4. Fels, I. G., Kaufman, M., *Nature*, 1959, v183, 392.
5. Dawkins, M. F. R., Judah, J. D., Rees, K. R., *Biochem. J.*, 1959, v72, 204.

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Digestion, Absorption and Metabolism of Chimyl Alcohol Fed as Free Alcohol or as Alkoxydiglyceride.* (25353)

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It was shown previously(1,2) that after feeding free chimyl alcohol dissolved in olive oil to rats or to human beings, the ether linkage was broken in the intestinal mucosa so that about 50% of radioactivity in lymph was associated with palmitic acid and the remainder of chimyl alcohol was present partly as chimyl alcohol and partly as esterified. This finding together with structural similarity of chimyl alcohol with a monoglyceride makes it possible to study whether a real synthesis of glycerides takes place in the intestinal contents and the forms in which dietary triglycerides are absorbed into the intestinal mucosa. In the present report the course of hydrolysis and synthesis of alkoxydiglycerides *in vivo* was followed after feeding labelled chimyl dioleate or free chimyl alcohol to rats. Furthermore metabolism and distribution of chimyl alcohol in the rat has been followed.

Methods. Labelled material: Preparation of α-(1-C^{14})-hexadecylglyceryl ether (chimyl alcohol) was carried out mainly according to Holmes *et al.*(3) as described earlier(1,2). Radioactive chimyl dioleate was prepared by esterification of α-(1-C^{14})-hexadecylglyceryl ether with oleic acid chloride in pyridine. Acid chloride was prepared from oxalyl chloride. Free fatty acids were removed by passing the mixture through Amberlite IRA-400. The neutral material was then applied in benzene to a silicic acid column and eluted with successive portions of benzene, benzene:chloroform (85:15) and chloroform. More than 95% of radioactive material was eluted with benzene. The diesterified chimyl alcohol (chimyl dioleate) thus behaved as a triglyceride. This chromatographic behavior of alkoxydiglyceride has been described(1,2). Labelled chimyl alcohol was fed as free alcohol or as chimyl dioleate dissolved in olive oil. In one series (A) intestinal contents were analyzed during absorption phase 2-4 hours after administration. In another series (B) distribution of activity in liver lipides and expired CO_2 was determined 24 hours after administration. Finally in some experiments (C) distribution of activity in lymph lipids was followed after feeding chimyl dioleate. A. Rats were fed 0.5 ml of 2% solution of chimyl dioleate or chimyl alcohol in olive oil by stomach tube. Animals were killed 2-4 hours after administration, and contents of small intestine were washed out with saline into dilute hydrochloric acid. Total fat was extracted 3 times with 2 volumes of ether. Separation of free fatty acids and glycerides of recovered lipides was performed on columns of Amberlite-IRA-400. A further separation of glycerides into tri-, di-, and monoglycerides was made on columns of silicic acid(4). In this separation chimyl dioleate followed the triglycerides, chimyl monooleate the diglycerides, and chimyl alcohol the monoglyceride fraction. B. In some experiments the same solutions as in Exp. A were fed to rats, expired CO_2 was collected and isotope content determined as described earlier(5). For comparison chimyl alcohol was injected intravenously and expired CO_2 collected. At different time intervals 24-72 hours, the rats were sacrificed and the organs analyzed. In the present paper only data on excretion of radioactivity in the breath are given. C. In a few experiments, labelled chimyl dioleate dissolved in olive oil was fed to rats with a thoracic duct fistula. The technic for collection of lymph

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TABLE I. Composition and Isotope Content in Lipides Recovered from Intestinal Washings after Feeding Free C^{14}-Labelled Chimyl Alcohol Dissolved in Olive Oil to 2 Rats. Animals were killed after 2 and 4 hr respectively. Exp. A and B. Free chimyl alcohol followed the monoglycerides in separation on silicic acid. Specific activity of fed mixture 569 cpm/mg.

Chromatographic fractions	% of total lipides		Specific activity, cpm/mg		% of total C^{14}-activity	
	A	B	A	B	A	B
Triglycerides	23.6	16.5	147	213	14.4	13.6
Diglycerides	23.6	13.2	240	836	16.3	31.0
Monoglycerides	22.4	20.0	1060	870	68.8	51.7
Free fatty acids	30.4	50.3	6	27	.5	3.7

and the analyses of lymph has been described (1).

Results. Composition of lipides recovered from contents of small intestine of rats fed free chimyl alcohol or chimyl dioleate dissolved in olive oil is summarized in Tables I and II. Although there are great variations, values for the different components agree well with those of Desnuelle and Constantine(6), Mattson *et al.*(7), and Borgström(8). Monoglyceride values represent total monoglycerides including free chimyl alcohol. Chimyl alcohol with one fatty acid attached is included in the diglyceride fraction and chimyl alcohol with 2 fatty acids attached follows the triglyceride fraction in separation on silicic acid.

In Table I results of activity determinations of different mono-, di-, and triglyceride fractions and of free fatty acids are summarized. No significant activity was associated with free fatty acids and none was found in the fatty acids of different glyceride fractions, indicating that radioactivity in the different glycerides is due to the chimyl alcohol component of these fractions.

When radioactivity of the glyceride fractions is considered (Table I), it appears that the monoglyceride fraction has the highest specific activity and the triglyceride fraction the lowest. However, the radioactivity associated with the di-, and triglyceride fractions is significant, and in Exp. B specific activity of the diglyceride fraction is even higher than that of the fed mixture, indicating a synthesis of glyceride ester bonds taking place simultaneously with hydrolysis.

Experiments in which chimyl dioleate dissolved in corn oil was fed are summarized in Table II. After feeding this mixture significant amounts of label were obtained in mono-, di-, and triglycerides. Because no significant activity was associated with the free fatty acids, no synthesis of glycerides from labelled fatty acids could have taken place. Of the glycerides the diglyceride fraction had the highest and the triglyceride fraction the lowest specific activity. The monoglyceride fraction, which includes free chimyl alcohol, had a relative specific activity of 158%, indicating a concentration of free chimyl alcohol, in which both original fatty acids had been released by enzymatic action. The results thus indicate that fatty acids in 1- and 2-position of the alkoxydiglyceride had been attacked by enzymes present in the intestinal content.

The results of some lymph experiments are given in Table III. About 75% of radioactivity in the lymph was present as glyceride fatty acids and about 2% as phospholipid fatty acids. About 25% of lymph activity was present as chimyl alcohol of which more

TABLE II. Summary of Analyses of Intestinal Contents of 2 Rats after Feeding C^{14}-Labelled Chimyl Alcohol Esterified with Oleic Acid (Chimyl Dioleate) and Dissolved in Olive Oil. Rats were sacrificed after 4 hr, and total fat separated into tri-, di-, and monoglycerides on silicic acid. In this separation chimyl dioleate followed the triglycerides and free chimyl alcohol the monoglyceride fraction. Specific activity of fed mixture 1747 cpm/mg.

Chromatographic fractions	Total wt of resp. fractions, mg	% of total fat	Spec. act., cpm/mg	% of total activity
Triglycerides	27.3	24.7	1748	33.0
Diglycerides	13.0	11.6	3571	32.1
Monoglycerides	18.0	16.4	2743	34.0
Free fatty acids	52.7	47.3	27	.9

TABLE III. Recovery and Distribution of Radioactivity in Lymph Lipids after Feeding C^{14}-Labelled Chimyl Dioleate Dissolved in Olive Oil to 2 Rats with a Thoracic Duct Fistula.

% of admin. activity absorbed	% of absorbed activity recovered in lymph lipids	% of activity in lymph lipids recovered as: Chimyl alcohol Free	Chimyl alcohol Esterified	Triglyceride fatty acids	Phospholipid fatty acids
90	60	6	18	74	2
85	45	5	20	73	2

than two-thirds was present as esterified chimyl alcohol. These results are similar to those obtained earlier(1,2) after feeding free chimyl alcohol to rats.

Appearance of C^{14} in expired CO_2 is shown in Table IV. A significant difference in rate of metabolism of chimyl alcohol and palmitic acid is demonstrated. Chimyl alcohol is metabolized to CO_2 only half as fast as palmitic acid. After intravenous injection of chimyl alcohol, there is a rapid appearance of labelled CO_2 demonstrating widespread occurrence of enzymes capable of splitting the ether bond.

Discussion. Our results with free chimyl alcohol (Table I) clearly show that incorporation of free fatty acids into chimyl alcohol with formation of alkoxydiglycerides is due to synthesis of new glyceride-ester bonds. These experiments thus with a different approach, support earlier findings of Borgström(7) that a resynthesis of glyceride ester bonds occurs simultaneously with the hydrolysis in the lumen of small intestine. Our results therefore give no support to the statement of Reiser and Dieckert(9) that no significant amount of fatty acid exchange occurs in the intestinal lumen of rat during fat digestion.

The results of analyses of intestinal contents together with lymph analyses of this and previous investigations(1,2) clearly indicate the dynamic situation in the intestine. In the intestinal lumen, ether linkage is intact during the whole absorption process, but once absorbed into the intestinal mucosa more than 50% of the compound is rapidly converted into palmitic acid, indicating a splitting of the ether bond.

TABLE IV. Excretion of Radioactivity in Breath by Rats after Feeding: A. Palmitic acid-1-C^{14} dissolved in olive oil, B. C^{14}-labelled chimyl alcohol esterified with oleic acid (chimyl dioleate) dissolved in olive oil, and C. Intrav. inj. of free C^{14}-labelled chimyl alcohol. Figures are the means of 2 exp.

Exp.	Hr after dose	Cumulative % of total radioactivity admin., excreted as $C^{14}O_2$
A	24	16
B	24	8
C	24	24

Our results (Table III) indicate that fatty acids in both 1- and 2-position in the alkoxydiglyceride molecule are exchanged during hydrolysis by pancreatic enzymes *in vivo*. Apparently an equilibrium is soon reached between synthesis and hydrolysis of mono- and di-esterified chimyl alcohol and free chimyl alcohol.

Earlier resynthesis of glyceride ester bonds during hydrolysis of glycerides has been evidenced for the step 1,2-diglyceride to triglyceride and a synthesis from a 2-monoglyceride has appeared likely(8,10). The present results clearly indicate that a synthesis from a 1-monoglyceride is possible, because significant amounts of radioactivity were associated with the triglyceride fraction after feeding free chimyl alcohol.

Our results must be considered as proof that glycerol monoethers can be absorbed unchanged. However, the results also indicate that chimyl alcohol is absorbed with one or 2 fatty acids attached. This finding indicates that dietary glycerides are absorbed as a mixture of mono-, di-, and triglycerides and free fatty acids(11). The important role of the intestinal mucosa in formation of final products which are fed into the intestinal lymph is emphasized in our investigation.

Summary. 1. C^{14}-labelled chimyl alcohol (α-/1-C^{14}/-hexadecylglyceryl ether) has been fed as free chimyl alcohol or as chimyl dioleate to rats. Intestinal contents and thoracic duct lymph lipids have been analyzed and expired CO_2 has been collected. 2. Mixed etherglycerides have been isolated from the intestinal contents after feeding free chimyl alcohol dissolved in olive oil, indicating a synthe-

sis of new glyceride ester bonds. 3. Free labelled chimyl alcohol has been isolated from the intestinal contents after feeding labelled chimyl dioleate indicating an exchange and release of fatty acids in both 1-, and 2-position in the mixed ether-glyceride. 4. In lymph lipids more than half the radioactivity was associated with palmitic acid, indicating a splitting of the ether bond in mucosa cells. 5. It is concluded that chimyl alcohol can be absorbed unchanged but that it is extensively metabolized already in the mucosa cells, whether it is fed as free chimyl alcohol or as an alkoxydiglyceride.

1. Bergström, S., Blomstrand, R., *Acta Physiol. Scand.,* 1956, v38, 166.

2. Blomstrand, R., Ahrens, E. H., Jr., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 802.

3. Holmes, H. N., Corbet, R. E., Geiger, W. B., Kornblum, N., Alexander, W., *J. Am. Chem. Soc.,* 1941, v63, 2607.

4. Borgström, B., *Acta Physiol. Scand.,* 1952, v25, 111.

5. Bergström, S., Blomstrand, R., Borgström, B., *Biochem. J.,* 1954, v58, 600.

6. Desnuelle, P., Constantin, M. J., *Biochim. et Biophys. Acta,* 1952, v9, 531.

7. Mattson, F. H., Benedict, J. H., Martin, J. B., Bech, L. W., *J. Nutrition,* 1952, v48, 335.

8. Borgström, B., *Arch. Biochem. and Biophys.,* 1954, v49, 268.

9. Reiser, R., Dieckert, J. W., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 649.

10. Borgström, B., *Biochim. et Biophys. Acta,* 1954, v13, 491.

11. Blomstrand, R., Tryding, N., Westöö, G., *Acta Physiol. Scand.,* 1956, v37, 91.

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Inhibition of Gastric Secretion in the Rat by Normal Human Gastric Juice.* (25354)

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Presence of a substance in normal human gastric juice which inhibits Heidenhain pouch secretion in dogs when administered intravenously has now been well established(1,2). This substance is not dialyzable, is resistant to boiling and is largely inactivated by drastic acidification(2). It appears to inhibit specifically hydrochloric acid and water secretion (2). Studies aimed at identification and isolation of the active substance have been hindered by certain disadvantages inherent to the Heidenhain pouch preparation. Of these, the foremost is gradual development of gastric atrophy after the animal received several injections of gastric juice(3). Our purpose was to determine the feasibility of using the pylorus ligated rat preparation(4) for determination of inhibitory activity of preparations of normal human gastric juice.

Materials and methods. Gastric juice was collected from hospital patients previously found free of any gastrointestinal pathology. Following an overnight fast, a 4 hour collection of insulin stimulated gastric secretion was carried out. Immediately after collection, the juice from several subjects was pooled, dialyzed 36 hours against distilled water and lyophilized. Approximately 1 mg of material/1 ml of gastric juice was obtained. Immediately prior to use, this material was resuspended in normal saline and brought to pH 7.0. Gastric secretion was measured by pylorus ligation technic(4) in 65 male Holtzman rats weighing 160 to 220 g. Prior to experiments, rats were fasted 48 hours in individual cages with wide mesh wire bottoms. Water was given *ad lib.* Rats were operated upon under light ether anesthesia, and pylorus was ligated with a 4-0 silk ligature. Rats were divided randomly into control group and 3 treatment groups. Control rats were given 1 ml of normal saline; treated rats received

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