glycerol moiety of carcass triglycerides of rats fed hydrogenated castor oil fatty acids and ricinoleic acid (Table IV). In spite of the great differences in degree of unsaturation of the hydrogenated castor oil fatty acid and triricinolein dietary supplement, almost identical proportions of individual acids were found in the carcass fats.

It is noteworthy that whereas percentages of individual fatty acids vary widely between samples, and between animals, these differences are less apparent if the fatty acids in both dietary and carcass fats are classified simply as saturated (S) and unsaturated (U) without regard to chain length or degree of unsaturation. A much simpler and unified picture of the positional distribution of saturated and unsaturated fatty acids in both dietary and depot fats is thus obtained (Table VI). The per cent of S in the whole dietary fats varied from 1.4-64.0%. Those of U varied correspondingly. The percentages of S and U in the 2- position also varied depending upon the type of fat ingested. The proportions of total S in carcass fats of all rats fed the test diets varied from 26.4-38.3%, while among the acids in the 1-, and 3- positions, the variation in S was from 35.4-46.5%. The percentages of U varied correspondingly. This rather limited variation of total S and U found in the carcass fats compared with a much greater variation in the dietary fats (corn oil and shortening) indicates that in the growing rat, carcass fat composition, in terms of U and S, is not greatly influenced by the S and U compositions of the dietary fat if it is not too different from the endogenous fat and not fed at an excessively high level. Apparently, the various saturated acids are equally acceptable to a considerable degree, and it appears that the same is true of unsaturated acids. It also appears that hydroxy acids as their tri-esters, as are fed here, are metabolized much the same as are normal triglycerides and exert no particular influence upon the body fat structure of the rat.

The distribution of total S and U in the 1-, 3-, and the 2- positions provides a measure of the animal's ability to preferentially arrange the fatty acids in its depot fat. In the present study the percentage of S in the 1-, and 3- positions varied from 35.4-46.6%, and in the 2- position from 0.7-29.9% (Table

TABLE VII
Triglyceride Types and Isomeric Forms of Diet Supplement Triglycerides, and Carcass Fats of Rats Calculated from Pancreatic Lipase Hydrolysis Data

Dietary group	Glyceride types				Isomeric forms			
	Ss ¹	SaU ²	SU ₂	U ₃	SUS	SSU	USU	UUS
Fresh corn oil	%	%	%	%	%	%	%	%
Diet supplement:	0.0	4.5	33.3	62.0	4.5	0.0	0.0	33.3
(Calculated random)	0.2	2.2	30.1	63.5	1.6	0.6	10.1	20.0
Carcass fat: 1	0.8	15.4	45.9	37.8	12.4	3.0	2.6	43.3
2	0.3	15.6	47.3	36.6	14.7	1.0	0.8	46.5
(Calculated random)	0.2	15.1	42.8	37.7	5.1	10.3	14.3	28.5
Shortening								
Diet supplement:	14.8	42.8	34.0	8.2	26.1	16.7	4.6	29.4
Carcass fat: 1	5.0	26.3	44.3	24.4	11.8	14.5	10.4	33.7
2	4.3	24.7	44.9	26.1	12.3	12.4	9.0	35.9
Triricinolein								
Diet supplement:	0.0	2.0	1.3	96.7	2.0	0.0	0.7	2.0
Carcass fat: 1	1.9	26.0	49.1	24.5	13.3	12.7	5.6	43.5
2	4.7	26.0	50.0	24.2	19.6	6.4	8.6	41.4
Fresh corn oil acids								
Carcass fat: 1	1.1	15.4	45.4	38.2	11.5	3.9	3.5	41.9
Ricinoleic acid								
Carcass fat: 1	3.6	25.4	47.0	25.3	16.0	9.1	4.8	42.2
2	3.6	25.4	47.6	24.8	18.0	7.9	5.7	41.9
Hydrogenated castor								
Oil fatty acids:								
Carcass fat: 1	2.9	26.0	47.0	24.2	19.6	6.4	3.5	43.5
2	3.6	26.3	46.2	23.9	17.9	8.4	4.8	41.4

¹S - Saturated fatty acids in the glycerides.

²U - Unsaturated fatty acids in the glycerides.

VI). The percentages of U varied correspondingly. It appears from these data that in the rat there may be wide variations in the percentages of S and U among the three positions of the glycerol molecule as long as restrictions as to the total S and U are adhered to; and the animal can preferentially deposit the various fatty acids as S or U into either the 1-, 3-, or 2- positions.

Examination of the triglyceride types and isomeric forms of triglycerides which make up the rat fat yields information concerning the influence of dietary fat on them and provides a picture of the whole carcass fat in broad terms (Table VII). When fresh corn oil is fed to a rat a small amount of S₃ is found (0.8%). Percentages of SU₂ and S₂U found are 45.9-47.3% and 15.4-15.6% respectively, and a small amount of S is placed in the 2- position to form 2.6% USU. A similar distribution of glyceride types and isomeric forms was found when the animals were fed free fatty acid-containing diets. Animals fed diets containing commercial hydrogenated shortening (Table VII) deposited 4.3-5% S₂; the amount of S₂U found was 26.3-24.7% while the percentages of S₂U and U₃ were 24.7-26.3, 26.4% and 24.4% respectively. The greatest changes were observed in the percentages of isomeric forms present; SUS was found to be from 11.8-12.3%, USU from 9.0-10.4%, and UUS from 33.7-35.9%. On triricinolein, the animals deposited a fat similar to that of animals fed shortening, except that the percentage of UUS increased somewhat. Animals fed the free fatty acids—ricinoleic and those of hydrogenated castor oil had carcass fats similar to those obtained by feeding the corresponding triglycerides. These results are similar to those of Craig et al. (21) who observed an apparent relationship between the U₃ in depot fat and the dietary unsaturated fatty acids, in that the U₃ increased in proportion to increased U in the dietary. The amounts of SUU found in the depot fats were related to the saturated acids of the dietary fat.

Regardless of the type of fat fed, the glyceride structure of the rat in terms of S and U was found to be variable only within narrow limits. A maximum trisaturated (S₃) content of 5% and ranges of 15-26% S₂U, of 45-50% SU₂, and of 26-38% U₃ were found to be the type of fat that the rat desired to have and attempted to achieve. Even less variation

was found between the various isomeric forms present in the carcass fats.

When the percentages of glyceride types found are compared to those calculated for a random distribution in the case of the animals fed corn oil (Table VII) fairly close agreement may be seen, and this fat might on this basis be designated as a randomly distributed fat. However, if one then examines the distribution of isomeric forms among the glycerides and compares these data with those calculated for a random distribution, the divergence of the carcass fat studied here from a purely random pattern is evident. Great differences in the individual percentages of SUS, SSU, USU, and UUS from the purely random pattern are found. The distribution of glycerides as found in the present study is therefore clearly not random.

ACKNOWLEDGMENTS

Assistance in obtaining analytical data from H. J. Ast and A. Amato; counsel throughout this work and assistance in manuscript preparation from R. J. VanderWal.

REFERENCES

1. Fiegenbaum, A. S., and H. Fisher, *Arch. Biochem. Biophys.* **79**, 302 (1959).
2. Brown, Myrtle L., *J. Nutri.* **71**, 235 (1960).
3. Machin, L. V., and R. S. Gordon, *Ibid.* **75**, 157 (1961).
4. Hilditch, T. P., "The Chemical Constitution of Natural Fats," 3rd rev. ed., John Wiley & Sons, New York, 1956, p. 402.
5. Kartha, A. R. S., "Studies on the Natural Fats," Vol. 1, Part 1, published by the author, Ernakulam, India, 1951.
6. Deuel, H. J., "The Lipids," Vol. 1, Interscience Publishers, New York, 1951.
7. VanderWal, R. J., "The Triglyceride Composition of Natural Fats. Progress in the Chemistry of Fats and Other Lipids," Pergamon Press, London, 1955, p. 329.
8. Reiser, R., and J. W. Dieckert, *JAACS* **31**, 625 (1954).
9. Tove, S. B., and F. H. Smith, *Fed. Proc.* **19**, 222A (1960).
10. Tove, S. B., and F. H. Smith, *J. Nutri.* **71**, 264 (1960).
11. Mattson, F. H., and L. W. Beck, *J. Biol. Chem.* **219**, 735 (1956).
12. Savary, P., J. Flanzky, and P. Desnuelle, *Biochim. Biophys. Acta* **24**, 414 (1957).
13. Mattson, F. H., and R. A. Volpenhein, *J. Biol. Chem.* **236**, 1891 (1961).
14. Mattson, F. H., and E. S. Lutton, *Ibid.*, **233**, 868 (1958).
15. VanderWal, R. J., *JAACS* **37**, 18 (1960).
16. Youngs, C. G., *Ibid.* **38**, 1 (1961).
17. Boatman, Carolyn, A. E. Decoteau, and E. F. Hammond, presented at the ADSA meeting in Logan, Utah, 1960.
18. Perkins, E. G., J. G. Endres, and F. A. Kummerow, *J. Nutri.* **73**, 291 (1961).
19. Johnston, P. V., O. C. Johnson, and F. A. Kummerow, *Ibid.* **65**, 13 (1958).
20. Ast, H. J., and R. J. VanderWal, *JAACS* **38**, 67 (1961).
21. Craig, B. M., C. G. Youngs, Joyce L. Beare, and J. A. Campbell, *Can. J. Biochem. Physiol.* **41**, 43 (1963).
22. Savary, P., M. J. Constantin, and P. Desnuelle, *Biochim. Biophys. Acta* **48**, 562 (1961).
23. Mattson, F. H., and R. A. Volpenhein, *J. Biol. Chem.* **237**, 53 (1962).
24. Tove, S. B., *J. Nutri.* **73**, 361 (1961).

[Received August 29, 1963—Accepted December 13, 1963]