Effect of ethanol on intestinal lipid absorption in the rat

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Abstract The effect of ethanol infusion on intestinal lipid absorption was studied in rats with a duodenal cannula. Rats were infused with ethanol overnight and ethanol was included in a trioleoylglycerol emulsion infusion given for 3 hr the next day. These rats were compared to control animals infused with glucose (isocalorically). The ethanol-infused rats had a greatly impaired lipid absorptive capacity. The monoacylglycerol and free fatty acid contents in the intestinal lumen in the ethanol-infused rats were 4- and 7-fold greater, respectively, than controls. The inhibition of absorption was not due to an effect of ethanol on lipolytic activity. The lipase content of the ethanol-infused rats was greater than controls and the separate infusion of monoacylglycerol and fatty acids demonstrated impaired absorption of these end products of lipolysis as compared to controls. To observe if these changes were due to an effect of ethanol on the enterocyte brush border membrane, the membrane lipids were analyzed. The phosphatidylcholine, lysophosphatidylcholine, and phosphatidylethanolanine content was reduced but not the neutral lipids, spingomyelin, or phosphatidylserine. The uptake of fatty acid into intestinal rings was also shown to be impaired by ethanol infusion. Lastly, the specific activity of the neutral lipids remaining in the intestinal lumen after [³H]glycerol-labeled trioleoylglycerol-infusion was similar to controls even though the mass was much greater. ■ It is concluded that ethanol impairs neutral lipid absorption due to an effect on the enterocyte brush border membrane and by increasing the efflux of low specific activity lipid from the enterocyte back out into the intestinal lumen. A potential pathway for this efflux is the recently described increased porosity of the apical junctional complex in response to ethanol infusion.—**Mansbach, C. M., II.** Effect of ethanol on intestinal lipid absorption in the rat. *J. Lipid Res.* 1983. **24:** 1310–1320.

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Alcohols have significant effects on cell membranes and detergent-mixed lipid micelles as might be expected from their structure. Either of these effects might be important in digestion and absorption of lipid in the presence of ethanol. In the human, approximately ⅓ of chronic alcoholic subjects admitted to the hospital malabsorb lipid (1). The reasons for this impaired absorption have in most instances been shown not to be related to the direct effects of alcohol. In two studies (2, 3) patients with steatorrhea on admission to the hospital were placed on a nutritious diet. Large doses of ethanol were then re-introduced, at which point lipid absorption was found to have normalized. Other patients have demonstrated indirect effects of ethanol on lipid absorption. Patients with ethanol-related chronic pancreatitis may have significant depression of lipase delivery to the intestine (4). The effect of ethanol on the liver may result in a reduction of bile acid delivery to the intestine (5). In other instances the administration of certain antibiotics that suppress urea-forming bacteria, also bind bile acids and distort intestinal morphology (6). However, ethanol has been shown to have a suppressive effect on a number of intestinal membrane functions. These have been recently reviewed (7).

In animal models the chronic feeding of ethanol has been shown to suppress the delivery of radiolabeled lipids from the intestinal tract to the lymph 4-fold, whereas the mass of transported lipid was not as significantly affected (8). These data suggest that the lipid mass delivered to the lymph may come from endogenous rather than exogenous sources. Such increases in endogenous sources might be expected in view of the known effects of ethanol on intestinal lipid metabolism. These effects, all of which would lead to enhanced TG synthesis, are a reduction in CO_2 formation from FFA (9), an increment in the activity of neutral lipid acyltransferases (10), and the incorporation of FFA into TG (11). Furthermore, the acute administration of ethanol in rats results in an increase in endogenous TG output into the lymph in the form of VLDL (12).

Interpretation of the studies in humans in terms of the overall effect of ethanol on lipid absorption is complicated by the fact that high ethanol concentrations are

only obtained in the proximal intestine, since ethanol is rapidly absorbed both in the stomach and the intestinal tract. When sampled, the more distal intestinal tract has ethanol levels similar to those found in blood, and therefore, considerably lower than that found in the upper intestine (3, 13). Since lipid absorption can occur in the distal as well as the proximal intestine, it might be expected that lipid not absorbed proximally would be absorbed more distally in the intestine where the ethanol concentration is lower. Therefore, to more adequately assess the acute effects of ethanol on lipid absorption, we designed studies in which large doses of ethanol were infused intraduodenally, thus eliminating the potential problem of delayed gastric emptying (14, 15). Additionally, these studies were stimulated by the recent findings that ethanol increases the permeability of apical junctional complexes in the ethanol-treated rat (16). This increased permeability might provide an avenue for the efflux of absorbed lipid back out into the intestinal lumen, which would then contribute toward the ultimate lipid load required to be absorbed (17). Also bearing on these studies were previous observations in which impaired uptake of FFA infused as a mixed micelle was demonstrated in patients with alcoholic cirrhosis suggesting an effect of ethanol on the enterocyte membrane (18).

## MATERIALS AND METHODS

Animal and tissue preparation

Male Sprague-Dawley rats (350–550 g) were given a duodenal cannula (PE 50, Clay Adams, Parsippany, NJ) the afternoon prior to an experiment. They were placed in restraining cages and infused overnight with 0.15 M NaCl containing ethanol or glucose at a rate of 3.4 ml hr^{-1}. Each rat received 10 mmol kg^{-1} hr^{-1} ethanol or an isocaloric amount of glucose overnight (15 hr). A total of 7 g kg^{-1} was infused. The next day the rats were infused with a triolein emulsion for 3 hr. Each hour of the 3-hr infusion the rat received either 13 mmol kg^{-1} ethanol or isocaloric glucose. The infusate also contained 30 mM triolein, 20 mM taurocholate, and 0.4–1 μCi of [^{3}H]glyceryl-labeled triolein. The infusate constituents were sonified with 0.15 M NaCl supplemented with 0.01 M Tris-HCl (pH 7.0) to a stable emulsion and was delivered at a rate of 4.5 ml hr^{-1}. After 3 hr of infusion, the rat was given an overdose of pentobarbitol. When nacrosis was induced, the rat was removed from the cage and heart blood was obtained. The proximal ¾ of the intestine was removed. Its contents were flushed with 15 ml of 0.15 M NaCl (2°C) into a tared beaker which was subsequently weighed to determine volume. Five ml was extracted as previously (17, 19) using 1 N HCL to break the phases. The remainder was stored frozen at −20°C. The intestine was opened on an iced glass plate and washed with a fine stream of 2% Triton-X 100 followed by 0.15 M NaCl. This washing procedure has been shown to remove 97% of adherant lipid radioactivity (17). The mucosa was scraped down to the level of the crypts, using a glass microscope slide, into 20 ml of 0.15 M NaCl at 2°C. The mucosa was homogenized in a glass-Teflon homogenizer and a 1-ml sample was extracted of its lipid content.

In some experiments brush border vesicles were prepared from rats that had been infused with ethanol (or glucose) and the triolein emulsion as above (20). In other experiments, intestinal rings from everted jejunum were prepared (21) from rats infused with either ethanol or glucose and subsequently with the trioleoylglycerol emulsion supplemented with ethanol (or glucose) for 3 hr. The rings, approximately 1 mm in width (wet weight was 300–400 mg) were incubated at 2°C in a histology cricket in a medium (20 ml) containing 10 mM taurocholate, 2 mM oleic acid, 0.15 M NaCl, and 10 mM Tris-HCL, pH 7.4. No ethanol (or glucose) was included in the incubation medium. The buffer was gassed with 95% O$_2$ and 5% CO$_2$ at 0°C for 30 min before the addition of oleic acid, which was supplemented with [^{14}C]oleate (New England Nuclear Corp., Boston, MA) to give approximately 8,000 cpm/ml of incubation fluid. The medium was stirred at 80 rpm with a magnetic stirring bar. The incubation was for 5 min at 2°C and was terminated by rinsing in three separate exchanges of cold 0.1 M NaHPO$_4$ buffer, pH 7.4. The rings were then extracted (19) and radioactivity was determined. In separate experiments, [^{14}C]mannitol was utilized to determine the amount of incubation fluid that was not removed by the washing procedure. This was equivalent to 34 μl. Uptake of [^{14}C]oleate at 0 time (dunking the cricket containing the tissue in the incubation medium followed by immediate extraction) was subtracted from uptake at 5 min of incubation. Under these conditions uptake was directly proportional for the time of incubation and quantity of tissue.

Enzyme assays

Sucrase was assayed by the two-step method of Dahlqvist (22). Alkaline phosphase activity was determined by the method of Bessey, Lowry, and Brock (23). Enzyme activities were performed in a thermostatic water bath (±0.5°) with heating and cooling capabilities (Fisher Scientific) using intestinal brush border vesicle preparations as protein sources. Lipase was measured using a Radiometer pH stat (Radiometer Corp., Co-
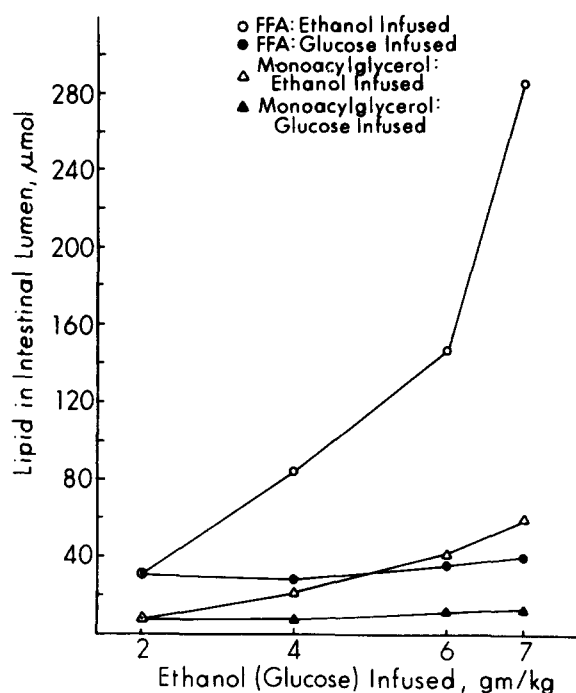
Fig. 1. The effect of increasing the amount of ethanol infused overnight on neutral lipid absorption. The data are the average for two rats at each infusion level. Ethanol, in g kg^{-1} body weight, was infused overnight (15 hr) as shown on the abscissa. During lipid infusion (3 hr), ethanol (glucose) delivery was increased 30% per hr. For example, the 7 g kg^{-1} infusion represents 10 mmol kg^{-1} hr^{-1} ethanol overnight and 13 mmol kg^{-1} hr^{-1} during the 3-hr lipid infusion. Data from ethanol-infused rats are shown by the open symbols and data from glucose-infused rats are shown by the closed symbols.

penhagen, Denmark) with tributyrin as substrate. 1 U of lipase activity is 1 μmol FFA released per min.

Chemical methods

Neutral lipids were separated on silica gel G layers using the solvent system hexanes–diethyl ether–acetic acid–methanol 80:20:2:6 (v/v). The lipids were identified by using I$_2$ vapors and the co-chromatography of authentic standards. The bands were scraped from the plate, the lipid was eluted, and the ester bonds were quantitated by the method of Snyder and Stevens (24). Recovery from the silica gel was 100% for TG and DG, 95% for MG. FFA was assayed phototitrametrically by the method of Mozinger (25).

Phospholipids were separated on silica gel G layers using TLC plates developed in chloroform–methanol–acetic acid–water 25:15:4:2 (v/v) except for sphingomyelin which was separated by a 25:15:9.5:4 (v/v) solvent mixture. The lipids were identified by the co-chromatography of authentic standards. They were quantitated as previously described (26). DNA, RNA, and protein were quantitated as before (17, 27). Alcohol was quantitated by the alcohol dehydrogenase method (28).

Radioactivity was determined as previously by liquid scintillation spectrometry (17).

Substrate preparation

Triolein (Sigma Chemical Co. St. Louis, MO), practical grade, was washed repetitively with ethanol. The ethanol was completely removed prior to use by evaporation in a N$_2$-containing atmosphere at reduced pressure using a rotary evaporator (Buchi, Rinco Instrument Co., Greenville, IL).

Synthesis of sn-2-monooleoylglycerol was started by the formation of 1,3 dibenzylidene glycerol from benzaldehyde and glycerol at 90°C under reduced pressure (60–70 mm Hg) (29). The final product was crystallized from ether in a dry ice-ethanol bath. It melted at 81°C (lit. 83°C). Oleoyl 1,3-dibenzylideneglycerol was synthesized by the method of Martin (30) using oleoylchloride (Sigma Chemical Co.) to acylate the 1,3 dibenzylideneglycerol. De-protection was accomplished using triethylborate-boric acid. The product was crystallized from petroleum ether in a dry ice-alcohol bath and filtered in a freezer locker to keep the product solid to enable its filtration. The final product chromatographed as a single spot on TLC, contained no fatty acid by phototitration, and was 90% as the sn-2 isomer as quantitated by the method of Pohle and Mehlenbacher (31).

Sodium oleate was from Sigma Chemical Co.

RESULTS

To determine the effect of increasing the amount of ethanol in the infusate on lipid absorption, a series of experiments was performed whose results are shown in **Fig. 1.** No effect was observed at the lowest infusion rate 3 μmol hr^{-1} (2 g kg^{-1}) but progressive difficulty in absorbing lipid was observed as the ethanol content of the infusion was increased. Both FFA and MG absorption were affected by ethanol with FFA absorption being more impaired. Because 7 g kg^{-1} (10 μmol h^{-1}) of ethanol was associated with the most severe impairment of lipid absorption, this level of ethanol infusion was used in subsequent experiments.

At infusion rates of 10 mmol kg^{-1} hr^{-1} for 15 hr and 13 mmol kg^{-1} hr^{-1} for 3 hr, blood ethanol levels were 574 ± 53 mg dl^{-1}. The rats were sleepy but easily aroused. The total gut fluid alcohol content was 1.69 ± 0.41 mmol. Since the average total volume of intestinal contents was 6.3 ± 0.5 ml for the alcohol-infused rats, the alcohol concentration in the intestinal fluid was 0.27 M or 1.3 g dl^{-1}. This concentration of alcohol is easily obtainable in the upper intestinal tract of man (13). Histologic sections of the intestine showed no gross

differences between the alcohol-infused and control groups. This was substantiated by quantitating cell slough as measured by intestinal luminal DNA content. This was the same in alcohol-infused rats (0.17 ± 0.06 mg) as in controls (0.25 ± 0.07 mg, $P = 0.61$). Total mucosal DNA was nearly the same in the alcohol-infused rats (19.9 ± 0.7 mg) as in controls (20.8 ± 1.1 mg, $P = 0.52$). Therefore only a small fraction (0.9% in the alcohol-infused group and 1.2% in controls) of the total mucosal cells had the potential of sloughing into the lumen during the experiment.

Lipase activity was significantly greater in the intestinal lumen of the alcohol group (4.4×10^3 U) than in the isocaloric glucose controls ($2.2 \pm 0.2 \times 10^3$ U, $P < 0.02$). Thus the ability to hydrolyze infused TG was equivalent in both groups.

As shown in **Fig. 2**, the amount of neutral lipids present at the end of the 3-hr TG infusion was greater for each neutral lipid species in the ethanol-infused rats as compared to those given glucose. This increment was especially notable for MG, which was increased 5-fold in the lumen of the ethanol-infused rats. The FFA content in the intestinal lumen of the ethanol-infused rats was also significantly (7-fold) greater than that found in controls. Also displayed in Fig. 2 is the neutral lipid content in the mucosa. As might be expected if less total lipid entered the mucosal cell in the ethanol-infused rat, the mucosal TG content was approximately ½ that of the rats receiving glucose. No differences were found between groups in the quantities of the partial glycer-

ides present in the mucosa. These partial glycerides are precursors of TG and were much nearer their fasting levels than was TG (17) after lipid infusion. Therefore they should be less responsive to differing rates of lipid intake than was the more slowly turning over final product, TG.

These data strongly suggested that there was an impairment of uptake by the intestinal mucosa of hydrolyzed TG in the alcohol-infused rats. In order to more clearly determine if this was the case, rats were infused with 2-monooleoylglycerol or, in separate experiments, sodium oleate in amounts approximating that expected to be generated from the trioleoylglycerol as previously infused. The results are given in **Table 1.** In the first set of experiments, 382 μmol of *sn*-2-monooleoylglycerol together with either ethanol or glucose was infused over a 3-hr period into rats that had been prepared by infusing either ethanol or isocaloric glucose overnight as before. At the end of the infusion there was a 3.8-fold increment (Table 1) in luminal MG content on alcohol infusion which should be compared to the 5-fold difference observed when trioleoylglycerol was infused. Secondly, when 811 μmol of sodium oleate was infused over a 3-hr period, 4.2-fold more FFA was recovered in the lumen of the ethanol-infused rats vs. controls (Table 1). This should be compared to the 7-fold difference found when trioleoylglycerol was infused. In sum, these experiments directly demonstrate that ethanol infusion inhibits the absorption of the end products of lipolysis of the original probe lipid, tri-
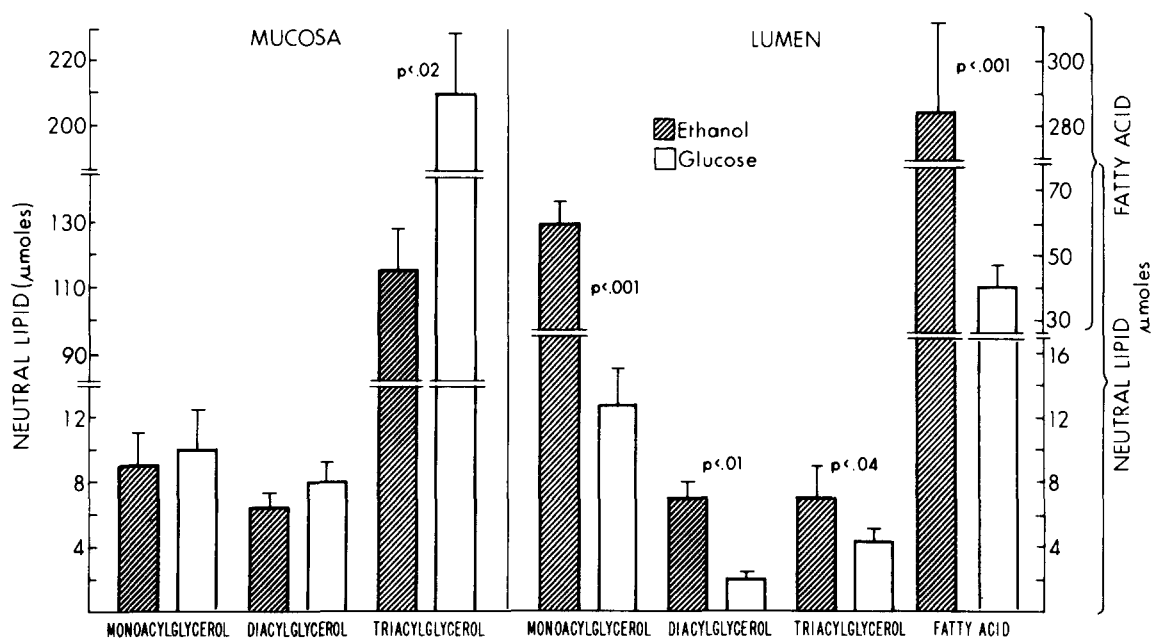
Fig. 2. The lipid content of the intestinal mucosa and lumen as affected by ethanol infusion. The lipid content of the mucosa is shown in the left panel and the luminal lipids in the right panel. Note the change in scale for FFA content. The specific neutral lipid species are given on the abscissa. The data are the mean ± 1 SEM. *P* values, where significant, are given at the top of the data bars.

TABLE 1. Luminal content of FFA and monoacylglycerol after their perfusion in concomitantly ethanol- or glucose-infused rats[a]

	Luminal Contents	
Lipid Infusion	Ethanol Rats	Glucose Rats
Monoacylglycerol (127 μmol hr^{-1}, N = 4)	14.0 ± 1.9 μmol	3.7 ± 0.3 μmol[b]
FFA (270 μmol hr^{-1}, N = 7)	162 ± 19.5 μmol	38.3 ± 6.7 μmol[c]

[a] Ethanol (glucose) 10 mmol kg^{-1} hr^{-1} overnight, or 13 mmol kg^{-1} hr^{-1} during lipid infusion (3 hr). The data are the means ± SEM of MG (FFA) recovered in the lumen of the six MG (FFA)-infused rats.
[b] $P < 0.01$, compares ethanol to glucose rats infused with MG.
[c] $P < 0.0001$, compares ethanol to glucose rats infused with FFA.

oleoylglycerol. Further, the data support the thesis that impaired lipolysis, either due to a lack of lipase within the lumen or an inhibitory effect of ethanol on lipase activity, is not the cause of the lipid malabsorption.

To observe whether or not ethanol could impair FFA uptake by mucosal cells more directly, experiments were performed using jejunal rings prepared from rats infused with either ethanol or isocaloric glucose overnight. The rings were incubated with [^{14}C]oleate for 4 min at 2°C in a medium containing neither ethanol nor glucose so that only the uptake step of the probe FFA would be observed. No conversion of FFA into partial or triacylglycerols occurred under these conditions. In these experiments it was found that intestinal rings prepared from in vivo ethanol-infused rats had a reduced uptake of FFA (2.0 ± 0.26 nmol/min) as compared to rings prepared from in vivo glucose-infused rats (2.9 ± 0.22 nmol/min $P < 0.05$). These data indicate that ethanol infusion makes the intestinal brush border less permeable to FFA and suggest that ethanol infusion might in some measurable way alter the microvillus membrane of the mucosal cells.

This supposition was tested by analyzing the phospholipids and neutral lipids in brush border vesicles prepared from alcohol- or glucose-infused rats. The brush border vesicles prepared from each group of rats was equally contaminated with other subcellular fractions. The RNA content was 1.6 μg ± 0.04 per mg of protein in the ethanol group and 1.7 ± 0.15 μg per mg of protein in the glucose group. The DNA content was 1.8 ± 0.2 μg per mg of protein in the ethanol group and 2.0 ± 0.2 μg per mg of protein for the glucose group. The total vesicle protein content was also similar, 19.8 ± 1.1 vs. 18.0 ± 2.2 mg in the ethanol and glucose groups, respectively. Furthermore, the vesicles demonstrated a high degree of purity as judged by the fact that sucrase specific activity in the vesicles was 23-fold that of the whole mucosa.

Phospholipid analysis of the vesicles is shown in **Table 2.** As can be seen, ethanol infusion significantly reduced

the vesicle content of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and lysophosphatidylcholine (LPC) while the other phospholipids measured remained unchanged. The increased amount of LPC present as compared to other studies (32, 33) is not entirely due to absorbed LPC since brush border vesicles isolated under similar conditions from non-TG-perfused rats had a LPC/LPC + PC ratio of 0.24 as compared to 0.25 and 0.28 in the data presented in Table 2. Nor is the increased LPC due to the fact that Ca^{2+} was used to precipitate the vesicles. When Mg^{2+} was substituted for Ca^{2+}, the LPC/LPC + PC ratio was 0.21. The most likely explanation is that a chelating agent was not used in the original homogenization of the mucosa. When

TABLE 2. Lipid content of rat intestinal brush border vesicles and whole mucosal cells from triolein-infused rats supplemented with ethanol or glucose

	Infusion	
	Ethanol	Glucose
	nmol / mg protein	
Brush border vesicles		
Phospholipid		
Phosphatidylcholine	77 ± 5.5	98 ± 1.9[a]
Phosphatidylserine	52 ± 6.8	62 ± 6.3
Phosphatidylethanolamine	48 ± 5.9	62 ± 5.5[a]
Sphingomyelin	66 ± 3.3	71 ± 5.0
Lysophosphatidylcholine	26 ± 2.3	38 ± 4.2[a]
Neutral lipid		
Monoacylglycerol	91 ± 9.6	108 ± 18
Diacylglycerol	30 ± 2.5	29 ± 6.0
Triacylglycerol	14 ± 3.1	14 ± 1.3
Cholesterol	198 ± 21	205 ± 15
Mucosa		
Phospholipid		
Phosphatidylcholine	142 ± 2.5	159 ± 14
Phosphatidylserine	48 ± 3.5	52 ± 6.8
Phosphatidylethanolamine	28 ± 1.8	35 ± 2.1[a]
Lysophosphatidylcholine	26 ± 0.4	26 ± 2.3

[a] $P < 0.05$.

this is done, considerably less LPC has been reported (32, 33). Therefore, the phospholipid data presented in Table 2 are to be viewed solely in a comparative sense as presented.

Also shown in Table 2 is the neutral lipid content of the vesicles. No differences were seen in any of the lipids measured. The neutral lipid content of the whole mucosa is given in Fig. 2 where TG is the predominant neutral glyceryl lipid species present.

Of additional interest is the phospholipid composition of the mucosa shown at the bottom of Table 2. There were no differences observed between the groups except for the PE content of the ethanol group which was reduced when compared to the control group. Furthermore, it should be noted that the whole cell homogenate contained more PC and less PE than brush border vesicles on a per mg of protein basis.

Because of the alterations in phospholipid composition of the brush border vesicles, experiments were performed to determine if this resulted in changes in the activation energy of alkaline phosphatase, an enzyme deeply embedded in the brush border membrane, which normally occurs in the 25–30°C range (34). The response of this enzyme to temperature change was compared to sucrase, another constituent enzyme of the brush border. This enzyme essentially lies free on the luminal side of the membrane being attached only through isomaltase to the brush border (35). Sucrase would not be expected to be affected by the melting point of the acyl groups of the vesicle's constituent phospholipids. This has been found to be the case (34). Shown in **Fig. 3** are data of both alkaline phosphatase and sucrase activities in ethanol- and glucose-infused rat brush border vesicles. The data are plotted as suggested by Arrhenius. As can be seen, the activation energy of alkaline phosphatase is bi-functional. The energy of activation of the enzyme on both sides of the presumed melting point of the vesicle acyl groups was unaffected by the inclusion of ethanol in the infusate. Thus the alteration in phospholipid composition of the brush border vesicles was not severe enough to change this parameter of vesicle function. In these studies ethanol was not included in the incubation medium so that the effects of phospholipid depletion itself could be observed. Sucrase, as might be expected, also was unaffected by ethanol infusion. The activation energies for both enzymes agree closely with those originally found by Brasitas, Schacher, and Mamouneus (34).

It had been noted in a previous study that the specific activity of the partial glycerides and TG was significantly
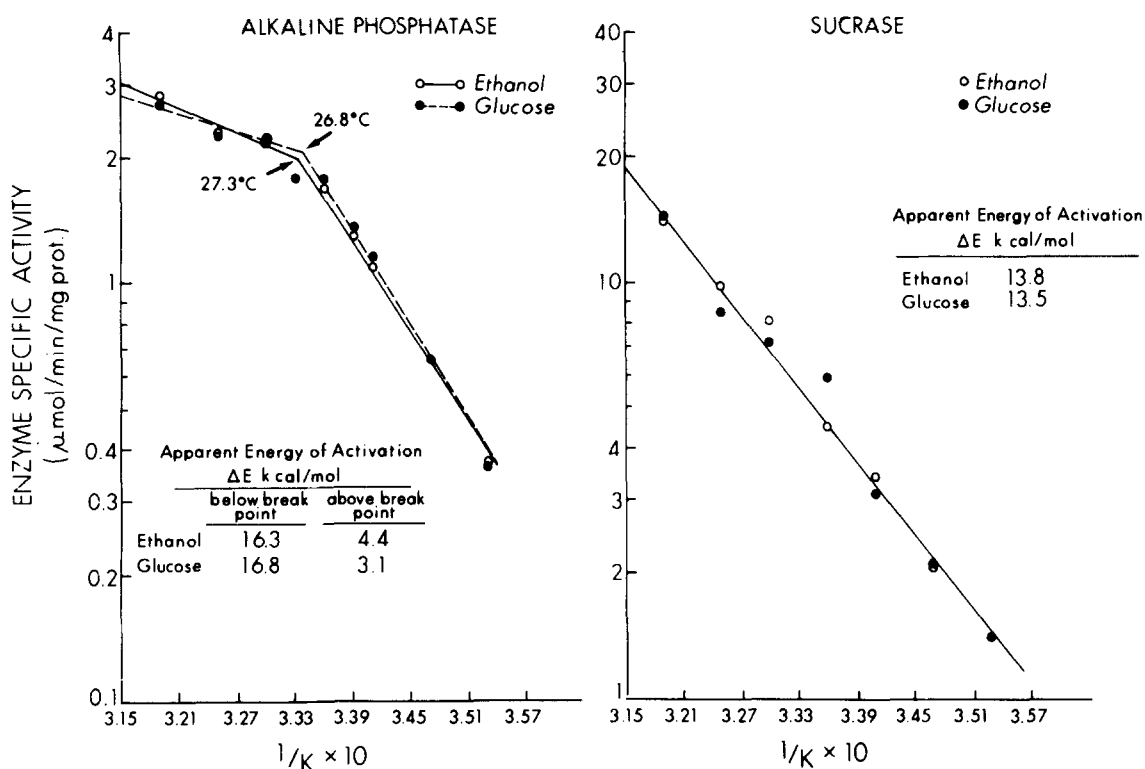

Fig. 3. Arrhenius plot of alkaline phosphatase (left panel) and sucrase (right panel) in brush border vesicle preparations of glucose- and ethanol-infused rats. Inserts in both panels give the apparent energy of activation of both enzymes. The data are the mean of four experiments.

lower in the intestinal lumen than the specific activity of constantly infused trioleoyl[^{3}H]glycerol (17). The reduction in luminal specific activity was documented to be secondary to effluxed neutral lipid from the intestinal mucosa. Therefore it was of interest to observe in the present studies whether ethanol administration influenced the efflux of neutral lipids from the mucosa into the lumen. The specific activity of neutral lipids after constant infusion of trioleoyl[^{3}H]glycerol in both the mucosa and lumen is shown in **Fig. 4.** As can be seen in the figure, the specific activities of the neutral lipid species are <46% of the infusate specific activity in both groups, both in the luminal fluid and mucosa. This suggests that in both groups significant amounts of endogenously synthesized glycerol contribute to glyceride-glycerol in the mucosa and subsequently, after its efflux, to the lumen. It should be noted that there are no significant differences in the specific activities between the control and experimental groups. However, since the quantity of neutral lipid in the intestinal lumen was significantly greater for the ethanol group as compared to controls, the quantity of lipid effluxed must also have been greater. It is evident that only by increasing the luminal efflux of low specific activity lipids could the large amount of luminal neutral lipid in the ethanol-

infused group have a specific activity similar to that of the controls.

The specific activity of the fatty acids in the lumen was also determined. It was found after the constant infusion of tri(^{14}C oleoyl)glycerol that the FFA in the lumen had a specific activity that was 90 ± 4% of the specific activity of the infusate's acyl groups. Interestingly, the specific activity of the luminal FFA in the ethanol-infused group was significantly less, 74 ± 6% (P < 0.05, n = 7). These data would suggest that significantly more endogenous acyl groups contributed to luminal FFA than were present in control animals.

Additional information provided by these studies was the observation that the vast majority of FFA radioactivity was in the TG fraction in the intestinal mucosa at the end of the infusion (90 ± 5% for glucose-infused, and 92 ± 3% in ethanol-infused rats). These data indicate that, as expected, FFA, once taken up by the enterocyte, is rapidly acylated to TG in normally functioning intestinal mucosa.

DISCUSSION

The experiments described in the present report demonstrate a severe impairment of lipid absorption in
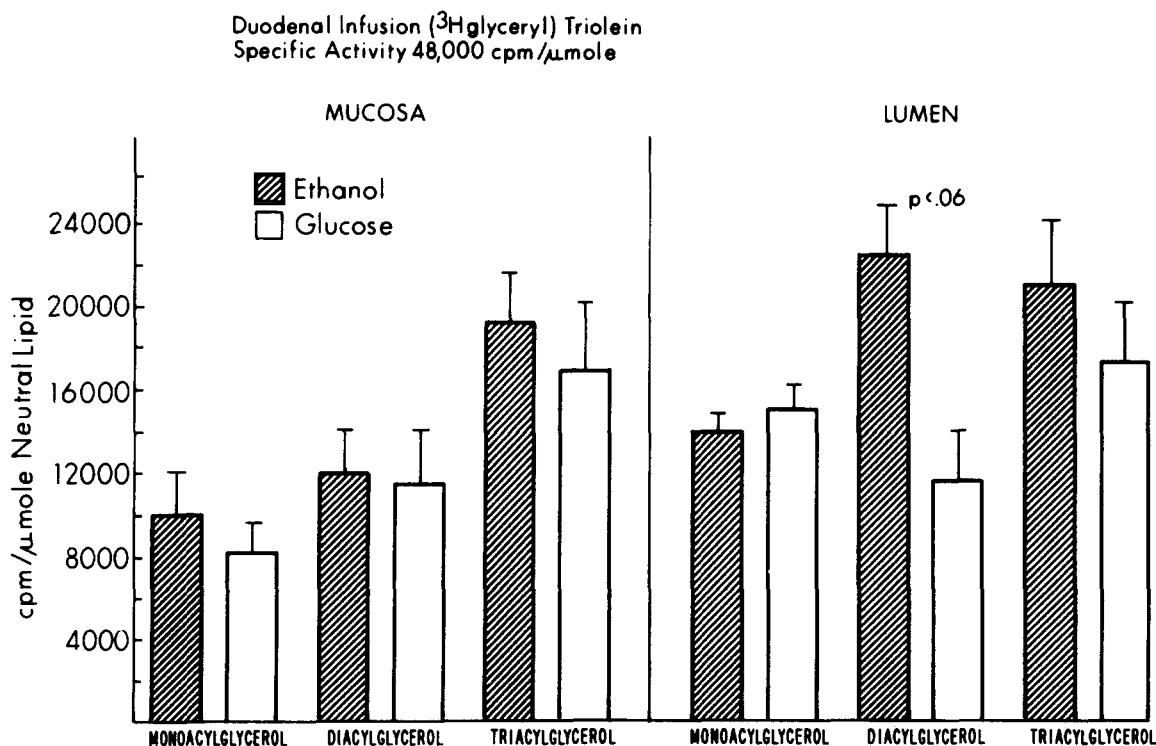

Fig. 4. Neutral lipid specific activity in intestinal mucosa (left panel) and intestinal lumen (right panel) in ethanol- and glucose-infused rats. The specific activity of the neutral lipids is given on the ordinate. The specific activity of the infused trioleoyl [^{3}H]glycerol was 48,000 cpm μmol^{-1} and is shown in the insert. The data are the mean ± 1 SEM.

the rat as a consequence of ethanol infusion. The experimental design allowed for observations to be made at large lipid loads, thus stressing the lipid absorptive system. Further, a constant perfusion technique through a duodenal cannula was utilized, thus bypassing the problem of gastric emptying. It is important to note that lipid malabsorption was found at concentrations of ethanol in the intestinal lumen similar to those found in the human after moderate ethanol intake (0.8 g/kg) (13). However, in the human, ethanol consumption was only intermittent whereas in the present studies in the rat, ethanol was administered constantly.

In fact, ethanol has been questioned as the causative agent of steatorrhea in man in the absence of chronic pancreatitis, neomycin administration, or bile acid deficiency secondary to alcoholic liver disease, (1, 2, 4, 5, 36). Nevertheless, steatorrhea without an obvious explanation has been demonstrated in man (1, 36).

Malabsorption of lipid in response to ethanol has also been difficult to demonstrate in the rat given ethanol either chronically (37) or acutely (38). However, when large amounts of corn oil and ethanol were given by gavage (15), a reduced efficiency of lipid absorption was suggested by the finding that although most (79%) of the oil remained in the stomach of the ethanol-infused rat as compared to 10% for controls, the same amount of lipid was found in the intestinal lumen in both groups of rats.

Possible explanations for the reduced uptake of lipid as shown in the present studies include a lipolytic defect, a problem of solubilizing the products of lipolysis, impaired entry of lipid into the mucosal cells, and enhanced efflux of lipid out of the mucosal cells into the lumen.

A potential lipolytic defect as a causative factor has been effectively eliminated by the present investigation. Lipase activity was found to be above control levels in the intestine and secondly, FFA was present in gross excess over controls. Thirdly, on infusion of the products of lipolysis of trioleoylglycerol, oleate, and sn-2-monooleoylglycerol, impaired absorption of these compounds was demonstrated as well. The reduction in absorption was nearly as great as when trioleoylglycerol itself was infused. These data support previous infusion data in humans (18).

As regards the solubilization of the products of lipolysis, taurocholate, the major bile acid of the rat, was supplied with the infusate at concentrations well above its critical micellar concentration (CMC). Ethanol's effects on the bile acid micelle have not been extensively studied. However, at high concentrations (7 M or greater), ethanol has been shown to completely suppress bile acid micelle formation (39). Ethanol's effect on both

anionic and cationic detergent micelles has been investigated in greater detail. In each instance (sodium dodecyl sulfate (40) and trimethylammonium bromide (41)) micelle formation was also suppressed. Of particular importance to the present investigation are the studies of Matuura et al. (42) in which the solubility of oleate in sodium dodecyl sulfate micelles was suppressed by a series of alcohols. This effect was more pronounced, however, in diect proportion to the chain length of the alcohol. In sum, although not well studied, ethanol at concentrations found in the intestinal lumen would be expected to have only a modest effect on oleate solubility in the taurocholate micelle.

A major result of this study was the finding that lipid, whether presented as TG or as the hydrolytic products of TG, was poorly absorbed in the presence of ethanol, thus strongly suggesting impaired entry of lipid into the mucosal cell. One potential explanation of this effect is that ethanol altered the brush border membrane composition in such a way that MG and FFA were less able to penetrate it than in the absence of ethanol. A direct example of this reduced penetrability was the finding that FFA uptake into intestinal rings was impaired by prior ethanol infusion. It is important to note that this effect of ethanol was unlikely to be due to the presence of ethanol itself in the membrane but rather to the longer term effects of ethanol as discussed below. Any ethanol present in the brush border membrane would likely have been lost to the isolation and incubation media.

As shown, ethanol did indeed alter the phospholipid composition of the brush border membrane in that several phospholipids (PC, LPC, and PE) were decreased in concentration by ethanol infusion as compared to controls. This reduction might well have been due to a local effect of ethanol on the membranous surface exposed to the highest concentrations of the drug and not a systemic effect, since only a minimal decrease in phospholipid content could be found in the whole intestinal cell preparation. The data on the whole mucosal cell agrees with the phospholipid content of erythrocyte membranes (43, 44) and brain synaptosomes (43) which is unaltered by ethanol feeding. By contrast, rats and mice on chronic ethanol diets have a reduced content of PC and PE in their hepatic membranes (45), i.e., the same phospholipids that were reduced in the brush border membranes. Ethanol feeding also alters the acyl group composition of the phospholipids of hepatic membranes to more saturated species (46) with consonant physicochemical changes related to their higher transition temperature (46, 47). The consequences of these demonstrated or potential alterations in membrane composition on the ability of lipids to penetrate the en-

terocyte microvillus membrane requires further investigation.

The data thus far presented most clearly suggest an impaired uptake of the products of TG lipolysis as a major cause for retained lipid within the lumen of the bowel. A second major cause for the increased mass of lipid found in the bowel documented in the present study is an enhanced efflux of lipid from the mucosa back into the lumen. This efflux has been previously documented from this laboratory (17). That the efflux of lipid is increased as compared to controls is shown by Fig. 4. Here, the mass of lipid in the bowel lumen was greatly increased, but the specific activity of the neutral lipids in the lumen was the same as controls. Therefore more nonradioactive lipid must have effluxed from the mucosa to dilute the specific activity of the large mass of lipid. These data strongly suggest that this effluxed lipid is another major contributor to the lipid content of the intestinal lumen in the ethanol-infused rat.

In considering which lipid species is likely to efflux, FFA and MG do not seem likely candidates. The absorption of FFA and MG is passive and has been demonstrated to be reduced in the present study. Thus the reverse movement (efflux) of lipid species would also be expected to be slowed as compared to controls. Therefore the most likely lipid species to efflux would be mucosal TG since mucosal DG is membrane-bound (48, 49). In support of this conclusion it should be noted that the FFA specific activity in the lumen was modestly reduced in the alcohol-infused rat as compared to controls. Mucosal TG, whose acyl group specific activity has been shown to be reduced as compared to the acyl group specific activity of the infusation (17), could provide a source for luminal nonradiolabeled FFA.

There are three possibilities to explain the manner in which TG might efflux from the mucosal cell. In the first, TG would dissolve in the brush border membrane and efflux back out into the intestinal lumen. TG has, in fact, been demonstrated to dissolve in model membranes, although to a minor extent (50), and to be present at the aqueous surface in the presence of PC monolayers (51). Even though the quantity of TG in the membrane is likely to be small, the intestinal surface area is large when the microvilli are considered, so that the potential remains that enough TG could exit via this route to account for the data. A second possibility is that the ruptured intestinal blebs seen on ethanol infusion in rats could allow chylomicrons destined for lymphatic absorption to escape instead into the lumen (52). Lastly, the tight junctions between epithelial cells, if loosened by ethanol infusion, could allow the exit of TG into the intestinal lumen. Support for this hypothesis comes from studies in which ethanol has been shown to increase the permeability for macromolecules through the apical tight junctional complexes (16). The present data do not allow for a choice between these possibilities.

When the amount of lipid necessary for efflux to account for the data shown in Fig. 2 and Fig. 4 is calculated, a minimum figure of 13% of the infused neutral lipid would be required. This figure is close to the 11% of FFA derived from infused TG which would be necessary to efflux from the mucosal cells in order to suppress the FFA specific activity to the level obtained in the experiments. These calculations assume that the effluxed lipids were of low specific activity (17). Further calculations reveal that effluxed TG-derived FFA from the mucosa $[288 \ \mu mol \times (1 - 0.74)]$ is 18% of the total acyl groups infused during the 3-hr period. This amount of FFA represents more FFA than was found in the lumen in the control rats (38 μmol).

Thus the acute ethanol-infused rat represents the first model where lipid absorbed from the lumen, but effluxed from the mucosa, contributes significantly to the malabsorption of lipid. Other models of lipid efflux are currently being investigated.∎

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