15. Kass, J. P., "Protective and Decorative Coatings," Vol. 4, pp. 362-405, McGraw Hill Book Company, New York (1944).
16. Robinson, G. N., J. Chem. Soc., 2204 (1926).
17. Braun, J. V., and Bayer, O., Ber., *58*, 2667 (1925).
18. Overbeck, V. O., Ann., *140*, 63 (1866).
19. Yasuda, M., J. Biol. Chem., *94*, 401 (1931-32).
20. Nystrom, R. F., and Brown, G. W., J. Am. Chem. Soc., *69*, 1197 (1947); *70*, 3738 (1948).
21. Morton, P., "Laboratory Techniques in Organic Chemistry," p. 64, McGraw Hill Book Company, New York (1938).
22. Arndt, F., "Organic Syntheses, Collective," Vol. 2, pp. 461 and 165, John Wiley and Sons inc., New York (1943).
23. Burr, G. O., and Lundberg, W. O., "Deterioration of Fats," Quartermaster Corps Manual, 17-7, p. 31 (1945).
24. Henne, A. L., and Perilstein, W. L., J. Am. Chem. Soc., *65*, 2183 (1943).
25. Gilman, H., *ibid.*, *55*, 3461 (1933).
26. Boeseken, J., Rec. trav. chim., *49*, 95 (1936).
27. Swern, D., Billen, G. N., Findley, T. W., and Scanlan, T. J., J. Am. Chem. Soc., *67*, 1786 (1945).
28. Joshel, L. M., Ind. Eng. Chem. (Anal. ed.), p. 590 (1943).
29. West, E. S., Hoagland, C. L., and Curtis, G. H., J. Biol. Chem., *104*, 627 (1934).

[Received July 10, 1950]

Molecularly Distilled Monoglycerides. III. Nutritional Studies on Monoglycerides Derived From Cottonseed Oil[1,2]

STANLEY R. AMES, M. PATRICIA O'GRADY, NORRIS D. EMBREE, and PHILIP L. HARRIS,
Research Laboratories, Distillation Products Industries, Division of Eastman Kodak Company, Rochester, New York

TRIGLYCERIDES, from a quantitative viewpoint, are the most important lipids of foods and of tissues and have been of prime interest to the lipid biochemist for a long time. However certain intermediate products of triglyceride metabolism, mono-, and di-glycerides, have received attention in recent years. Monoglycerides, in particular, have been studied with respect to their beneficial role in fat absorption from the intestinal tract (1) and to their presence normally in pancreas and other tissues (2). Furthermore Tidwell (3) has shown that monoglycerides of olive oil fatty acids were more efficiently absorbed than olive oil. Also the nutritive properties of monostearin and monolinolein were investigated by Braun and Shrewsbury (4), who indicated that these two monoglycerides were "practically equal" to lard in promoting the growth of young rats.

These experiments were designed to investigate the problem of whether relatively large amounts of monoglycerides of a natural mixture of fatty acids would be utilized *in vivo*, as efficiently as natural fats composed of triglycerides of the same mixture of fatty acids. Monoglycerides of the fatty acids of cottonseed oil have been compared with cottonseed oil itself at 15% and at 25% levels in the diet of three generations of rats, for adequacy in supporting growth, reproduction, and lactation. Results indicate that for all these functions cottonseed oil monoglycerides are equal to the fat from which they were prepared.

Experimental

Diets. The detailed composition of the diets is given in Table I. The additional fat was added at the expense of a portion of both cerelose and starch. The cottonseed oil was deodorized, refined salad oil (Wesson oil), and the cottonseed oil monoglycerides were a commercial product (Myverol Type 18-85 Monoglyceride) made by molecular distillation (5). Each diet was prepared to contain 400 mg. of vitamin E/kg. The cottonseed oil contained 0.75 mg./gm. of vitamin E and the cottonseed oil monoglyceride, 0.22 mg./gm.; the extra amount needed was furnished as distilled d,α-tocopherol concentrate. Fresh diets were prepared weekly, and the prepared diets were stored in the refrigerator until used. No increase in peroxide value (about 2) of the diet was observed on storage for one week. All diets were fed *ad libitum*.

TABLE I

Composition of Diets

Ingredient	Content (%)
Casein, crude	16.0
Casein, vitaminized	2.0
Salt mixture, U.S.P. No. 2	4.0
Yeast, dried brewers	7.8
Liver extract, Wilson's 1-10	0.2
Lipid, cottonseed oil or monoglycerides	15.0 or 25.0
Corn starch	36.7 or 30.0
Dextrose (Cerelose)	18.3 or 15.0

Vitamins: per 10 grams of diet—

A—400 I.U.	B_1—100 μg.	Ca-pantothenate—250 μg.
D— 40 I.U.	B_2—100 μg.	Niacin—200 μg.
E— 4 mg.	B_6—100 μg.	Choline—10 mg.
K—500 μg.		Inositol—5 mg.

Treatment of Animals. Eighty albino rats, forty males and forty females, were selected at time of weaning from our stock colony. The animals (10 per group) were placed immediately on the four diets previously described; 15% and 25% cottonseed oil, and 15% and 25% cottonseed oil monoglycerides. The growth of these animals was observed for an 8-week period, at which time reproduction and digestibility experiments were started. Six females and two males were selected from each dietary group for breeding purposes. Throughout the breeding and lactation period the females were maintained on the same diets previously fed. The males were interchanged between breeding cages and subsisted for short periods on diets other than those to which they were originally assigned. The young of the original ani-

TABLE II

Growth Response of Male Rats (8-Week Period)

Level of Fat	Generation	Mean Gain ± S.E. (gms.)	
		Cottonseed Oil	Monoglycerides
15%	1	219.3± 9.5	213.9±6.5
	2	251.1± 9.4	220.5±5.9
	3	225.7± 2.7	235.9±6.7
25%	1	217.3± 9.5	228.0±8.5
	2	226.9± 4.2	219.0±7.7
	3	226.5±10.5	219.8±5.3
Over-all Mean		227.8± 4.0	222.9±2.8

Diff. of Means = 4.9; S.E. of Diff. = 5.0; t = 0.98; p = 0.3-0.4.

Each group contained approximately 10 animals. The over-all mean gain for cottonseed oil is based on 61 animals and for monoglycerides on 59 animals.

[1] Presented at San Francisco fall meeting, American Oil Chemists' Society, Sept. 26-28, 1950.
[2] Communication No. 174.

TABLE III

Growth Response of Female Rats (8-Week Period)

Level of Fat	Generation	Mean Gain ± S.E. (gms.)	
		Cottonseed Oil	Monoglycerides
15%	1	151.1±6.0	139.9±4.7
	2	152.3±3.8	144.9±2.7
	3	143.9±4.5	152.0±6.0
25%	1	143.6±4.4	136.8±3.6
	2	149.7±5.7	141.5±2.3
	3	147.3±6.7	147.3±2.4
Over-all Mean		149.2±2.5	143.6±1.5

Diff. of Means =5.6; S.E. of Diff. = 2.9; t = 1.96; p = 0.05-0.06.

Each group contained approximately 10 animals. The over-all mean gain for cottonseed oil is based on 58 animals and for monoglycerides on 67 animals.

mals were termed second generation animals. They were treated in a similar manner throughout growth, breeding, and lactation. The young of the second generation (or third-generation animals) were treated in similar manner throughout the 8 weeks' growth study period. No attempt was made to breed these animals, and they are being retained for survival tests.

Growth. The results of the 8 weeks' growth studies of the three generations of rats fed the four diets under examination are summarized in Tables II and III. It was found by statistical analysis that there was practically no difference in rate of growth between diets containing 15% and 25% fat or between generations of animals. The data are therefore summarized so that a direct comparison can be made between the diets containing cottonseed oil on one hand and those containing cottonseed oil monoglycerides. The results of the conventional "t" test indicate that there was no significant difference in the over-all mean weight gains of the male rats between the diets containing the two types of fat (p = 0.3-0.4). The difference in mean weight gains for the female rats was slightly greater but was not statistically significant (p = 0.05-0.06).

Reproduction. The reproductive performance of the females of the first- and second-generation animals is summarized in Table IV. Comparable fertility was observed irrespective of type of fat, level of fat, or generation. The mean size of the litters and the mean weight of the young at birth are also comparable, irrespective of treatment. No differences in reproductive performance were observed in females fed the different diets under consideration.

Lactation. The body weight of the young at weaning was used as the criterion of lactation. Lactation performance is summarized in Table V. There was no significant difference in the weaning weights between sex or between level of fat. The data are therefore summarized so that a direct comparison can be made between the cottonseed oil and cottonseed oil monoglyceride diets. The results of the conventional "t" test indicate that diets containing the two fats are comparable in their effect on the weaning weight of the young (p = 0.1-0.2).

TABLE IV

Reproductive Performance

Diet	Generation	Females Pregnant /Mated	Litters		
			Total Live Young	Mean Litter Size	Mean Wt. at Birth (gms.)
15% Fat Cottonseed Oil	1	6/6	52	8.7	....
	2	6/6	41	6.9	5.6
Monoglycerides	1	5/6	42	8.4	....
	2	6/6	54	9.0	6.1
25% Fat Cottonseed Oil	1	4/4	27	6.8	....
	2	6/6	50	8.3	5.8
Monoglycerides	1	5/6	39	7.8	....
	2	6/6	55	9.2	6.7

TABLE V

Lactation (Body Weight of Young at Weaning, 21 Days)

Sex	Level of Fat	Mean Weight at Weaning ± S.E. (gms.)	
		Cottonseed Oil	Monoglycerides
Male	15%	50.4±2.2	43.1±0.9
Male	25%	43.2±2.2	49.1±1.8
Female	15%	49.9±2.3	41.7±1.3
Female	25%	43.8±3.3	47.0±1.6
Over-all Mean		47.4±1.3	45.4±0.8

Diff. of Means = 1.4; S.E. of Diff. = 2.0; t = 1.40; p = 0.1-0.2.

Digestibility. Eight male rats on each of the two diets containing 25% fat, one containing cottonseed oil, the other cottonseed oil monoglycerides, were segregated following the first generation, 8 weeks' growth experiment. The feces were collected, and food consumption was determined daily for a period of seven days. In order to determine the excretion of metabolic fat the animals were then placed on the corresponding fat-free diet. After an equilibration period of seven days feces were again collected and food consumption determined on a daily basis for seven days. The fat content of the feces and the digestive coefficient were determined according to the procedures described by Hoagland and Snider (8). The results of food consumption and digestibility studies are given in Table VI. There was no difference in the mean digestive coefficient between the rats fed the diet containing 25% cottonseed oil and the diet containing 25% cottonseed oil monoglycerides. In view of the high digestive coefficients observed with the diets containing 25% fat, no experiments were performed with the 15% fat diets.

There was likewise no difference in the mean daily food consumption between rats on the two diets.

TABLE VI

Food Consumption and Digestibility
(Male Rats; Diets Containing 25% Fat)

Type of Fat	Mean Daily Food Consumption (9th experimental week)	Coefficient of Digestibility
	(gms.±S.E.)	*(%±S.E.)*
Cottonseed Oil	13.8±0.4	96.8±0.5
Monoglycerides	13.6±0.5	97.8±0.4

Food Consumption: Diff. = 0.14; S.E. of Diff. = 0.6; t = 0.22; p = 0.8-0.9.

Coef. of Digestibility: Diff. = 0.94; S.E. of Diff. = 0.6; t = 1.52; p = 0.1-0.2.

Discussion

Prior to this study it was interesting to speculate that monoglycerides of the fatty acids of cottonseed oil would be superior, nutritionally, to cottonseed oil

itself. This speculation was based on the concept of Frazer and co-workers (1) that monoglycerides are essential elements in the mechanism of intestinal absorption of lipids and on the results obtained by Tidwell (3), showing that monoglycerides of olive oil fatty acids were more efficiently absorbed than olive oil. These facts and the successful use of monoglycerides in intravenous feeding experiments (6, 7) are compatible with the concept that monoglycerides of natural fats might be superior in nutritive value to the original fat. However from the results obtained in the present study it is evident that monoglycerides of fatty acids of cottonseed oil are not significantly better than cottonseed oil itself and the two types of lipids must be considered nutritionally equal.

Summary and Conclusions

Monoglycerides, prepared from cottonseed oil, were fed to three generations of rats at a 15% and a 25% level as the sole source of fat in the diet. Refined cottonseed oil was fed to comparable groups of rats at the same levels.

No significant differences were found between the monoglycerides and the cottonseed oil in their nutritive value as measured by growth response, reproduction ability, and lactation performance. Absorption of fatty acids, either as monoglycerides or as the original oil, from the intestinal tract was the same as shown by essentially equal coefficients of absorption for the two types of lipid at a 25% level in the diet.

Acknowledgment

Appreciation is expressed to Hugh Risley, who performed the analyses associated with the digestibility experiments.

REFERENCES

1. Frazer, A. C., Chem. and Ind., *27*, 379-82 (1947).
2. Jones, M. E., Koch, F. C., Heath, A. E., and Munson, P. L., J. Biol. Chem., *181*, 755-60 (1949).
3. Tidwell, H. C., J. Biol. Chem., *182*, 405-14 (1950).
4. Braun, W. Q., and Shrewsbury, C. L., Oil and Soap, *18*, 249-50 (1941).
5. Kuhrt, N. H., Welch, E. A., and Kovarik, F. J., J. Am. Oil Chemists' Soc., *27*, 310-13 (1950).
6. Meng, H. C., and Freeman, S., J. Lab. and Clin. Med., *33*, 689-707 (1948).
7. Lerner, S. R., Chaikoff, I. L., and Entenman, C., Proc. Soc. Exptl. Biol. Med., *70*, 388-91 (1949).
8. Hoagland, R., and Snider, G. G., U. S. Dept. Agr. Tech. Bull. 821, March 1942.

[Received October 5, 1950]

ABSTRACTS . . . . . Don Whyte, Editor

• Oils and Fats

R. A. Reiners, Abstractor

ADSORPTION SEPARATIONS IN THE FAT FIELD. VI. PAPER CHROMATOGRAPHY. H. P. Kaufmann (Univ., Münster, Ger.). *Fette u. Seifen* **52**, 331-43(1950). The literature on paper chromatography is thoroughly reviewed (81 references). Carotene is determined by capillarizing a 1-g. sample in 100 ml. petroleum ether or hexane, developing with isopropyl alcohol, and testing for β-carotene by the Carr-Price reaction. Fatty acids were separated by dissolving in pure hexane and developing with methanol containing 1% water. Mixtures of stearic and oleic acid, stearic and linoleic acid, and oleic and linoleic acid were separated in this manner. Mixtures of glycerides and fatty acids were separated by capillarizing a 2% petroleum ether solution and developing with methanol containing 1% water. Mixtures of glycerides were separated by using a 2% petroleum ether solution and developing with aqueous methanol containing 1% iso-butyl alcohol or 1% acetic acid. (*Chem. Abs.* **44**, 10351)

DISTRIBUTION OF THE FATTY ACIDS FORMED BY THE OXIDATION OF PARAFFINS [IN THE FISCHER-TROPSCH PROCESS]. H. Pardun (Ölfabriken Noblée u, Thörl G.m.b.H., Hamburg-Harburg, Ger.). *Fette u. Seifen* **52**, 290-5(1950). The influence of the degree of oxidation on the distribution of the fatty acids formed in the Fischer-Tropsch process was investigated by oxidizing samples of a paraffin fraction b_{13} 168-233°C. at temperatures of 110°, 120°, 130°, and 140° so as to yield products of acid nos. 10, 20, 40, and 80. The distribution of fatty acids formed a maximum in all cases. With decrease in degree of oxidation this maximum migrates toward fatty acids of higher molecular weight. It is probable that the primary reaction takes place at the end of the paraffin chain and that the higher-molecular weight compounds formed first are split further on continuing oxidation. (*Chem. Abs.* **44**, 10351)

AUTOXIDATION OF LINOLEIC ACID IN AQUEOUS COLLOIDAL SOLUTION. S. Bergstrom, E. Blomstrand, and S. Laurell (Univ. of Lund, Sweden). *Acta Chem. Scand.* **4**, 245-50(1950). Autoxidation of Na linoleate colloidally suspended in an aqueous pH 9 buffer seemed to yield products that were not polymerized and of a fairly reproducible composition. A maximum of 2 moles of oxygen per mole of Na linoleate was absorbed. Ultraviolet absorption rose to a maximum at one mole oxygen absorbed, then dropped to almost zero at 2 moles oxygen absorbed. (*Chem. Abs.* **44**, 9165)

SOLID SOLUTIONS OF HIGHER FATTY ACIDS AND TRIGLYCERIDES. N. N. Efremov, G. B. Ravich, and V. A. Vol'nova. *Izvest, Sektora Fiz.-Khim. Anal., Inst. Obshchei i Neorg. Khim., Akad. Nauk. S.S.S.R.* **16**, No. 3, 142-55(1948). The system's stearic acid-palmitic acid and tristearin-tripalmitin were subjected to thermal analysis, microstructure, hardness, and efflux pressure studies. At a rapid cooling rate the system stearic-palmitic acids formed a continuous row of solid solutions. At a slow cooling rate molecular compounds were formed. The latter combined with the components of the system to form some solid solutions. Microstructure studies revealed a eutectic at 70% of palmitic acid. The triglycerides have three similar temperature-concentration curves, each within a different temperature range. The curves of composition-hardness of the triglycerides system depended on the rate of cooling. The solid solutions formed within this system partially decomposed with time. The solid solutions with up to 30% of tristearin were more stable than the others. The triglycerides did not form molecular compounds. (*Chem. Abs.* **44**, 10352)

METHODS OF EXTRACTING VITAMIN A AND OIL FROM FISHERY PRODUCTS. III. EXPERIMENTS ON THE EXTRACTION OF LOW-OIL-CONTENT LIVERS WITH PETROLEUM ETHER BY THE SHAKING METHOD. F. B. Sanford and Neva L. Karrick (Tech. Lab., U. S. Fish and Wildlife Service, Seattle, Wash.). *Com. Fisheries Rev.* **12**, No. 6, 4-8(1950). In a study to determine the efficiency of oil extraction from low-oil-content fish livers with petroleum ether it was found that at a ratio of sample to solvent of 1 to 50, the sample was more completely extracted than at the lower ratios of 5 to 50 or 18 to 50. Pumice added in amount equal to volume of extracting solvent proved to be a better dispersing agent than anhydrous Na_2SO_4. (*Chem. Abs.* **44**, 9634)

THE MECHANISM OF OXIDATION OF MONOETHENOID FATTY ACIDS. FACTORS INFLUENCING HYDROPEROXIDE FORMATION AND TRANSITION IN CATALYTIC AUTOXIDATIONS. J. H. Skellon (Acton Tech. Coll.). *J. Chem. Soc.* **1950**, 2020. Oxidation of oleic acid by air at room temperature in the presence of strong ultraviolet light results in rapid formation of peroxides which then undergo transition or decomposition. Metals such as lead, aluminum, and barium were shown to accelerate, during the aerial oxidation of oleic acid at 120°, the primary stage of peroxidation but to have only a moderate effect on the transition to ketonic derivatives.

MEASUREMENT OF THE COLOR OF FATS, OILS, AND THE RESINS WITH THE HELLIGE COLORIMETER, F. Pallauf (Fabrik Abshagen & Co., Hamburg-Wandsbek, Ger.). *Fette u. Seifen* **52**, 370-2