thin appeared to increase it. The addition of penicillin to tetracycline or chloramphenicol reduced the hemolytic inhibitory activity of these compounds. Combining cephalothin with these broad-spectrum antibiotics shortened the time required for inhibition of hemolysis to develop.

1. Pfizer and Co., "Pfizer Handbook of microbial metabolites," Miller, M. W., ed., p. 772. McGraw-Hill, New York, 1967.

2. Syphred, P. S., Strauss, N., and Treffers, H. P., Biochem. Biophys. Res. Commun. **7**, 447 (1962).

3. Michael, T. M., Michael, J. G., and Massell, B. F., J. Bacteriol. **93**, 1749 (1967).

4. Magnusson, B. J. and Gulasekharam, J., Nature **206**, 728 (1965).

5. Watanebe, Y. and Verway, W. F., J. Infect. Diseases **116**, 363 (1966).

6. Watanebe, Y. and Seeman, G. R., Arch. Biochem. Biophys. **97**, 393 (1962).

7. Hallander, H. O., Laurell, G., and Löfström, G., Acta Pathol. Microbiol. Scand. **68**, 142 (1966).

8. Chang, T. W. and Weinstein, L., Nature **221**, 763 (1966).

9. Chang, T. W. and Weinstein, L., Proc. Soc. Exptl. Biol. Med. **124**, 980 (1967).

10. Chang, T. W. and Weinstein, L., Science **143**, 807 (1964).

11. Chang, T. W. and Weinstein, L., J. Bacteriol. **88**, 1790 (1964).

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The 1- and 2-Octadecyl Glyceryl Ethers as Model Compounds for Study of Triglyceride Resynthesis in Cell Fractions of Intestinal Mucosa* (32645)

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It has been well documented that dietary triglycerides are split in the lumen of the intestine into 2-monoglycerides and free fatty acids. Further, it has been shown that the monoglyceride penetrates the intestinal mucosa and in the mucosal cell is resynthesized to triglyceride (1). In the hamster intestine, according to Johnston (2), both the 1- and 2-monoglycerides are readily converted into triglyceride via the 1,3- and 1,2-diglyceride intermediates, respectively. In the rat intestine, the 2-monoglyceride has been reported to be the preferred acyl acceptor in the formation of triglyceride (3,4), but such data is difficult to evaluate since the 2-monoglyceride rapidly undergoes isomerization to the 1-monoglyceride. Likewise, the ease with which the 1,2-diglyceride isomerizes to the 1,3-diglyceride has made determination of the intermediate diglyceride difficult.

Swell *et al.* (5) have shown that both the 1- and 2-octadecyl glyceryl ethers are absorbed by rat intestine *in vivo* and appear in the lymph as alkoxydiglycerides. Sherr and Treadwell (6), using everted rat intestinal sacs, have demonstrated the conversion of 2-octadecyl glyceryl ether into alkoxymono- and alkoxydiglycerides. Kern and Börgstrom (7), using hamster intestinal rings, have shown that both the 1- and 2-monoglyceryl ethers are converted into alkoxydiglycerides as readily as monoolein is converted into triglyceride. The purpose of the work presented here was to establish (a) which monoglyceride isomer is preferred for triglyceride resynthesis, and (b) the positional specificity of the diglyceride intermediate for its acylation to triglyceride using octadecyl glyceryl ethers as model compounds. The glyceryl ethers are useful since they fail to undergo isomerization or significant splitting in the *in vitro* system but are converted to alkoxydiglycerides apparently by the same mucosal transacylases which convert monoglyceride into triglyceride (7).

Experimental. Male Wistar rats from Carworth Farms, weighing between 100 and

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200 gm, were sacrificed by decapitation. The upper two thirds of the small intestine was removed, washed with ice cold isotonic saline, and everted. The mucosa was scraped off, weighed, and placed in 0.278 *M* mannitol; this was homogenized, and fractionated into subcellular fractions according to Senior and Isselbacher (8). The microsomes were resuspended by gentle homogenization just prior to use in two volumes of 1.1% KCl in 0.01 M potassium phosphate buffer per gram of original wet weight of mucosa. The 1- and 2-octadecyl-(1-^{14}C) glyceryl ethers were gifts of Dr. Leon Swell. The ethers were solubilized on fatty acid-poor bovine albumin (Fraction V) according to Senior and Isselbacher (8). Palmityl- and linoleyl-CoA were prepared chemically according to the procedure of Goldman and Vagelos (9). The specific extinction coefficient at 260 mμ was used to determine the percentage of acyl-CoA derivative in the preparations.

Each incubation flask contained octadecyl glyceryl ether (1 μmole), acyl-CoA (2 μmoles), and microsomes from 0.25 gm of mucosa in a total volume of 2 ml. Incubations were carried out in a Dubnoff shaker, under air, for 20 or 45 min at 37°C, as indicated in the individual tables. The reactions were stopped by addition of 25 vol. of ethanol–ether (3:1 v/v) and the solutions were brought to a boil to extract the lipid. After filtration, the lipid extract was evaporated to dryness at 40°C under a stream of nitrogen, and the lipids were redissolved in 5 ml of hexane. Aliquots of the lipid extract were spotted on silicic acid plates and the lipids separated with a solvent system of hexane, acetone, and acetic acid (89:11:3, v/v) (6) or chloroform. The ether analogs migrated slightly ahead of their glyceride counterparts in chloroform, but the monoglyceride and the free fatty acids moved as one band. Utilization of the two solvent systems independently gave clear separation of each lipid component. The lipids were identified by comparison with known standards. The 1- and 2-monoethers and alkoxydiglyceride were obtained from Dr. Leon Swell; 1,2- and 1,3-dioleins from Dr. Fred Mattson. The monoglyceride, free fatty acid and triglyceride standards were purchased from Hormel Institute. The 1,3- and 1,2-alkoxymonoglycerides were synthesized from the 1- or 2-octadecyl glyceryl ether and palmityl chloride. The individual lipid fractions were scraped from the plates into vials, 10 ml of liquid scintillate (100 mg of 1,4-di-2(5 phenyl oxazolyl)-benzene plus 4 gm of 2,5-diphenyl oxazole per 1000 ml toluene) added, and the radioactivity measured in a Nuclear Chicago liquid scintillation counter. The data shown in each of the tables are from a single experiment and are representative of 3–6 separate experiments.

Results. Positional specificity of mono- and diglycerides for triglyceride resynthesis. It is evident from Table I that rat mucosal microsomes are capable of readily converting both the 1- and 2-octadecyl glyceryl ethers into alkoxymonoglycerides. The 2-octadecyl glyceryl ether is converted into 1,2-alkoxymonoglyceride (44.7%) followed by further conversion into alkoxydiglyceride (19.7%), while the 1-octadecyl glyceryl ether is converted mainly to 1,3-alkoxymonoglyceride (38.6%), with small proportions of the 1,2-isomer (5.9%) and the alkoxydiglyceride (3.1%). The amount of the 1,2-isomer synthesized may be adequate to account for the alkoxydiglyceride in the system. It can also be seen from Table I that the 2-octadecyl glyceryl ether is a more favorable substrate than the 1-octadecyl glyceryl ether for conversion into higher glyceride analog (64.5% vs 41.7%).

Utilization of saturated vs unsaturated acyl-CoA in synthesis of higher glycerides. Table II shows that the relative utilization of palmityl-CoA and linoleyl-CoA as sources of activated fatty acid in the resynthesis of higher glyceride analogs. Palmityl-CoA is a more effective acylating agent in the conversion of both glyceryl ethers to their corresponding alkoxymonoglycerides. Due to the low yield of alkoxydiglyceride in this experiment, no conclusion can be drawn as to the relative utilization of the saturated and unsaturated acyl-CoA's in the second acylation. However, in an additional experiment, 2-octadecyl glyceryl ether was incubated in the presence of either 2 μmoles of palmityl-CoA, 2 μmoles linoleyl-CoA, or a mixture of 1

TABLE I. Glyceride Formation from 1- and 2-Octadecyl-[1-^{14}C]-glyceryl Ether by Rat Mucosal Microsomes.

Each incubation flask contained 1 μmole of octadecyl-[1-^{14}C]-glyceryl ether dispersed with 100 mg bovine serum albumin, 200 μmoles of potassium phosphate buffer (pH 6.4), 80 μmoles KCl, 70 μmoles NaCl, 25 μmoles KF, 5 μmoles $MgCl_2$, 2 μmoles palmityl-CoA, and freshly prepared microsomes (from 0.25 gm mucosa) in a total volume of 2 ml. Incubations were at 37°C for 20 min.

	Conversion of octadecyl glyceryl ethers to higher glycerides			
	1-Monoether		2-Monoether	
Lipid fraction[a]	Boiled microsomes (%)	Fresh microsomes (%)	Boiled microsomes (%)	Fresh microsomes (%)
PL	0.6	2.1	2.8	3.1
ME	94.0	49.5	92.3	28.3
1,2-AMG	0.9	5.9	1.6	44.7
1,3-AMG	2.3	38.6	1.3	2.2
FFA	1.2	0.8	0.3	1.8
ADG	1.0	3.1	1.7	19.7

[a] PL = phospholipid; ME = monoether; AMG = alkoxymonoglyceride; FFA = free fatty acid; ADG = alkoxydiglyceride.

TABLE II. Utilization of Palmityl- and Linoleyl-CoA in Glyceride Resynthesis.

	Conversion of monoethers to higher glycerides[a]			
	1-Monoether		2-Monoether	
Lipid fractions[b]	Linoleyl-CoA (%)	Palmityl-CoA (%)	Linoleyl-CoA (%)	Palmityl-CoA (%)
ME	74.8	58.3	63.6	44.7
1,2-AMG	3.4	0.5	28.6	50.1
1,3-AMG	19.4	36.4	0.3	0.5
FFA	0.7	1.8	0.6	0.6
ADG	1.7	2.0	6.0	4.1

[a] The incubation mixture and conditions are given in Table I.

[b] See Table I.

μmole each of palmityl and linoleyl CoA. The results indicate that palmityl-CoA is a better acylating agent for both the first and second acylation.

Triglyceride resynthesis in mucosal homogenates. Previous *in vitro* studies (8,10) of the monoglyceride pathway in rat mucosal microsomes have consistently reported high yields of diglyceride and low yields of triglyceride. The data given in Tables I and II confirm this. Experiments were carried out in which microsomes were preincubated with palmtiyl-CoA for 15 min in order that endogenous fatty acid acceptors might be utilized before addition of the glyceryl ether. This did not increase the conversion of glyceryl ether to alkoxydiglyceride. However, whole homogenates were found to be capable of synthesizing considerably larger amounts of the triglyceride analog than microsomes prepared from a comparable amount of whole homogenate. Table III shows the conversion of the 1- and 2-octadecyl glyceryl ethers into higher glycerides by a mucosal homogenate. The conversion of the 2-glyceryl ether to the 1,2-diglyceride analog (38.2%) and the tri-

TABLE III. Glyceride Formation from 1- and 2-Octadecyl-(1-^{14}C) Glyceryl Ethers by Mucosal Homogenates.

Lipid fraction[b]	Conversion of monoethers to higher glycerides[a]	
	1-Monoether (%)	2-Monoether (%)
ME	51.3	14.0
1,2-AMG	1.8	38.2
1,3-AMG	30.5	0
ADG	5.9	47.8

[a] Incubation conditions given in Table I + 20 μmoles ATP and 20 μmoles $MgCl_2$. Flasks incubated 45 min.

[b] See Table I.

glyceride analog (47.8%) was significantly higher than the conversion of the 1-glyceryl ether to its corresponding higher glyceride analogs (30.5% and 5.9%).

Discussion. Assuming the 1- and 2-octadecyl glyceryl ethers are acylated by the mucosal enzymes without distinction between ether and ester linkages, then the ether analogs are excellent models for studying triglyceride resynthesis. The ethers do not isomerize and are not split significantly by mucosal enzymes in the *in vitro* system. Based on the assumption that the ether analogs are handled as the monoglycerides, then the data suggest that the 2-monoglyceride is acylated preferentially by mucosal transacylases for triglyceride synthesis via a 1,2-diglyceride intermediate. The 1-monoglyceride that is converted to triglyceride most probably proceeds via a 1,3-diglyceride followed by isomerization to the 1,2-diglyceride, which in turn is acylated to form triglyceride. The failure of the rat mucosa to readily utilize 1-monoglyceride, while the hamster mucosa converts both the 1- and 2-monoglyceride equally well into triglyceride suggests that the rat possesses either a more specific diglyceride transacylase, or that the hamster possesses an additional enzyme necessary for the direct conversion of 1,3-diglyceride into triglyceride.

Summary. The 1- and 2-octadecyl-(1-^{14}C) glyceryl ethers have been used as model compounds to study triglyceride synthesis. The data suggest that: (a) The rat mucosa utilizes 2-monoglyceride for the formation of triglyceride; (b) the 1,2-diglyceride is the intermediate which is acylated to form triglyceride in the monoglyceride pathway; (c) and that utilization of the 1,3-diglyceride in triglyceride synthesis probably involves preliminary isomerization to the 1,2-diglyceride.

1. Senior, J. R., J. Lipid Res. **5**, 495 (1964).

2. Johnston, J. M. and Brown, J. L., in "Biochemical Problems of Lipids," Frazer, A. C. and Library, B. B. A., eds., Vol. 1, p. 211. Elsevier, Amsterdam, 1963.

3. Johnston, J. M., Rao, G. A., and Reistad, R., Biochim. Biophys. Acta **98**, 432 (1965).

4. Ailhaud, G., Samuel, D., Lazdunski, M., and Desnuelle, P., Biochim. Biophys. Acta **84**, 643 (1964).

5. Swell, Leon, Law, M. D., and Treadwell, C. R., Arch. Biochem. Biophys. **110**, 231 (1965).

6. Sherr, S. I. and Treadwell, C. R., Biochim. Biophys. Acta **98**, 539 (1965).

7. Kern, F., Jr., and Börgstrom, B., Biochim. Biophys. Acta **98**, 520 (1965).

8. Senior, J. R. and Isselbacher, K. J., J. Biol. Chem. **237**, 1454 (1962).

9. Goldman, P. and Vagelos, P. R., J. Biol. Chem. **236**, 2620 (1961).

10. Clark, B. and Hubscher, G., Biochim. Biophys. Acta **70**, 43 (1963).

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